US 20020115081A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2002/0115081 A1

Lee et al. (43) Pub. Date: Aug. 22, 2002

(54) DIAGNOSIS AND TREATMENT OF CARDIOVASCULAR CONDITIONS

(76) Inventors: **Richard T. Lee**, Weston, MA (US); **Katherine T. Landschulz**, East Lyme, CT (US); **Scott P. Kennedy**, Old Lyme, CT (US); **John F. Thompson**, Warwick, RI (US); **Thomas G. Turi**, Groton, CT (US)

Correspondence Address:
Elizabeth R. Plumer
Wolf, Greenfield & Sacks, P.C.
Federal Reserve Plaza
600 Atlantic Avenue
Boston, MA 02210 (US)

(21) Appl. No.: **09/934,249**

(22) Filed: **Aug. 21, 2001**

Related U.S. Application Data

(60) Provisional application No. 60/227,159, filed on Aug. 22, 2000.

Publication Classification

(51) **Int. Cl.**[7] **C12Q 1/68**; G01N 33/567; A61K 38/17

(52) **U.S. Cl.** **435/6**; 435/7.21; 514/12

(57) **ABSTRACT**

This invention pertains to methods and compositions for the diagnosis and treatment of cardiovascular conditions. More specifically, the invention relates to diagnostics and therapeutics involving isolated molecules that can be used to inhibit cardiac apoptotic cell-death.

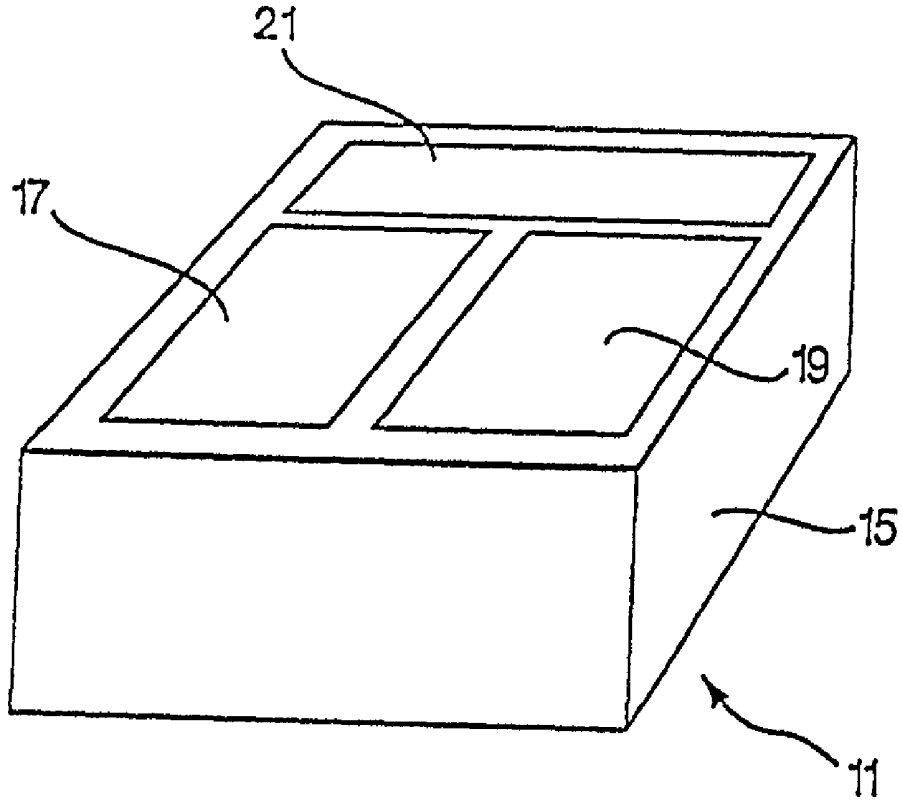

Fig. 1

DIAGNOSIS AND TREATMENT OF CARDIOVASCULAR CONDITIONS

RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. §119(e) from Provisional U.S. patent application Ser. No. 60/227,159 filed on Aug. 22, 2000, entitled DIAGNOSIS AND TREATMENT OF CARDIOVASCULAR CONDITIONS. The contents of the provisional application are hereby expressly incorporated by reference.

FIELD OF THE INVENTION

[0002] This invention relates to methods and compositions for the diagnosis and treatment of cardiovascular conditions. More specifically, the invention relates to isolated molecules that can be used to inhibit cardiac apoptotic cell-death, and in particular, to the treatment of vascular and cardiovascular conditions including myocardial infarction, stroke, arteriosclerosis, and heart failure.

BACKGROUND OF THE INVENTION

[0003] Despite significant advances in therapy, cardiovascular disease remains the single most common cause of morbidity and mortality in the developed world. Thus, prevention and therapy of cardiovascular conditions such as myocardial infarction and stroke is an area of major public health importance. Currently, several risk factors for future cardiovascular disorders have been described and are in wide clinical use in the detection of individuals at high risk. Such screening tests include evaluations of total and HDL cholesterol levels. However, a large number of cardiovascular disorders occur in individuals with apparently low to moderate risk profiles, and ability to identify such patients is limited. Moreover, accumulating data suggests that the beneficial effects of certain preventive and therapeutic treatments for patients at risk for or known to have cardiovascular disorders differs in magnitude among different patient groups. At this time, however, data describing diagnostic tests to determine whether certain therapies can be expected to be more or less effective are lacking.

SUMMARY OF THE INVENTION

[0004] This invention provides methods and compositions for the diagnosis and treatment of cardiovascular conditions. More specifically, we have identified a number of genes that are upregulated in cardiac cells when the cells are subjected to mechanically-induced deformation. It has been discovered, unexpectedly, that such upregulation leads to inhibition of cardiac cell apoptosis. In view of these discoveries, it is believed that the molecules of the present invention can be used to inhibit cardiac cell-death, and in particular, to treat conditions that are characterized by cardiac cell apoptotic cell-death, such as vascular and cardiovascular conditions including myocardial infarction, stroke, arteriosclerosis, and heart failure.

[0005] Additionally, methods for using these molecules in the diagnosis of any of the foregoing vascular and cardiovascular conditions as well as cardiac hypertrophy, are also provided.

[0006] Furthermore, methods for using these molecules in vivo or in vitro for the purpose of modulating apoptotic cell-death, methods for treating conditions associated with such cell-death, and compositions useful in the preparation of therapeutic preparations for the treatment of the foregoing conditions, are also provided.

[0007] The present invention thus involves, in several aspects, polypeptides modulating cardiac cell apoptotic activity, isolated nucleic acids encoding those polypeptides, functional modifications and variants of the foregoing, useful fragments of the foregoing, as well as therapeutics and diagnostics, research methods, compositions and tools relating thereto.

[0008] According to one aspect of the invention, an isolated nucleic acid molecule selected from the group consisting of: (a) nucleic acid molecules which hybridize under stringent conditions to a molecule consisting of a nucleotide sequence set forth as SEQ ID NO:1 and which code for a Mechanically Induced Vascular Receptor 1 (MIVR-1) polypeptide having cardiac cell anti-apoptotic activity, (b) nucleic acid molecules that differ from the nucleic acid molecules of (a) or (b) in codon sequence due to the degeneracy of the genetic code, and (c) complements of (a) or (b), is provided. In certain embodiments, the isolated nucleic acid molecule comprises the nucleotide sequence set forth as SEQ ID NO:1. In some embodiments, the isolated nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:3 or a fragment thereof.

[0009] The invention in another aspect provides an isolated nucleic acid molecule selected from the group consisting of (a) unique fragments of a nucleotide sequence set forth as SEQ ID NO:1, and (b) complements of (a), provided that a unique fragment of (a) includes a sequence of contiguous nucleotides which is not identical to any sequence selected from the sequence group consisting of: (1) sequences having SEQ ID NOs. 14-16, or 17, (2) complements of (1), and (3) fragments of (1) and (2). In any of the foregoing embodiments, complements refer to full-length complements.

[0010] In one embodiment, the sequence of contiguous nucleotides is selected from the group consisting of (1) at least two contiguous nucleotides nonidentical to the sequence group, (2) at least three contiguous nucleotides nonidentical to the sequence group, (3) at least four contiguous nucleotides nonidentical to the sequence group, (4) at least five contiguous nucleotides nonidentical to the sequence group, (5) at least six contiguous nucleotides nonidentical to the sequence group, and (6) at least seven contiguous nucleotides nonidentical to the sequence group.

[0011] In another embodiment, the fragment has a size selected from the group consisting of at least:8 nucleotides, 10 nucleotides, 12 nucleotides, 14 nucleotides, 16 nucleotides, 18 nucleotides, 20, nucleotides, 22 nucleotides, 24 nucleotides, 26 nucleotides, 28 nucleotides, 30 nucleotides, 40 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, 200 nucleotides, 1000 nucleotides and every integer length therebetween.

[0012] According to another aspect, the invention provides expression vectors, and host cells transformed or transfected with such expression vectors, comprising the nucleic acid molecules described above.

[0013] According to another aspect of the invention, an isolated polypeptide is provided. The isolated polypeptide is encoded by the foregoing nucleic acid molecules of the

invention. In some embodiments, the isolated polypeptide is encoded by the nucleic acid of SEQ ID NO:1, giving rise to a polypeptide having the sequence of SEQ ID NO:2 that has cardiac cell anti-apoptotic activity. In other embodiments, the isolated polypeptide may be a fragment or variant of the foregoing of sufficient length to represent a sequence unique within the human genome, and identifying with a polypeptide that has cardiac cell anti-apoptotic activity, provided that the fragment includes a sequence of contiguous amino acids which is not identical to any sequence encoded for by a nucleic acid sequence selected from the group consisting of SEQ ID NOs. 14-16, and 17. In another embodiment, immunogenic fragments of the polypeptide molecules described above are provided. The immunogenic fragments may or may not have cardiac cell anti-apoptotic activity.

[0014] According to another aspect of the invention, isolated binding polypeptides are provided which selectively bind a polypeptide encoded by the foregoing nucleic acid molecules of the invention. Preferably the isolated binding polypeptides selectively bind a polypeptide which comprises the sequence of SEQ ID NO:2, or fragments thereof. In preferred embodiments, the isolated binding polypeptides include antibodies and fragments of antibodies (e.g., Fab, $F(ab)_2$, Fd and antibody fragments which include a CDR3 region which binds selectively to the MIVR-1 polypeptide). In certain embodiments, the antibodies are human. In some embodiments, the antibodies are monoclonal antibodies. In one embodiment, the antibodies are polyclonal antisera. In further embodiments, the antibodies are humanized. In yet further embodiments, the antibodies are chimeric.

[0015] According to a further aspect of the invention, a method for determining the level of MIVR-1 expression in a subject, is provided. The method involves measuring expression of MIVR -1 in a test sample from a subject to determine the level of MIVR-1 expression in the subject. In certain embodiments, the measured MIVR-1 expression in the test sample is is compared to MIVR-1 expression in a control containing a known level of MIVR-1 expression. Expression is defined as MIVR-1 mRNA expression, MIVR-1 polypeptide expression, or MIVR-1 cardiac cell anti-apoptotic activity as defined elsewhere herein. Various methods can be used to measure expression. Preferred embodiments of the invention include PCR and Northern blotting for measuring mRNA expression, MIVR-1 monoclonal antibodies or MIVR-1 polyclonal MIVR-1 antisera as reagents to measure MIVR-1 polypeptide expression, as well as methods for measuring MIVR-1 cardiac cell anti-apoptotic activity.

[0016] In certain embodiments, test samples such as biopsy samples, and biological fluids such as blood, are used as test samples. MIVR-1 expression in a test sample of a subject is compared to MIVR-1 expression in control.

[0017] According to another aspect of the invention, a method for identifying an agent useful in modulating cardiac cell anti-apoptotic activity of a molecule, is provided. The method involves (a) contacting a molecule having cardiac cell anti-apoptotic activity with a candidate agent, (b) measuring cardiac cell anti-apoptotic activity of the molecule, and (c) comparing the measured cardiac cell anti-apoptotic activity of the molecule to a control to determine whether the candidate agent modulates cardiac cell anti-apoptotic activity of the molecule, wherein the molecule is a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1 (SEQ ID NO. 4), VDUP-1 (SEQ ID NO. 6), BTG-2 (SEQ ID NO. 8), and TIS-11d (SEQ ID NO. 10), or an expression product thereof (e.g., SEQ ID NOs. 2, 5, 7, 9, and 11, respectively). In certain embodiments, the control is cardiac cell anti-apoptotic activity of the molecule measured in the absence of the candidate agent.

[0018] According to still another aspect of the invention, a method of diagnosing a condition characterized by aberrant expression of a nucleic acid molecule or an expression product thereof, is provided. The method involves contacting a biological sample from a subject with an agent, wherein said agent specifically binds to said nucleic acid molecule, an expression product thereof, or a fragment of an expression product thereof, and measuring the amount of bound agent and determining therefrom if the expression of said nucleic acid molecule or of an expression product thereof is aberrant, aberrant expression being diagnostic of the disorder, wherein the nucleic acid molecule is at least one nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d. In some embodiments, the disorder is a cardiovascular condition selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure. In one embodiment, the disorder is cardiac hypertrophy.

[0019] According to still another aspect of the invention, a method for determining regression, progression or onset of a vascular condition in a subject characterized by aberrant expression of a nucleic acid molecule or an expression product thereof, is provided. The method involves monitoring a sample from a patient for a parameter selected from the group consisting of (i) a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, (ii) a polypeptide encoded by the nucleic acid molecule, (iii) a peptide derived from the polypeptide, and (iv) an antibody which selectively binds the polypeptide or peptide, as a determination of regression, progression or onset of said vascular condition in the subject. In some embodiments, the sample is a biological fluid or a tissue as described in any of the foregoing embodiments. In certain embodiments, the step of monitoring comprises contacting the sample with a detectable agent selected from the group consisting of (a) an isolated nucleic acid molecule which selectively hybridizes under stringent conditions to the nucleic acid molecule of (i), (b) an antibody which selectively binds the polypeptide of (ii), or the peptide of (iii), and (c) a polypeptide or peptide which binds the antibody of (iv). The antibody, polypeptide, peptide, or nucleic acid can be labeled with a radioactive label or an enzyme. In further embodiments, the method further comprises assaying the sample for the peptide. In still further embodiments, monitoring the sample occurs over a period of time.

[0020] According to another aspect of the invention, a kit is provided. The kit comprises a package containing an agent that selectively binds to any of the foregoing MIVR-1 isolated nucleic acids, or expression products thereof, and a control for comparing to a measured value of binding of said agent any of the foregoing MIVR-1 isolated nucleic acids or expression products thereof. In some embodiments, the control is a predetermined value for comparing to the measured value. In certain embodiments, the control comprises an epitope of the expression product of any of the foregoing MIVR-1 isolated nucleic acids. In one embodi-

ment, the kit further comprises a second agent that selectively binds to an isolated nucleic acid molecule selected from the group consisting of IEX-1, VDUP-1, BTG-2, TIS-11d, and/or an expression product thereof, and a control for comparing to a measured value of binding of said second agent to said isolated nucleic acid molecule or expression product thereof.

[0021] According to a further aspect of the invention, a method of treating apoptotic cell-death of a cardiac cell in a subject, is provided. The method involves administering to a subject in need of such treatment an agent that modulates expression of a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, in an effective amount to inhibit apoptotic cell-death of the cardiac cell in the subject. In certain embodiments, the cardiovascular condition is selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure. In some embodiments, the method further comprises co-administering an agent selected from the group consisting of an anti-inflammatory agent, an anti-thrombotic agent, an anti-platelet agent, a fibrinolytic agent, a lipid reducing agent, a direct thrombin inhibitor, a glycoprotein IIb/IIIa receptor inhibitor, an agent that binds to cellular adhesion molecules and inhibits the ability of white blood cells to attach to such molecules, a calcium channel blocker, a beta-adrenergic receptor blocker, a cyclooxygenase-2 inhibitor, or an angiotensin system inhibitor.

[0022] According to one aspect of the invention, a method for treating a cardiovascular condition, is provided. The method involves administering to a subject in need of such treatment a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, in an amount effective to treat the cardiovascular condition. In certain embodiments, the cardiovascular condition is selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure. In some embodiments, the method further comprises co-administering an agent selected from the group consisting of an anti-inflammatory agent, an anti-thrombotic agent, an anti-platelet agent, a fibrinolytic agent, a lipid reducing agent, a direct thrombin inhibitor, a glycoprotein IIb/IIIa receptor inhibitor, an agent that binds to cellular adhesion molecules and inhibits the ability of white blood cells to attach to such molecules, a calcium channel blocker, a beta-adrenergic receptor blocker, a cyclooxygenase-2 inhibitor, or an angiotensin system inhibitor.

[0023] According to one aspect of the invention, a method for inhibiting apoptotic cell-death of a cell, is provided. The method involves contacting an isolated nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, with a cell under conditions that permit entry of the nucleic acid molecule into the cell, in an amount effective to inhibit apoptotic cell-death of the cell. In some embodiments, the cell is selected from the group consisting of a cardiomyocyte and a vascular endothelial cell.

[0024] According to still another aspect of the invention, a method for treating a condition mediated by increased apoptotic cell-death of vascular endothelial cells in a subject, is provided. The method involves administering to a subject in need of such treatment a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, in an amount effective to inhibit increased apoptotic cell-death of vascular endothelial cells. In some embodiments, the molecule is a nucleic acid. In certain embodiments, the molecule is a polypeptide.

[0025] According to another aspect of the invention, a method for treating cardiac hypertrophy, is provided. The method involves administering to a subject in need of such treatment an agent that increases cardiac cell-death, in an amount effective to treat cardiac hypertrophy in the subject, wherein the agent that increases cardiac cell-death is an inhibitor of a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof.

[0026] According to a further aspect of the invention, a method for treating a subject to reduce the risk of a cardiovascular condition developing in the subject, is provided. The method involves administering to a subject who is known to express decreased levels of a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, an agent for reducing the risk of the cardiovascular disorder in an amount effective to lower the risk of the subject developing a future cardiovascular disorder, wherein the agent is an anti-inflammatory agent, an anti-thrombotic agent, an anti-platelet agent, a fibrinolytic agent, a lipid reducing agent, a direct thrombin inhibitor, a glycoprotein IIb/IIIa receptor inhibitor, an agent that binds to cellular adhesion molecules and inhibits the ability of white blood cells to attach to such molecules, a calcium channel blocker, a beta-adrenergic receptor blocker, a cyclooxygenase-2 inhibitor, or an angiotensin system inhibitor, or an agent that modulates expression of a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

[0027] According to one aspect of the invention, a method for identifying a candidate agent useful in the treatment of a cardiovascular condition, is provided. The method involves determining expression of a set of nucleic acid molecules in a cardiac cell or tissue under conditions which, in the absence of a candidate agent, permit a first amount of expression of the set of nucleic acid molecules, wherein the set of nucleic acid molecules comprises at least one nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, contacting the cardiac cell or tissue with the candidate agent, and detecting a test amount of expression of the set of nucleic acid molecules, wherein an increase in the test amount of expression in the presence of the candidate agent relative to the first amount of expression indicates that the candidate agent is useful in the treatment of the cardiovascular condition. In certain embodiments, the cardiovascular condition is selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure. In some embodiments, the set of nucleic acid molecules comprises at least two, at least three, at least four, or even at least five nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

[0028] According to another aspect of the invention, a method for identifying a candidate agent useful in the treatment of cardiac hypertrophy, is provided. The method involves determining expression of a set of nucleic acid molecules in a cardiac cell or tissue under conditions which, in the absence of a candidate agent, permit a first amount of

expression of the set of nucleic acid molecules, wherein the set of nucleic acid molecules comprises at least one nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, contacting the cardiac cell or tissue with the candidate agent, and detecting a test amount of expression of the set of nucleic acid molecules, wherein a decrease in the test amount of expression in the presence of the candidate agent relative to the first amount of expression indicates that the candidate agent is useful in the treatment of cardiac hypertrophy. In certain embodiments, the set of nucleic acid molecules comprises at least two, at least three, at least four, or even at least five nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

[0029] According to another aspect of the invention, a pharmaceutical composition is provided. The composition comprises an agent comprising an isolated nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, preferably in a pharmaceutically effective amount to treat a cardiovascular condition, and a pharmaceutically acceptable carrier. In some embodiments, the agent is an expression product of the isolated nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d. In certain embodiments, the cardiovascular condition is selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure.

[0030] According to yet another aspect of the invention, a pharmaceutical composition for treating cardiac hypertrophy, is provided. The composition comprises an agent that increases cardiac cell-death in a pharmaceutically effective amount to treat cardiac hypertrophy, wherein the agent that increases cardiac cell-death is an inhibitor of a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, and a pharmaceutically acceptable carrier. In some embodiments, the subjects in need of such treatment are known to express increased levels of of a nucleic is acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof.

[0031] According to a further aspect of the invention, methods for preparing medicaments useful in the treatment of a cardiovascular condition and/or cardiac hypertrophy, are provided.

[0032] According to still another aspect of the invention, a solid-phase nucleic acid molecule array, is provided. The array consists essentially of a set of nucleic acid molecules, expression products thereof, or fragments (of either the nucleic acid or the polypeptide molecule) thereof, each nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, fixed to a solid substrate. In some embodiments, the solid-phase array further comprises at least one control nucleic acid molecule. In certain embodiments, the set of nucleic acid molecules comprises at least one, at least two, at least three, at least four, or even at least five nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

[0033] These and other objects of the invention will be described in further detail in connection with the detailed description of the invention.

BRIEF DESCRIPTION OF THE SEQUENCES

[0034] SEQ ID NO:1 is the nucleotide sequence of the human MIVR-1 cDNA.

[0035] SEQ ID NO:2 is the predicted amino acid sequence of the translation product of human MIVR-1 cDNA (SEQ ID NO:1).

[0036] SEQ ID NO:3 is the nucleotide sequence of the human MIVR-1 cDNA encoding the polypeptide of SEQ ID NO:2 (i.e., nucleotides 413-1273 of SEQ ID NO:1).

[0037] SEQ ID NO:4 is the nucleotide sequence of the human IEX-1 cDNA.

[0038] SEQ ID NO:5 is the predicted amino acid sequence of the translation product of human IEX-1 cDNA (SEQ ID NO:4).

[0039] SEQ ID NO:6 is the nucleotide sequence of the human VDUP-1 cDNA.

[0040] SEQ ID NO:7 is the predicted amino acid sequence of the translation product of human VDUP-1 cDNA (SEQ ID NO:6).

[0041] SEQ ID NO:8 is the nucleotide sequence of the human BTG-2 cDNA.

[0042] SEQ ID NO:9 is the predicted amino acid sequence of the translation product of human BTG-2 cDNA (SEQ ID NO:8).

[0043] SEQ ID NO:10 is the nucleotide sequence of the human TIS-11d cDNA.

[0044] SEQ ID NO:11 is the predicted amino acid sequence of the translation product of human TIS-11d cDNA (SEQ ID NO:10).

[0045] SEQ ID NO:12 is the nucleotide sequence of the mouse MIVR-1 cDNA.

[0046] SEQ ID NO:13 is the predicted amino acid sequence of the translation product of mouse MIVR-1 cDNA (SEQ ID NO:12).

[0047] SEQ ID NO:14 is the nucleotide sequence of GenBank Acc. No. AI761441.1, having partial homology to SEQ ID NO:1.

[0048] SEQ ID NO:15 is the nucleotide sequence of GenBank Acc. No. AI594390, having partial homology to SEQ ID NO:1.

[0049] SEQ ID NO:16 is the nucleotide sequence of GenBank Acc. No. NM_{13} 004338, having partial homology to SEQ ID NO:1.

[0050] SEQ ID NO:17 is the nucleotide sequence of GenBank Acc. No. AQ177461, having partial homology to SEQ ID NO:1.

[0051]

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] **FIG. 1** depicts a kit embodying features of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0053] The invention involves the discovery of a number of genes that are upregulated in cardiac cells when the cells

are subjected to a mechanically-induced strain deformation. It has been discovered that such upregulation leads, unexpectedly, to inhibition of cardiac cell apoptosis. In view of these discoveries, it is believed that the molecules of the present invention can be used to inhibit cardiac cell-death, and in particular, to treat conditions that are characterized by cardiac cell apoptotic cell-death, such as vascular and cardiovascular conditions including myocardial infarction, stroke, arteriosclerosis, and heart failure.

[0054] Additionally, methods for using these molecules in the diagnosis of any of the foregoing vascular and cardiovascular conditions as well as cardiac hypertrophy, are also provided.

[0055] Furthermore, methods for using these molecules in vivo or in vitro for the purpose of modulating apoptotic cell-death, methods for treating conditions associated with such cell-death, and compositions useful in the preparation of therapeutic preparations for the treatment of the foregoing conditions, are also provided.

[0056] Apoptosis (also known as programmed cell-death) is a form of cell-death defined by morphological and biochemical characteristics. Apoptosis is a characteristic of the normal developmental process as well as a response of cells to stress or other environmental insults. Apoptosis is characterized by membrane blebbing, cellular and cytoplasmic shrinkage, chromosome fragmentation and condensation, and endonuclease activation resulting in the characteristic 180 bp DNA ladder. During this process, the nuclear lamins are cleaved inducing their disassembly. Apoptosis does not induce an inflammatory response because cells form apoptotic bodies which are phagocytozed by neighboring cells. A number of stresses can induce apoptosis in vitro and in vivo. The administration of glucocorticoids, reduction of hormone and/or growth factor levels, chemotherapy (toxic agents), mechanical injury and DNA damage can all result in apoptosis. Apoptosis is also induced by aberrant cell cycle activity, and it can be triggered in cells that express the Fas receptor with crosslinking antibodies or the natural Fas ligand. High frequencies of apoptotic cell-death are associated in a diverse array of pathological disorders. A number of standard tests are known in the art for detecting cell death in cells and/or tissue. For example, TdT-mediated biotin-dUDP nick-end labeling (TUNEL) staining, and the appearance of condensed chromatin and other morphological features characteristic of apoptosis in electron micrographs can be used to assess apoptosis in the cells of the invention and other cell types (see Examples section).

[0057] "Upregulated," as used herein, refers to increased expression of a gene and/or its encoded polypeptide. Increased expression refers to increasing (i.e., to a detectable extent) replication, transcription, and/or translation of any of the nucleic acids of the invention (MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d), since upregulation of any of these processes results in concentration/amount increase of the polypeptide encoded by the gene (nucleic acid). Conversely, downregulation or decreased expression refers to decreased expression of a gene and/or its encoded polypeptide. The upregulation or downregulation of gene expression can be directly determined by detecting an increase or decrease, respectively, in the level of mRNA for the gene, or the level of protein expression of the gene-encoded polypeptide, using any suitable means known to the art, such as nucleic acid hybridization or antibody detection methods, respectively, and in comparison to controls. Upregulation or downregulation of gene expression can also be determined indirectly by detecting a change in cardiac cell anti-apoptotic activity of the gene.

[0058] "Cardiac cell anti-apoptotic activity" refers to the ability of a molecule to prevent or inhibit apoptotic cell-death of a cardiac cell and can be determined using, for example, standard tests known in the art (e.g., TUNEL staining, -see Examples section). A cardiac cell includes a a cardiomyocyte and a vascular endothelial cell (including a smooth muscle cell). A "molecule," as used herein, embraces both "nucleic acids" and "polypeptides." MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d molecules are capable of inhibiting apoptotic cell-death of a cell such as a cardiomyocyte, and/or a vascular endothelial cell (including a smooth muscle cell) both in vivo and in vitro.

[0059] "Expression," as used herein, refers to nucleic acid and/or polypeptide expression, as well as to activity of the polypeptide molecule (e.g., cardiac cell anti-apoptotic activity of the molecule).

[0060] One aspect of the invention involves the cloning of a cDNA encoding MIVR-1. MIVR-1 according to the invention is an isolated nucleic acid molecule that comprises a nucleic acid molecule of SEQ ID NO:1, and codes for a polypeptide with cardiac cell anti-apoptotic activity. The sequence of the human MIVR-1 cDNA is presented as SEQ ID NO:1, and the predicted amino acid sequence of this cDNA's encoded protein product is presented as SEQ ID NO:2.

[0061] As used herein, a subject is a mammal or a non-human mammal. In all embodiments human MIVR-1 and human subjects are preferred.

[0062] The invention thus involves in one aspect an isolated MIVR-1 polypeptide, the cDNA encoding this polypeptide, functional modifications and variants of the foregoing, useful fragments of the foregoing, as well as diagnostics and therapeutics relating thereto.

[0063] As used herein with respect to nucleic acids, the term "isolated" means: (i) amplified in vitro by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid is one which is readily manipulated by recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulated by standard techniques known to those of ordinary skill in the art.

[0064] As used herein with respect to polypeptides, the term "isolated" means separated from its native environment in sufficiently pure form so that it can be manipulated or

used for any one of the purposes of the invention. Thus, isolated means sufficiently pure to be used (i) to raise and/or isolate antibodies, (ii) as a reagent in an assay, (iii) for sequencing, (iv) as a therapeutic, etc.

[0065] According to the invention, isolated nucleic acid molecules that code for a MIVR-1 polypeptide having cardiac cell anti-apoptotic activity include: (a) nucleic acid molecules which hybridize under stringent conditions to a molecule consisting of a nucleic acid of SEQ ID NO:1 and which code for a MIVR-1 polypeptide having cardiac cell anti-apoptotic activity, (b) deletions, additions and substitutions of (a) which code for a respective MIVR-1 polypeptide having cardiac cell anti-apoptotic activity, (c) nucleic acid molecules that differ from the nucleic acid molecules of (a) or (b) in codon sequence due to the degeneracy of the genetic code, and (d) complements of (a), (b) or (c). "Complements," as used herein, includes "full-length complements or 100% complements of (a), (b) or (c).

[0066] Homologs and alleles of the MIVR-1 nucleic acids of the invention can be identified by conventional techniques. Thus, an aspect of the invention is those nucleic acid sequences which code for MIVR-1 polypeptides and which hybridize to a nucleic acid molecule consisting of the coding region of SEQ ID NO:1, under stringent conditions. The term "stringent conditions," as used herein, refers to parameters with which the art is familiar. With nucleic acids, hybridization conditions are said to be stringent typically under conditions of low ionic strength and a temperature just below the melting temperature (T_m) of the DNA hybrid complex (typically, about 3° C. below the T_m of the hybrid). Higher stringency makes for a more specific correlation between the probe sequence and the target. Stringent conditions used in the hybridization of nucleic acids are well known in the art and may be found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual,* J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, or *Current Protocols in Molecular Biology,* F. M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. An example of "stringent conditions" is hybridization at 65° C. in 6×SSC. Another example of stringent conditions is hybridization at 65° C. in hybridization buffer that consists of 3.5×SSC, 0.02% Ficoll, 0.02% polyvinyl pyrolidone, 0.02% Bovine Serum Albumin, 2.5 mM NaH_2PO_4[pH7], 0.5% SDS, 2 mM EDTA. (SSC is 0.15M sodium chloride/0.15M sodium citrate, pH7; SDS is sodium dodecyl sulphate; and EDTA is ethylenediaminetetracetic acid). After hybridization, the membrane upon which the DNA is transferred is washed at 2×SSC at room temperature and then at 0.1×SSC/0.×SDS at temperatures up to 68° C. In a further example, an alternative to the use of an aqueous hybridization solution is the use of a formamide hybridization solution. Stringent hybridization conditions can thus be achieved using, for example, a 50% formamide solution and 42° C. There are other conditions, reagents, and so forth which can be used, and would result in a similar degree of stringency. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of MIVR-1 nucleic acids of the invention. The skilled artisan also is familiar with the methodology for screening cells and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

[0067] In general homologs and alleles typically will share at least 40% nucleotide identity and/or at least 50% amino acid identity to SEQ ID NO:1 and SEQ ID NO:2, respectively, in some instances will share at least 50% nucleotide identity and/or at least 65% amino acid identity and in still other instances will share at least 60% nucleotide identity and/or at least 75% amino acid identity. In further instances, homologs and alleles typically will share at least 90%, 95%, or even 99% nucleotide identity and/or at least 95%, 98%, or even 99% amino acid identity to SEQ ID NO:1 and SEQ ID NO:2, respectively. The homology can be calculated using various, publicly available software tools developed by NCBI (Bethesda, Md.). Exemplary tools include the heuristic algorithm of Altschul S F, et al., (*J Mol Biol,* 1990, 215:403-410), also known as BLAST. Pairwise and ClustalW alignments (BLOSUM30 matrix setting) as well as Kyte-Doolittle hydropathic analysis can be obtained using public (EMBL, Heidelberg, Germany) and commercial (e.g., the MacVector sequence analysis software from Oxford Molecular Group/enetics Computer Group, Madison, Wis.). Watson-Crick complements of the foregoing nucleic acids also are embraced by the invention.

[0068] In screening for MIVR-1 related genes, such as homologs and alleles of MIVR-1, a Southern blot may be performed using the foregoing conditions, together with a radioactive probe. After washing the membrane to which the DNA is finally transferred, the membrane can be placed against X-ray film or a phosphoimager plate to detect the radioactive signal.

[0069] Given the teachings herein of a full-length human MIVR-1 cDNA clone, other mammalian sequences such as the mouse cDNA clone corresponding to the human MIVR-1 gene can be isolated from a cDNA library, using standard colony hybridization techniques.

[0070] The invention also includes degenerate nucleic acids which include alternative codons to those present in the native materials. For example, serine residues are encoded by the codons TCA, AGT, TCC, TCG, TCT and AGC. Thus, it will be apparent to one of ordinary skill in the art that any of the serine-encoding nucleotide triplets may be employed to direct the protein synthesis apparatus, in vitro or in vivo, to incorporate a serine residue into an elongating MIVR-1 polypeptide. Similarly, nucleotide sequence triplets which encode other amino acid residues include, but are not limited to: CCA, CCC, CCG and CCT (proline codons); CGA, CGC, CGG, CGT, AGA and AGG (arginine codons); ACA, ACC, ACG and ACT (threonine codons); AAC and AAT (asparagine codons); and ATA, ATC and ATT (isoleucine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acids that differ from the biologically isolated nucleic acids in codon sequence due to the degeneracy of the genetic code.

[0071] The invention also provides isolated unique fragments of SEQ ID NO:1 or SEQ ID NO:3 or complements of thereof. A unique fragment is one that is a 'signature' for the larger nucleic acid. For example, the unique fragment is long enough to assure that its precise sequence is not found in molecules within the human genome outside of the MIVR-1

nucleic acids defined above (and human alleles). Those of ordinary skill in the art may apply no more than routine procedures to determine if a fragment is unique within the human genome. Unique fragments, however, exclude fragments completely composed of the nucleotide sequences selected from the group consisting of SEQ ID NOs. 14-16, and 17, and/or other previously published sequences as of the filing date of this application (e.g., GenBank Acc. No. AL035541).

[0072] A fragment which is completely composed of the sequence described in the foregoing GenBank deposits is one which does not include any of the nucleotides unique to the sequences of the invention. Thus, a unique fragment according to the invention must contain a nucleotide sequence other than the exact sequence of those in the GenBank deposits or fragments thereof. The difference may be an addition, deletion or substitution with respect to the GenBank sequence or it may be a sequence wholly separate from the GenBank sequence.

[0073] Unique fragments can be used as probes in Southern and Northern blot assays to identify such nucleic acids, or can be used in amplification assays such as those employing PCR. As known to those skilled in the art, large probes such as 200, 250, 300 or more nucleotides are preferred for certain uses such as Southern and Northern blots, while smaller fragments will be preferred for uses such as PCR. Unique fragments also can be used to produce fusion proteins for generating antibodies or determining binding of the polypeptide fragments, as demonstrated in the Examples, or for generating immunoassay components. Likewise, unique fragments can be employed to produce nonfused fragments of the MIVR-1 polypeptides, useful, for example, in the preparation of antibodies, immunoassays or therapeutic applications. Unique fragments further can be used as antisense molecules to inhibit the expression of MIVR-1 nucleic acids and polypeptides respectively.

[0074] As will be recognized by those skilled in the art, the size of the unique fragment will depend upon its conservancy in the genetic code. Thus, some regions of SEQ ID NO:1 or SEQ ID NO:3 and complements will require longer segments to be unique while others will require only short segments, typically between 12 and 32 nucleotides long (e.g. 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32 bases) or more, up to the entire length of the disclosed sequence. As mentioned above, this disclosure intends to embrace each and every fragment of each sequence, beginning at the first nucleotide, the second nucleotide and so on, up to 8 nucleotides short of the end, and ending anywhere from nucleotide number 8, 9, 10 and so on for each sequence, up to the very last nucleotide, (provided the sequence is unique as described above). Virtually any segment of the region of SEQ ID NO:1 beginning at nucleotide 1 and ending at nucleotide 1321, or SEQ ID NO:3 beginning at nucleotide 1 and ending at nucleotide 861, or complements thereof, that is 20 or more nucleotides in length will be unique. Those skilled in the art are well versed in methods for selecting such sequences, typically on the basis of the ability of the unique fragment to selectively distinguish the sequence of interest from other sequences in the human genome of the fragment to those on known databases typically is all that is necessary, although in vitro confirmatory hybridization and sequencing analysis may be performed.

[0075] As mentioned above, the invention embraces antisense oligonucleotides that selectively bind to a nucleic acid molecule encoding a MIVR-1 polypeptide, to decrease MIVR-1 activity.

[0076] As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridization with the target gene or transcript. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions. Based upon SEQ ID NO:1 or upon allelic or homologous genomic and/or cDNA sequences, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the present invention. In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 10 and, more preferably, at least 15 consecutive bases which are complementary to the target, although in certain cases modified oligonucleotides as short as 7 bases in length have been used successfully as antisense oligonucleotides (Wagner et al., *Nat. Med,* 1995, 1(11):1116-1118; *Nat. Biotech.,* 1996, 14:840-844). Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases. Although oligonucleotides may be chosen which are antisense to any region of the gene or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3' -untranslated regions may be targeted by antisense oligonucleotides. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al., *Cell Mol. Neurobiol.* 14(5):439-457, 1994) and at which proteins are not expected to bind. Finally, although, SEQ ID No:1 discloses a cDNA sequence, one of ordinary skill in the art may easily derive the genomic DNA corresponding to this sequence. Thus, the present invention also provides for antisense oligonucleotides which are complementary to the genomic DNA corresponding to SEQ ID NO:1. Similarly, antisense to allelic or homologous MIVR-1 cDNAs and genomic DNAs are enabled without undue experimentation.

[0077] In one set of embodiments, the antisense oligonucleotides of the invention may be composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5' end of one native nucleotide and the 3' end of another native nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside

linkage. These oligonucleotides may be prepared by art recognized methods which may be carried out manually or by an automated synthesizer. They also may be produced recombinantly by vectors.

[0078] In preferred embodiments, however, the antisense oligonucleotides of the invention also may include "modified" oligonucleotides. That is, the oligonucleotides may be modified in a number of ways which do not prevent them from hybridizing to their target but which enhance their stability or targeting or which otherwise enhance their therapeutic effectiveness.

[0079] The term "modified oligonucleotide" as used herein describes an oligonucleotide in which (1) at least two of its nucleotides are covalently linked via a synthetic internucleoside linkage (i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide) and/or (2) a chemical group not normally associated with nucleic acids has been covalently attached to the oligonucleotide. Preferred synthetic internucleoside linkages are phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, carboxymethyl esters and peptides.

[0080] The term "modified oligonucleotide" also encompasses oligonucleotides with a covalently modified base and/or sugar. For example, modified oligonucleotides include oligonucleotides having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified oligonucleotides may include a 2'-O-alkylated ribose group. In addition, modified oligonucleotides may include sugars such as arabinose instead of ribose. The present invention, thus, contemplates pharmaceutical preparations containing modified antisense molecules that are complementary to and hybridizable with, under physiological conditions, nucleic acids encoding MIVR-1 polypeptides, together with pharmaceutically acceptable carriers. Antisense oligonucleotides may be administered as part of a pharmaceutical composition. Such a pharmaceutical composition may include the antisense oligonucleotides in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain a therapeutically effective amount of the antisense oligonucleotides in a unit of weight or volume suitable for administration to a patient. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Physiologically and pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art.

[0081] The invention also involves expression vectors coding for MIVR-1 proteins and fragments and variants thereof and host cells containing those expression vectors. Virtually any cells, prokaryotic or eukaryotic, which can be transformed with heterologous DNA or RNA and which can be grown or maintained in culture, may be used in the practice of the invention. Examples include bacterial cells such as Escherichia coli and mammalian cells such as mouse, hamster, pig, goat, primate, etc. They may be of a wide variety of tissue types, including mast cells, fibroblasts, oocytes and lymphocytes, and they may be primary cells or cell lines. Specific examples include CHO cells and COS cells. Cell-free transcription systems also may be used in lieu of cells.

[0082] As used herein, a "vector" may be any of a number of nucleic acids into which a desired sequence may be inserted by restriction and ligation for transport between different genetic environments or for expression in a host cell. Vectors are typically composed of DNA although RNA vectors are also available. Vectors include, but are not limited to, plasmids, phagemids and virus genomes. A cloning vector is one which is able to replicate in a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases in copy number within the host bacterium or just a single time per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase. An expression vector is one into which a desired DNA sequence may be inserted by restriction and ligation such that it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art (e.g., β-galactosidase or alkaline phosphatase), and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (e.g., green fluorescent protein). Preferred vectors are those capable of autonomous replication and expression of the structural gene products present in the DNA segments to which they are operably joined.

[0083] As used herein, a coding sequence and regulatory sequences are said to be "operably" joined when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

[0084] The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribed and 5' non-translated sequences involved with the initiation of transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribed regulatory sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined gene. Regulatory sequences may also include enhancer sequences or upstream activator sequences as desired. The vectors of the invention may optionally include 5' leader or signal sequences. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art.

[0085] Expression vectors containing all the necessary elements for expression are commercially available and known to those skilled in the art. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Second Edition, Cold Spring Harbor Laboratory Press, 1989. Cells are genetically engineered by the introduction into the cells of heterologous DNA (RNA) encoding MIVR-1 polypeptide or fragment or variant thereof. That heterologous DNA (RNA) is placed under operable control of transcriptional elements to permit the expression of the heterologous DNA in the host cell.

[0086] Preferred systems for mRNA expression in mammalian cells are those such as pRc/CMV (available from Invitrogen, Carlsbad, Calif.) that contain a selectable marker such as a gene that confers G418 resistance (which facilitates the selection of stably transfected cell lines) and the human cytomegalovirus (CMV) enhancer-promoter sequences. Additionally, suitable for expression in primate or canine cell lines is the pCEP4 vector (Invitrogen, Carlsbad, Calif.), which contains an Epstein Barr virus (EBV) origin of replication, facilitating the maintenance of plasmid as a multicopy extrachromosomal element. Another expression vector is the pEF-BOS plasmid containing the promoter of polypeptide Elongation Factor 1α, which stimulates efficiently transcription in vitro. The plasmid is described by Mishizuma and Nagata (*Nuc. Acids Res.* 18:5322, 1990), and its use in transfection experiments is disclosed by, for example, Demoulin (*Mol. Cell. Biol.* 16:4710-4716, 1996). Still another preferred expression vector is an adenovirus, described by Stratford-Perricaudet, which is defective for E1 and E3 proteins (*J Clin. Invest.* 90:626-630, 1992). The use of the adenovirus as an Adeno.P1A recombinant is disclosed by Warnier et al., in intradermal injection in mice for immunization against P1A (*Int. J Cancer,* 67:303-310, 1996).

[0087] The invention also embraces so-called expression kits, which allow the artisan to prepare a desired expression vector or vectors. Such expression kits include at least separate portions of each of the previously discussed coding sequences. Other components may be added, as desired, as long as the previously mentioned sequences, which are required, are included.

[0088] It will also be recognized that the invention embraces the use of the above described, MIVR-1 cDNA sequence containing expression vectors, to transfect host cells and cell lines, be these prokaryotic (e.g., *Escherichia coli*), or eukaryotic (e.g., CHO cells, COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). Especially useful are mammalian cells such as mouse, hamster, pig, goat, primate, etc. They may be of a wide variety of tissue types, and include primary cells and cell lines. Specific examples include dendritic cells, U293 cells, peripheral blood leukocytes, bone marrow stem cells and embryonic stem cells. The invention also permits the construction of MIVR-1 gene "knock-outs" in cells and in animals, providing materials for studying certain aspects of MIVR-1 activity.

[0089] The invention also provides isolated polypeptides (including whole proteins and partial proteins), encoded by the foregoing MIVR-1 nucleic acids, and include the polypeptide of SEQ ID NO:2 and unique fragments thereof. Such polypeptides are useful, for example, alone or as part of fusion proteins to generate antibodies, as components of an immunoassay, etc. Polypeptides can be isolated from biological samples including tissue or cell homogenates, and can also be expressed recombinantly in a variety of prokaryotic and eukaryotic expression systems by constructing an expression vector appropriate to the expression system, introducing the expression vector into the expression system, and isolating the recombinantly expressed protein. Short polypeptides, including antigenic peptides (such as are presented by MHC molecules on the surface of a cell for immune recognition) also can be synthesized chemically using well-established methods of peptide synthesis.

[0090] A unique fragment of a MIVR-1 polypeptide, in general, has the features and characteristics of unique fragments as discussed above in connection with nucleic acids. As will be recognized by those skilled in the art, the size of the unique fragment will depend upon factors such as whether the fragment constitutes a portion of a conserved protein domain. Thus, some regions of SEQ ID NO:2 will require longer segments to be unique while others will require only short segments, typically between 5 and 12 amino acids (e.g. 5, 6, 7, 8, 9, 10, 11 and 12 amino acids long or more, including each integer up to the full length, 287 amino acids long).

[0091] Unique fragments of a polypeptide preferably are those fragments which retain a distinct functional capability of the polypeptide. Functional capabilities which can be retained in a unique fragment of a polypeptide include interaction with antibodies, interaction with other polypeptides or fragments thereof, interaction with other molecules, etc. One important activity is the ability to act as a signature for identifying the polypeptide. Those skilled in the art are well versed in methods for selecting unique amino acid sequences, typically on the basis of the ability of the unique fragment to selectively distinguish the sequence of interest from non-family members. A comparison of the sequence of the fragment to those on known databases typically is all that is necessary.

[0092] The invention embraces variants of the MIVR-1 polypeptides described above. As used herein, a "variant" of a MIVR-1 polypeptide is a polypeptide which contains one or more modifications to the primary amino acid sequence of a MIVR-1 polypeptide. Modifications which create a MIVR-1 polypeptide variant are typically made to the nucleic acid which encodes the MIVR-1 polypeptide, and can include deletions, point mutations, truncations, amino acid substitutions and addition of amino acids or non-amino

acid moieties to:1) reduce or eliminate an activity of a MIVR-1 polypeptide; 2) enhance a property of a MIVR-1 polypeptide, such as protein stability in an expression system or the stability of protein-ligand binding; 3) provide a novel activity or property to a MIVR-1 polypeptide, such as addition of an antigenic epitope or addition of a detectable moiety; or 4) to provide equivalent or better binding to a MIVR-1 polypeptide receptor or other molecule. Alternatively, modifications can be made directly to the polypeptide, such as by cleavage, addition of a linker molecule, addition of a detectable moiety, such as biotin, addition of a fatty acid, and the like. Modifications also embrace fusion proteins comprising all or part of the MIVR-1 amino acid sequence. One of skill in the art will be familiar with methods for predicting the effect on protein conformation of a change in protein sequence, and can thus "design" a variant MIVR-1 polypeptide according to known methods. One example of such a method is described by Dahiyat and Mayo in *Science* 278:82-87, 1997, whereby proteins can be designed de novo. The method can be applied to a known protein to vary only a portion of the polypeptide sequence. By applying the computational methods of Dahiyat and Mayo, specific variants of the MIVR-1 polypeptide can be proposed and tested to determine whether the variant retains a desired conformation.

[**0093**] Variants can include MIVR-1 polypeptides which are modified specifically to alter a feature of the polypeptide unrelated to its physiological activity. For example, cysteine residues can be substituted or deleted to prevent unwanted disulfide linkages. Similarly, certain amino acids can be changed to enhance expression of a MIVR-1 polypeptide by eliminating proteolysis by proteases in an expression system (e.g., dibasic amino acid residues in yeast expression systems in which KEX2 protease activity is present).

[**0094**] Mutations of a nucleic acid which encodes a MIVR-1 polypeptide preferably preserve the amino acid reading frame of the coding sequence, and preferably do not create regions in the nucleic acid which are likely to hybridize to form secondary structures, such a hairpins or loops, which can be deleterious to expression of the variant polypeptide.

[**0095**] Mutations can be made by selecting an amino acid substitution, or by random mutagenesis of a selected site in a nucleic acid which encodes the polypeptide. Variant polypeptides are then expressed and tested for one or more activities to determine which mutation provides a variant polypeptide with the desired properties. Further mutations can be made to variants (or to non-variant MIVR-1 polypeptides) which are silent as to the amino acid sequence of the polypeptide, but which provide preferred codons for translation in a particular host. The preferred codons for translation of a nucleic acid in, e.g., *Escherichia coli,* are well known to those of ordinary skill in the art. Still other mutations can be made to the noncoding sequences of a MIVR-1 gene or cDNA clone to enhance expression of the polypeptide.

[**0096**] The skilled artisan will realize that conservative amino acid substitutions may be made in MIVR-1 polypeptides to provide functionally equivalent variants of the foregoing polypeptides, i.e., the variants retain the functional capabilities of the MIVR-1 polypeptides. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution which does not significantly alter the the tertiary structure and/or activity of the polypeptide. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art, and include those that are found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual,* J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, or *Current Protocols in Molecular Biology,* F. M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Exemplary functionally equivalent variants of the MIVR-1 polypeptides include conservative amino acid substitutions of SEQ ID NO:2. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

[**0097**] Thus functionally equivalent variants of MIVR-1 polypeptides, i.e., variants of MIVR-1 polypeptides which retain the function of the natural MIVR-1 polypeptides, are contemplated by the invention. Conservative amino-acid substitutions in the amino acid sequence of MIVR-1 polypeptides to produce functionally equivalent variants of MIVR-1 polypeptides typically are made by alteration of a nucleic acid encoding MIVR-1 polypeptides (SEQ ID NOs:1,3). Such substitutions can be made by a variety of methods known to one of ordinary skill in the art. For example, amino acid substitutions may be made by PCR-directed mutation, site-directed mutagenesis according to the method of Kunkel (Kunkel, *Proc. Nat. Acad. Sci. U.S.A.* 82: 488-492, 1985), or by chemical synthesis of a gene encoding a MIVR-1 polypeptide. The activity of functionally equivalent fragments of MIVR-1 polypeptides can be tested by cloning the gene encoding the altered MIVR-1 polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the altered MIVR-1 polypeptide, and testing for a functional capability of the MIVR-1 polypeptides as disclosed herein (e.g., cardiac cell anti-apoptotic activity, etc.).

[**0098**] The invention as described herein has a number of uses, some of which are described elsewhere herein. First, the invention permits isolation of MIVR-1 polypeptides. A variety of methodologies well-known to the skilled practitioner can be utilized to obtain isolated MIVR-1 molecules. The polypeptide may be purified from cells which naturally produce the polypeptide by chromatographic means or immunological recognition. Alternatively, an expression vector may be introduced into cells to cause production of the polypeptide. In another method, mRNA transcripts may be microinjected or otherwise introduced into cells to cause production of the encoded polypeptide. Translation of MIVR-1 mRNA in cell-free extracts such as the reticulocyte lysate system also may be used to produce MIVR-1 polypeptides. Those skilled in the art also can readily follow known methods for isolating MIVR-1 polypeptides. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography and immune-affinity chromatography.

[**0099**] The invention also provides, in certain embodiments, "dominant negative" polypeptides derived from MIVR-1 polypeptides. A dominant negative polypeptide is an inactive variant of a protein, which, by interacting with the cellular machinery, displaces an active protein from its

interaction with the cellular machinery or competes with the active protein, thereby reducing the effect of the active protein. For example, a dominant negative receptor which binds a ligand but does not transmit a signal in response to binding of the ligand can reduce the biological effect of expression of the ligand. Likewise, a dominant negative catalytically-inactive kinase which interacts normally with target proteins but does not phosphorylate the target proteins can reduce phosphorylation of the target proteins in response to a cellular signal. Similarly, a dominant negative transcription factor which binds to a promoter site in the control region of a gene but does not increase gene transcription can reduce the effect of a normal transcription factor by occupying promoter binding sites without increasing transcription.

[0100] The end result of the expression of a dominant negative polypeptide in a cell is a reduction in function of active proteins. One of ordinary skill in the art can assess the potential for a dominant negative variant of a protein, and use standard mutagenesis techniques to create one or more dominant negative variant polypeptides. See, e.g., U.S. Pat. No. 5,580,723 and Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Second Edition, Cold Spring Harbor Laboratory Press, 1989. The skilled artisan then can test the population of mutagenized polypeptides for diminution in a selected activity and/or for retention of such an activity. Other similar methods for creating and testing dominant negative variants of a protein will be apparent to one of ordinary skill in the art.

[0101] The isolation of the MIVR-1 cDNA also makes it possible for the artisan to diagnose a disorder characterized by an aberrant expression of MIVR-1. These methods involve determining expression of the MIVR-1 gene, and/or MIVR-1 polypeptides derived therefrom. In the former situation, such determinations can be carried out via any standard nucleic acid determination assay, including the polymerase chain reaction, or assaying with labeled hybridization probes as exemplified below. In the latter situation, such determination can be carried out via any standard immunological assay using, for example, antibodies which bind to the secreted MIVR-1 protein.

[0102] The invention also embraces isolated peptide binding agents which, for example, can be antibodies or fragments of antibodies ("binding polypeptides"), having the ability to selectively bind to MIVR-1 polypeptides. Antibodies include polyclonal and monoclonal antibodies, prepared according to conventional methodology. In certain embodiments, the invention excludes binding agents (e.g., antibodies) that bind to the polypeptides encoded by the nucleic acids having sequences selected from the group consisting of SEQ ID NOs. 14-17.

[0103] Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W. R. (1986) *The Experimental Foundations of Modern Immunology* Wiley & Sons, Inc., New York; Roitt, I. (1991) *Essential Immunology,* 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an $F(ab')_2$ fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

[0104] Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

[0105] It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody. See, e.g., U.S. Pat. Nos. 4,816,567, 5,225,539, 5,585,089, 5,693,762 and 5,859,205. Thus, for example, PCT International Publication Number WO 92/04381 teaches the production and use of humanized murine RSV antibodies in which at least a portion of the murine FR regions have been replaced by FR regions of human origin. Such antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as "chimeric" antibodies.

[0106] Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for $F(ab')_2$, Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric $F(ab')_2$ fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

[0107] Thus, the invention involves polypeptides of numerous size and type that bind specifically to MIVR-1 polypeptides, and complexes of both MIVR-1 polypeptides and their binding partners. These polypeptides may be derived also from sources other than antibody technology. For example, such polypeptide binding agents can be pro-

vided by degenerate peptide libraries which can be readily prepared in solution, in immobilized form, as bacterial flagella peptide display libraries or as phage display libraries. Combinatorial libraries also can be synthesized of peptides containing one or more amino acids. Libraries further can be synthesized of peptides and non-peptide synthetic moieties.

[0108] Phage display can be particularly effective in identifying binding peptides useful according to the invention. Briefly, one prepares a phage library (using e.g. m13, fd, or lambda phage), displaying inserts from 4 to about 80 amino acid residues using conventional procedures. The inserts may represent, for example, a completely degenerate or biased array. One then can select phage-bearing inserts which bind to the MIVR-1 polypeptide or a complex of MIVR-1 and a binding partner. This process can be repeated through several cycles of reselection of phage that bind to the MIVR-1 polypeptide or complex. Repeated rounds lead to enrichment of phage bearing particular sequences. DNA sequence analysis can be conducted to identify the sequences of the expressed polypeptides. The minimal linear portion of the sequence that binds to the MIVR-1 polypeptide or complex can be determined. One can repeat the procedure using a biased library containing inserts containing part or all of the minimal linear portion plus one or more additional degenerate residues upstream or downstream thereof. Yeast two-hybrid screening methods also may be used to identify polypeptides that bind to the MIVR-1 polypeptides. Thus, the MIVR-1 polypeptides of the invention, or a fragment thereof, or complexes of MIVR-1 and a binding partner can be used to screen peptide libraries, including phage display libraries, to identify and select peptide binding partners of the MIVR-1 polypeptides of the invention. Such molecules can be used, as described, for screening assays, for purification protocols, for interfering directly with the functioning of MIVR-1 and for other purposes that will be apparent to those of ordinary skill in the art.

[0109] An MIVR-1 polypeptide, or a fragment thereof, also can be used to isolate their native binding partners. Isolation of binding partners may be performed according to well-known methods. For example, isolated MIVR-1 polypeptides can be attached to a substrate, and then a solution suspected of containing a MIVR-1 binding partner may be applied to the substrate. If the binding partner for MIVR-1 polypeptides is present in the solution, then it will bind to the substrate-bound MIVR-1 polypeptide. The binding partner then may be isolated. Other proteins which are binding partners for MIVR-1, may be isolated by similar methods without undue experimentation.

[0110] The invention also provides methods to measure the level of MIVR-1 expression in a subject. This can be performed by first obtaining a test sample from the subject. The test sample can be tissue or biological fluid. Tissues include brain, heart, serum, breast, colon, bladder, uterus, prostate, stomach, testis, ovary, pancreas, pituitary gland, adrenal gland, thyroid gland, salivary gland, mammary gland, kidney, liver, intestine, spleen, thymus, blood vessels, bone marrow, trachea, and lung. In certain embodiments, test samples originate from heart and blood vessel tissues, and biological fluids include blood, saliva and urine. Both invasive and non-invasive techniques can be used to obtain such samples and are well documented in the art. At the molecular level both PCR and Northern blotting can be used to determine the level of MIVR-1 mRNA using products of this invention described herein, and protocols well known in the art that are found in references which compile such methods. At the protein level, MIVR-1 expression can be determined using either polyclonal or monoclonal anti-MIVR-1 sera in combination with standard immunological assays. The preferred methods will compare the measured level of MIVR-1 expression of the test sample to a control. A control can include a known amount of a nucleic acid probe, a MIVR-1 epitope (such as a MIVR-1 expression product), or a similar test sample of a subject with a control or 'normal' level of MIVR-1 expression.

[0111] MIVR-1 polypeptides preferably are produced recombinantly, although such polypeptides may be isolated from biological extracts. Recombinantly produced MIVR-1 polypeptides include chimeric proteins comprising a fusion of a MIVR-1 protein with another polypeptide, e.g., a polypeptide capable of providing or enhancing protein-protein binding, sequence specific nucleic acid binding (such as GAL4), enhancing stability of the MIVR-1 polypeptide under assay conditions, or providing a detectable moiety, such as green fluorescent protein. A polypeptide fused to a MIVR-1 polypeptide or fragment may also provide means of readily detecting the fusion protein, e.g., by immunological recognition or by fluorescent labeling.

[0112] The invention also is useful in the generation of transgenic non-human animals. As used herein, "transgenic non-human animals" includes non-human animals having one or more exogenous nucleic acid molecules incorporated in germ line cells and/or somatic cells. Thus the transgenic animals include "knockout" animals having a homozygous or heterozygous gene disruption by homologous recombination, animals having episomal or chromosomally incorporated expression vectors, etc. Knockout animals can be prepared by homologous recombination using embryonic stem cells as is well known in the art. The recombination may be facilitated using, for example, the cre/lox system or other recombinase systems known to one of ordinary skill in the art. In certain embodiments, the recombinase system itself is expressed conditionally, for example, in certain tissues or cell types, at certain embryonic or post-embryonic developmental stages, is induced by the addition of a compound which increases or decreases expression, and the like. In general, the conditional expression vectors used in such systems use a variety of promoters which confer the desired gene expression pattern (e.g., temporal or spatial). Conditional promoters also can be operably linked to MIVR-1 nucleic acid molecules to increase expression of MIVR-1 in a regulated or conditional manner. Trans-acting negative regulators of MIVR-1 activity or expression also can be operably linked to a conditional promoter as described above. Such trans-acting regulators include antisense MIVR-1 nucleic acids molecules, nucleic acid molecules which encode dominant negative MIVR-1 molecules, ribozyme molecules specific for MIVR-1 nucleic acids, and the like. The transgenic non-human animals are useful in experiments directed toward testing biochemical or physiological effects of diagnostics or therapeutics for conditions characterized by increased or decreased MIVR-1 expression. Other uses will be apparent to one of ordinary skill in the art.

[0113] The invention also contemplates gene therapy. The procedure for performing ex vivo gene therapy is outlined in U.S. Pat. No. 5,399,346 and in exhibits submitted in the file history of that patent, all of which are publicly available documents. In general, it involves introduction in vitro of a functional copy of a gene into a cell(s) of a subject which contains a defective copy of the gene, and returning the genetically engineered cell(s) to the subject. The functional copy of the gene is under operable control of regulatory elements which permit expression of the gene in the genetically engineered cell(s). Numerous transfection and transduction techniques as well as appropriate expression vectors are well known to those of ordinary skill in the art, some of which are described in PCT application WO95/00654. In vivo gene therapy using vectors such as adenovirus, retroviruses, herpes virus, and targeted liposomes also is contemplated according to the invention.

[0114] The invention further provides efficient methods of identifying agents or lead compounds for agents active at the level of a MIVR-1 or MIVR-1 fragment dependent cellular function. In particular, such functions include interaction with other polypeptides or fragments. Generally, the screening methods involve assaying for compounds which interfere with MIVR-1 activity (such as MIVR-1 cardiac cell anti-apoptotic activity), although compounds which enhance MIVR-1 cardiac cell anti-apoptotic activity also can be assayed using the screening methods. Such methods are adaptable to automated, high throughput screening of compounds. Target indications include cellular processes modulated by MIVR-1 such as cardiac cell anti-apoptotic activity.

[0115] A wide variety of assays for candidate (pharmacological) agents are provided, including, labeled in vitro protein-ligand binding assays, electrophoretic mobility shift assays, immunoassays, cell-based assays such as two-or three-hybrid screens, expression assays, etc. The transfected nucleic acids can encode, for example, combinatorial peptide libraries or cDNA libraries. Convenient reagents for such assays, e.g., GAL4 fusion proteins, are known in the art. An exemplary cell-based assay involves transfecting a cell with a nucleic acid encoding a MIVR-1 polypeptide fused to a GAL4 DNA binding domain and a nucleic acid encoding a reporter gene operably linked to a gene expression regulatory region, such as one or more GAL4 binding sites. Activation of reporter gene transcription occurs when the MIVR-1 and reporter fusion polypeptide binds such as to enable transcription of the reporter gene. Agents which modulate a MIVR-1 polypeptide mediated cell function are then detected through a change in the expression of reporter gene. Methods for determining changes in the expression of a reporter gene are known in the art.

[0116] MIVR-1 fragments used in the methods, when not produced by a transfected nucleic acid are added to an assay mixture as an isolated polypeptide. MIVR-1 polypeptides preferably are produced recombinantly, although such polypeptides may be isolated from biological extracts. Recombinantly produced MIVR-1 polypeptides include chimeric proteins comprising a fusion of a MIVR-1 protein with another polypeptide, e.g., a polypeptide capable of providing or enhancing protein-protein binding, sequence specific nucleic acid binding (such as GAL4), enhancing stability of the MIVR-1 polypeptide under assay conditions, or providing a detectable moiety, such as green fluorescent protein or Flag epitope.

[0117] The assay mixture is comprised of a natural intracellular MIVR-1 binding target capable of interacting with MIVR-1. While natural MIVR-1 binding targets may be used, it is frequently preferred to use portions (e.g., peptides or nucleic acid fragments) or analogs (i.e., agents which mimic the MIVR-1 binding properties of the natural binding target for purposes of the assay) of the MIVR-1 binding target so long as the portion or analog provides binding affinity and avidity to the MIVR-1 fragment measurable in the assay.

[0118] The assay mixture also comprises a candidate agent. Typically, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration of agent or at a concentration of agent below the limits of assay detection. Candidate agents encompass numerous chemical classes, although typically they are organic compounds. Preferably, the candidate agents are small organic compounds, i.e., those having a molecular weight of more than 50 yet less than about 2500, preferably less than about 1000 and, more preferably, less than about 500. Candidate agents comprise functional chemical groups necessary for structural interactions with polypeptides and/or nucleic acids, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups and more preferably at least three of the functional chemical groups. The candidate agents can comprise cyclic carbon or heterocyclic structure and/or aromatic or polyaromatic structures substituted with one or more of the above-identified functional groups. Candidate agents also can be biomolecules such as peptides, saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs of the above, or combinations thereof and the like. Where the agent is a nucleic acid, the agent typically is a DNA or RNA molecule, although modified nucleic acids as defined herein are also contemplated.

[0119] Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides, synthetic organic combinatorial libraries, phage display libraries of random peptides, and the like. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural and synthetically produced libraries and compounds can be modified through conventional chemical, physical, and biochemical means. Further, known (pharmacological) agents may be subjected to directed or random chemical modifications such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs of the agents.

[0120] A variety of other reagents also can be included in the mixture. These include reagents such as salts, buffers, neutral proteins (e.g., albumin), detergents, etc. which may be used to facilitate optimal protein-protein and/or protein-nucleic acid binding. Such a reagent may also reduce non-specific or background interactions of the reaction components. Other reagents that improve the efficiency of the assay such as protease, inhibitors, nuclease inhibitors, antimicrobial agents, and the like may also be used.

[0121] The mixture of the foregoing assay materials is incubated under conditions whereby, but for the presence of the candidate agent, the MIVR-1 polypeptide specifically binds a cellular binding target, a portion thereof or analog thereof. The order of addition of components, incubation temperature, time of incubation, and other parameters of the assay may be readily determined. Such experimentation merely involves optimization of the assay parameters, not the fundamental composition of the assay. Incubation temperatures typically are between 4° C. and 40° C. Incubation times preferably are minimized to facilitate rapid, high throughput screening, and typically are between 0.1 and 10 hours.

[0122] After incubation, the presence or absence of specific binding between the MIVR-1 polypeptide and one or more binding targets is detected by any convenient method available to the user. For cell free binding type assays, a separation step is often used to separate bound from unbound components. The separation step may be accomplished in a variety of ways. Conveniently, at least one of the components is immobilized on a solid substrate, from which the unbound components may be easily separated. The solid substrate can be made of a wide variety of materials and in a wide variety of shapes, e.g., microtiter plate, microbead, dipstick, resin particle, etc. The substrate preferably is chosen to maximum signal to noise ratios, primarily to minimize background binding, as well as for ease of separation and cost.

[0123] Separation may be effected for example, by removing a bead or dipstick from a reservoir, emptying or diluting a reservoir such as a microtiter plate well, rinsing a bead, particle, chromatograpic column or filter with a wash solution or solvent. The separation step preferably includes multiple rinses or washes. For example, when the solid substrate is a microtiter plate, the wells may be washed several times with a washing solution, which typically includes those components of the incubation mixture that do not participate in specific bindings such as salts, buffer, detergent, non-specific protein, etc. Where the solid substrate is a magnetic bead, the beads may be washed one or more times with a washing solution and isolated using a magnet.

[0124] Detection may be effected in any convenient way for cell-based assays such as two- or three-hybrid screens. The transcript resulting from a reporter gene transcription assay of MIVR-1 polypeptide interacting with a target molecule typically encodes a directly or indirectly detectable product, e.g., β-galactosidase activity, luciferase activity, and the like. For cell free binding assays, one of the components usually comprises, or is coupled to, a detectable label. A wide variety of labels can be used, such as those that provide direct detection (e.g., radioactivity, luminescence, optical or electron density, etc.), or indirect detection (e.g., epitope tag such as the FLAG epitope, enzyme tag such as horseseradish peroxidase, etc.). The label may be bound to a MIVR-1 binding partner, or incorporated into the structure of the binding partner.

[0125] A variety of methods may be used to detect the label, depending on the nature of the label and other assay components. For example, the label may be detected while bound to the solid substrate or subsequent to separation from the solid substrate. Labels may be directly detected through optical or electron density, radioactive emissions, nonradiative energy transfers, etc. or indirectly detected with antibody conjugates, streptavidin-biotin conjugates, etc. Methods for detecting the labels are well known in the art.

[0126] The invention provides MIVR-1-specific binding agents, methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. For example, MIVR-1-specific pharmacological agents are useful in a variety of diagnostic and therapeutic applications, especially where disease or disease prognosis is associated with altered MIVR-1 binding characteristics. Novel MIVR-1-specific binding agents include MIVR-1-specific antibodies, cell surface receptors, and other natural intracellular and extracellular binding agents identified with assays such as two hybrid screens, and non-natural intracellular and extracellular binding agents identified in screens of chemical libraries and the like.

[0127] In general, the specificity of MIVR-1 binding to a specific molecule is determined by binding equilibrium constants. Targets which are capable of selectively binding a MIVR-1 polypeptide preferably have binding equilibrium constants of at least about 10^7 M^{-1}, more preferably at least about 10^8 M^{-1}, and most preferably at least about 10^9 M^{-1}. A wide variety of cell based and cell free assays may be used to demonstrate MIVR-1-specific binding. Cell based assays include one, two and three hybrid screens, assays in which MIVR-1-mediated transcription is inhibited or increased, etc. Cell free assays include MIVR-1-protein binding assays, immunoassays, etc. Other assays useful for screening agents which bind MIVR-1 polypeptides include fluorescence resonance energy transfer (FRET), and electrophoretic mobility shift analysis (EMSA).

[0128] According to another aspect of the invention, a method for identifying an agent useful in modulating cardiac cell anti-apoptotic activity of a molecule of the invention, is provided. The method involves (a) contacting a molecule having cardiac cell anti-apoptotic activity with a candidate agent, (b) measuring cardiac cell anti-apoptotic activity of the molecule, and (c) comparing the measured cardiac cell anti-apoptotic activity of the molecule to a control to determine whether the candidate agent modulates cardiac cell anti-apoptotic activity of the molecule, wherein the molecule is a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof. "Contacting" refers to both direct and indirect contacting of a molecule having cardiac cell anti-apoptotic activity with the candidate agent. "Indirect" contacting means that the candidate agent exerts its effects on the cardiac cell anti-apoptotic activity of the molecule via a third agent (e.g., a messenger molecule, a receptor, etc.). In certain embodiments, the control is cardiac cell anti-apoptotic activity of the molecule measured in the absence of the candidate agent. Assaying methods and candidate agents are as described above in the foregoing embodiments with respect to MIVR-1.

[0129] According to still another aspect of the invention, a method of diagnosing a disorder characterized by aberrant expression of a nucleic acid molecule, an expression product thereof, or a fragment of an expression product thereof, is provided. The method involves contacting a biological sample isolated from a subject with an agent that specifically binds to the nucleic acid molecule, an expression product

thereof, or a fragment of an expression product thereof, and determining the interaction between the agent and the nucleic acid molecule or the expression product as a determination of the disorder, wherein the nucleic acid molecule is selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d. In some embodiments, the disorder is a cardiovascular condition selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure. In one embodiment, the disorder is cardiac hypertrophy.

[0130] In the case where the molecule is a nucleic acid molecule, such determinations can be carried out via any standard nucleic acid determination assay, including the polymerase chain reaction, or assaying with labeled hybridization probes as exemplified herein. In the case where the molecule is an expression product of the nucleic acid molecule, or a fragment of an expression product of the nucleic acid molecule, such determination can be carried out via any standard immunological assay using, for example, antibodies which bind to any of the polypeptide expression products.

[0131] "Aberrant expression" refers to decreased expression (underexpression) or increased expression (overexpression) of any of the foregoing molecules (MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, nucleic acids and/or polypeptides) in comparison with a control (i.e., expression of the same molecule in a healthy or "normal" subject). A "healthy subject", as used herein, refers to a subject who is not at risk for developing a future cardiovascular condition (see earlier discussion and Harrison's). Healthy subjects also do not otherwise exhibit symptoms of disease. In other words, such subjects, if examined by a medical professional, would be characterized as healthy and free of symptoms of a cardiovascular disorder or at risk of developing a cardiovascular disorder.

[0132] When the disorder is a cardiovascular condition selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure, decreased expression of any of the foregoing molecules in comparison with a control (e.g., a healthy individual) is indicative of the presence of the disorder, or indicative of the risk for developing such disorder in the future.

[0133] When the disorder is cardiac hypertrophy, increased expression of any of the foregoing molecules in comparison with a control (e.g., a healthy individual) is indicative of the presence of the disorder, or indicative of the risk for developing such disorder in the future.

[0134] The invention also provides novel kits which could be used to measure the levels of the nucleic acids of the invention, or expression products of the invention.

[0135] In one embodiment, a kit comprises a package containing an agent that selectively binds to any of the foregoing MIVR-1 isolated nucleic acids, or expression products thereof, and a control for comparing to a measured value of binding of said agent any of the foregoing MIVR-1 isolated nucleic acids or expression products thereof. In some embodiments, the control is a predetermined value for comparing to the measured value. In certain embodiments, the control comprises an epitope of the expression product of any of the foregoing MIVR-1 isolated nucleic acids. In one embodiment, the kit further comprises a second agent that selectively binds to an isolated nucleic acid molecule selected from the group consisting of IEX-1, VDUP-1, BTG-2, TIS-11d, and/or an expression product thereof, and a control for comparing to a measured value of binding of said second agent to said isolated nucleic acid molecule or expression product thereof.

[0136] In the case of nucleic acid detection, pairs of primers for amplifying a nucleic acid molecule of the invention can be included. The preferred kits would include controls such as known amounts of nucleic acid probes, epitopes (such as IEX-1, VDUP-1, BTG-2, TIS-11d expression products) or anti-epitope antibodies, as well as instructions or other printed material. In certain embodiments the printed material can characterize risk of developing a cardiovascular condition based upon the outcome of the assay. The reagents may be packaged in containers and/or coated on wells in predetermined amounts, and the kits may include standard materials such as labeled immunological reagents (such as labeled anti-IgG antibodies) and the like. One kit is a packaged polystyrene microtiter plate coated with MIVR-1 protein and a container containing labeled anti-human IgG antibodies. A well of the plate is contacted with, for example, a biological fluid, washed and then contacted with the anti-IgG antibody. The label is then detected. A kit embodying features of the present invention, generally designated by the numeral **11**, is illustrated in **FIG. 1**. Kit **11** is comprised of the following major elements: packaging **15**, an agent of the invention **17**, a control agent **19** and instructions **21**. Packaging **15** is a box-like structure for holding a vial (or number of vials) containing an agent of the invention **17**, a vial (or number of vials) containing a control agent **19**, and instructions **21**. Individuals skilled in the art can readily modify packaging **15** to suit individual needs.

[0137] According to another aspect of the invention, a method for treating cardiac hypertrophy, is provided. The method involves administering to a subject in need of such treatment an agent that increases cardiac cell-death, in an amount effective to treat cardiac hypertrophy in the subject, wherein the agent that increases cardiac cell-death is an inhibitor of a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof.

[0138] Types of inhibitors of nucleic acid molecules or expression products thereof, are well known in the art. These include antisense oligonucleotides and nucleotides, promoter decoys, and dominant negatives, that can be used to downregulate expression of the desired molecule.

[0139] As used herein, "downregulating expression" refers to inhibiting (i.e., reducing to a detectable extent) replication, transcription, and/or translation of a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, since inhibition of any of these processes results in a decrease in the concentration/amount of the polypeptide encoded by the gene. The term also refers to inhibition of post-translational modifications on the polypeptide (e.g., in its phosphorylation), since inhibition of such modifications may also prevent proper expression (i.e., expression as in a wild type cell) of the encoded polypeptide. The term also refers to an increase in, or facilitation of, polypeptide degradation (e.g., via increased ubiquitinization). Polypeptide turnover can be determined using meth-

ods well known in the art and described elsewhere herein. The inhibition of gene expression can be directly determined by detecting a decrease in the level of mRNA for the gene, or the level of protein expression of the gene, using any suitable means known to the art, such as nucleic acid hybridization or antibody detection methods, respectively. Inhibition of gene expression can also be determined indirectly by detecting a change in cardiac cell anti-apoptotic activity of the molecule as a whole.

[0140] The invention also embraces methods for treating a cardiovascular condition. In some embodiments, the method involves administering to a subject in need of such treatment a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-1 d, in an amount effective to treat the cardiovascular condition. In certain embodiments, the method involves administering to a subject in need of such treatment an agent that modulates expression of any of the foregoing molecules (MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d), in an amount effective to treat the cardiovascular condition.

[0141] "Agents that modulate expression" of a nucleic acid or a polypeptide, as used herein, are known in the art, and refer to sense and antisense nucleic acids, dominant negative nucleic acids, antibodies to the polypeptides, and the like. Any agents that modulate exression of a molecule (and as described herein, modulate its activity), are useful according to the invention.

[0142] According to a further aspect of the invention, a method of treating apoptotic cell-death of a cardiac cell in a subject, is provided. The method involves administering to a subject in need of such treatment an agent that modulates expression of a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, in an effective amount to inhibit apoptotic cell-death of the cardiac cell in the subject.

[0143] In certain embodiments, the molecule is a nucleic acid. In some embodiments the nucleic acid is operatively coupled to a gene expression sequence which directs the expression of the nucleic acid molecule within a eukaryotic cell such as a cardiomyocyte and/or a vascular endothelial cell (including a smooth muscle cell). The "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the nucleic acid to which it is operably linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPTR), adenosine deaminase, pyruvate kinase, α-actin promoter and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the simian virus, papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of moloney leukemia virus and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are activated in the presence of an inducing agent. For example, the metallothionein promoter is activated to increase transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

[0144] In general, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined nucleic acid. The gene expression sequences optionally includes enhancer sequences or upstream activator sequences as desired.

[0145] Preferably, any of the nucleic acid molecules of the invention (e.g., MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d) is linked to a gene expression sequence which permits expression of the nucleic acid molecule in a cell such as a cardiomyocyte and/or a vascular endothelial cell (including a smooth muscle cell). More preferably, the gene expression sequence permits expression of the nucleic acid molecule in a cell (such as a cardiomyocyte and/or a vascular endothelial cell) and does not permit expression of the molecule in a cell selected from the group consisting of a neuronal cell, a fibroblasts and a cell of hematopoietic origin. A sequence which permits expression of the nucleic acid molecule in a cell such as a cardiomyocyte and/or a vascular endothelial cell, is one which is selectively active in such a cell type, thereby causing expression of the nucleic acid molecule in these cells. The cardiac myosin heavy chain gene promoter, for example, can be used to express any of the foregoing nucleic acid molecules of the invention in a cardiomyocyte; and the von Willebrand factor gene promoter, for example, can be used to express a nucleic acid molecule in a vascular endothelial cell. Those of ordinary skill in the art will be able to easily identify alternative promoters that are capable of expressing a nucleic acid molecule in any of the preferred cells of the invention.

[0146] The nucleic acid sequence and the gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the transcription and/or translation of the nucleic acid coding sequence (e.g., in the case of MIVR-1, SEQ ID NO. 3) under the influence or control of the gene expression sequence. If it is desired that the nucleic acid sequence be translated into a functional protein, two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the nucleic acid sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the nucleic acid sequence, and/or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that nucleic acid sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

[0147] The molecules of the invention can be delivered to the preferred cell types of the invention alone or in association with a vector. In its broadest sense, a "vector" is any

vehicle capable of facilitating: (1) delivery of a molecule to a target cell and/or (2) uptake of the molecule by a target cell. Preferably, the vectors transport the molecule into the target cell with reduced degradation relative to the extent of degradation that would result in the absence of the vector. Optionally, a "targeting ligand" can be attached to the vector to selectively deliver the vector to a cell which expresses on its surface the cognate receptor for the targeting ligand. In this manner, the vector (containing a nucleic acid or a protein) can be selectively delivered to a cardiomyocyte cell in, e.g., the myocardium. Methodologies for targeting include conjugates, such as those described in U.S. Pat. No. 5,391,723 to Priest. Another example of a well-known targeting vehicle is a liposome. Liposomes are commercially available from Gibco BRL. Numerous methods are published for making targeted liposomes. Preferably, the molecules of the invention are targeted for delivery to cardiomyocytes, and/or vascular endothelial cells.

[0148] In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the nucleic acid sequences of the invention, and additional nucleic acid fragments (e.g., enhancers, promoters) which can be attached to the nucleic acid sequences of the invention. Viral vectors are a preferred type of vector and include, but are not limited to, nucleic acid sequences from the following viruses: adenovirus; adeno-associated virus; retrovirus, such as moloney murine leukemia virus; harvey murine sarcoma virus; murine mammary tumor virus; rouse sarcoma virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known in the art.

[0149] A particularly preferred virus for certain applications is the adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus is capable of infecting a wide range of cell types and species and can be engineered to be replication-deficient. It further has advantages, such as heat and lipid solvent stability, high transduction frequencies in cells of diverse lineages, including hematopoietic cells, and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

[0150] In general, other preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Adenoviruses and retroviruses have been approved for human gene therapy trials. In general, the retroviruses are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., "Gene Transfer and Expression, A Laboratory Manual," W.H. Freeman Co., New York (1990) and Murry, E. J. Ed. "Methods in Molecular Biology," vol. 7, Humana Press, Inc., Cliffton, N.J. (1991).

[0151] Another preferred retroviral vector is the vector derived from the moloney murine leukemia virus, as described in Nabel, E. G., et al., *Science,*1990, 249:1285-1288. These vectors reportedly were effective for the delivery of genes to all three layers of the arterial wall, including the media. Other preferred vectors are disclosed in Flugelman, et al., *Circulation,* 1992, 85:1110-1117. Additional vectors that are useful for delivering molecules of the invention are described in U.S. Patent No. 5,674,722 by Mulligan, et. al.

[0152] In addition to the foregoing vectors, other delivery methods may be used to deliver a molecule of the invention to a cell such as a cardiomyocyte and/or a vascular endothelial cell, and facilitate uptake thereby.

[0153] A preferred such delivery method of the invention is a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system of the invention is a liposome. Liposomes are artificial membrane vessels which are useful as a delivery vector in vivo or in vitro. It has been shown that large unilamellar vessels (LUV), which range in size from 0.2-4.0 μm can encapsulate large macromolecules. RNA, DNA, and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.,* 1981, 6:77). In order for a liposome to be an efficient gene transfer vector, one or more of the following characteristics should be present: (1) encapsulation of the gene of interest at high efficiency with retention of biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information.

[0154] Liposomes may be targeted to a particular tissue, such as the myocardium or the vascular cell wall, by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Ligands which may be useful for targeting a liposome to the vascular wall include, but are not limited to the viral coat protein of the Hemagglutinating virus of Japan. Additionally, the vector may be coupled to a nuclear targeting peptide, which will direct the nucleic acid to the nucleus of the host cell.

[0155] Liposomes are commercially available from Gibco BRL, for example, as LIPOFECTIN™ and LIPOFECTACE™, which are formed of cationic lipids such as N-[(1-2, 3 dioleyloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium

bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Liposomes also have been reviewed by Gregoriadis, G. in *Trends in Biotechnology,* V. 3, p. 235-241 (1985). Novel liposomes for the intracellular delivery of macromolecules, including nucleic acids, are also described in PCT International application no. PCT/US96/07572 (Publication No. WO 96/40060, entitled "Intracellular Delivery of Macromolecules").

[0156] In one particular embodiment, the preferred vehicle is a biocompatible micro particle or implant that is suitable for implantation into the mammalian recipient. Exemplary bioerodible implants that are useful in accordance with this method are described in PCT International application no. PCT/US/03307 (Publication No. WO 95/24929, entitled "Polymeric Gene Delivery System", claiming priority to U.S. patent application Ser. No. 213,668, filed Mar. 15, 1994). PCT/US/0307 describes a biocompatible, preferably biodegradable polymeric matrix for containing an exogenous gene under the control of an appropriate promoter. The polymeric matrix is used to achieve sustained release of the exogenous gene in the patient. In accordance with the instant invention, the nucleic acids described herein are encapsulated or dispersed within the biocompatible, preferably biodegradable polymeric matrix disclosed in PCT/US/03307. The polymeric matrix preferably is in the form of a micro particle such as a micro sphere (wherein a nucleic acid is dispersed throughout a solid polymeric matrix) or a microcapsule (wherein a nucleic acid is stored in the core of a polymeric shell). Other forms of the polymeric matrix for containing the nucleic acids of the invention include films, coatings, gels, implants, and stents. The size and composition of the polymeric matrix device is selected to result in favorable release kinetics in the tissue into which the matrix device is implanted. The size of the polymeric matrix devise further is selected according to the method of delivery which is to be used, typically injection into a tissue or administration of a suspension by aerosol into the nasal and/or pulmonary areas. The polymeric matrix composition can be selected to have both favorable degradation rates and also to be formed of a material which is bioadhesive, to further increase the effectiveness of transfer when the devise is administered to a vascular surface. The matrix composition also can be selected not to degrade, but rather, to release by diffusion over an extended period of time.

[0157] Both non-biodegradable and biodegradable polymeric matrices can be used to deliver the nucleic acids of the invention to the subject. Biodegradable matrices are preferred. Such polymers may be natural or synthetic polymers. Synthetic polymers are preferred. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable. The polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multi-valent ions or other polymers.

[0158] In general, the nucleic acids of the invention are delivered using the bioerodible implant by way of diffusion, or more preferably, by degradation of the polymeric matrix.

[0159] Exemplary synthetic polymers which can be used to form the biodegradable delivery system include: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, poly-vinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, poly vinyl chloride, polystyrene and polyvinylpyrrolidone.

[0160] Examples of non-biodegradable polymers include ethylene vinyl acetate, poly(meth) acrylic acid, polyamides, copolymers and mixtures thereof.

[0161] Examples of biodegradable polymers include synthetic polymers such as polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water in vivo, by surface or bulk erosion.

[0162] Bioadhesive polymers of particular interest include bioerodible hydrogels described by H. S. Sawhney, C. P. Pathak and J. A. Hubell in Macromolecules, 1993, 26, 581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate). Thus, the invention provides a composition of the above-described molecules of the invention for use as a medicament, methods for preparing the medicament and methods for the sustained release of the medicament in vivo.

[0163] Compaction agents also can be used in combination with a vector of the invention. A "compaction agent"as used herein, refers to an agent, such as a histone, that neutralizes the negative charges on the nucleic acid and thereby permits compaction of the nucleic acid into a fine granule. Compaction of the nucleic acid facilitates the uptake of the nucleic acid by the target cell. The compaction

agents can be used alone, i.e., to deliver an isolated nucleic acid of the invention in a form that is more efficiently taken up by the cell or, more preferably, in combination with one or more of the above-described vectors.

[0164] Other exemplary compositions that can be used to facilitate uptake by a target cell of the nucleic acids of the invention include calcium phosphate and other chemical mediators of intracellular transport, microinjection compositions, electroporation and homologous recombination compositions (e.g., for integrating a nucleic acid into a preselected location within the target cell chromosome).

[0165] The invention also provides methods for the diagnosis and therapy of vascular and cardiovascular disorders. Such disorders include myocardial infarction, stroke, arteriosclerosis, heart failure, and cardiac hypertrophy.

[0166] The methods of the invention are useful in both the acute and the prophylactic treatment of any of the foregoing conditions. As used herein, an acute treatment refers to the treatment of subjects having a particular condition. Prophylactic treatment refers to the treatment of subjects at risk of having the condition, but not presently having or experiencing the symptoms of the condition.

[0167] In its broadest sense, the terms "treatment" or "to treat" refer to both acute and prophylactic treatments. If the subject in need of treatment is experiencing a condition (or has or is having a particular condition), then treating the condition refers to ameliorating, reducing or eliminating the condition or one or more symptoms arising from the condition. In some preferred embodiments, treating the condition refers to ameliorating, reducing or eliminating a specific symptom or a specific subset of symptoms associated with the condition. If the subject in need of treatment is one who is at risk of having a condition, then treating the subject refers to reducing the risk of the subject having the condition.

[0168] Stroke (also referred to herein as ischemic stroke and/or cerebrovascular ischemia) is often cited as the third most common cause of death in the industrial world, ranking behind ischemic heart disease and cancer. Strokes are responsible for about 300,000 deaths annually in the United States and are a leading cause of hospital admissions and long-term disabilities. Accordingly, the socioeconomic impact of stroke and its attendant burden on society is practically immeasurable.

[0169] "Stroke" is defined by the World Health Organization as a rapidly developing clinical sign of focal or global disturbance of cerebral function with symptoms lasting at least 24 hours. Strokes are also implicated in deaths where there is no apparent cause other than an effect of vascular origin.

[0170] Strokes are typically caused by blockages or occlusions of the blood vessels to the brain or within the brain. With complete occlusion, arrest of cerebral circulation causes cessation of neuronal electrical activity within seconds. Within a few minutes after the deterioration of the energy state and ion homeostasis, depletion of high energy phosphates, membrane ion pump failure, efflux of cellular potassium, influx of sodium chloride and water, and membrane depolarization occur. If the occlusion persists for more than five to ten minutes, irreversible damage results. With incomplete ischemia, however, the outcome is difficult to evaluate and depends largely on residual perfusion and the availability of oxygen. After a thrombotic occlusion of a cerebral vessel, ischemia is rarely total. Some residual perfusion usually persists in the ischemic area, depending on collateral blood flow and local perfusion pressure.

[0171] Cerebral blood flow can compensate for drops in mean arterial blood pressure from 90 to 60 mm Hg by autoregulation. This phenomenon involves dilatation of downstream resistant vessels. Below the lower level of autoregulation (about 60 mm Hg), vasodilatation is inadequate and the cerebral blood flow falls. The brain, however, has perfusion reserves that can compensate for the fall in cerebral blood flow. This reserve exists because under normal conditions only about 35% of the oxygen delivered by the blood is extracted. Therefore, increased oxygen extraction can take place, provided that normoxia and normocapnea exist. When distal blood pressure falls below approximately 30 mm Hg, the two compensatory mechanisms (autoregulation and perfusion reserve) are inadequate to prevent failure of oxygen delivery.

[0172] As blood flow drops below the ischemic threshold of 23 ml/100 g/minute, symptoms of tissue hypoxia develop. Severe ischemia may be lethal. When the ischemia is moderate, it will result in "penumbra." In the neurological context, penumbra refers to a zone of brain tissue with moderate ischemia and paralyzed neuronal function, which is reversible with restoration of adequate perfusion. The penumbra forms a zone of collaterally perfused tissue surrounding a core of severe ischemia in which an infarct has developed. In other words, the penumbra is the tissue area that can be saved, and is essentially in a state between life and death.

[0173] Although an ischemic event can occur anywhere in the vascular system, the carotid artery bifurcation and the origin of the internal carotid artery are the most frequent sites for thrombotic occlusions of cerebral blood vessels, which result in cerebral ischemia. The symptoms of reduced blood flow due to stenosis or thrombosis are similar to those caused by middle cerebral artery disease. Flow through the ophthalmic artery is often affected sufficiently to produce amaurosis fugax or transient monocular blindness. Severe bilateral internal carotid artery stenosis may result in cerebral hemispheric hypoperfusion. This manifests with acute headache ipsilateral to the acutely ischemic hemisphere. Occlusions or decrease of the blood flow with resulting ischemia of one anterior cerebral artery distal to the anterior communicating artery produces motor and cortical sensory symptoms in the contralateral leg and, less often, proximal arm. Other manifestations of occlusions or underperfusion of the anterior cerebral artery include gait ataxia and sometimes urinary incontinence due to damage to the parasagital frontal lobe. Language disturbances manifested as decreased spontaneous speech may accompany generalized depression of psychomotor activity.

[0174] Most ischemic strokes involve portions or all of the territory of the middle cerebral artery with emboli from the heart or extracranial carotid arteries accounting for most cases. Emboli may occlude the main stem of the middle cerebral artery, but more frequently produce distal occlusion of either the superior or the inferior branch. Occlusions of the superior branch cause weakness and sensory loss that are greatest in the face and arm. Occlusions of the posterior

cerebral artery distal to its penetrating branches cause complete contralateral loss of vision. Difficulty in reading (dyslexia) and in performing calculations (dyscalculia) may follow ischemia of the dominant posterior cerebral artery. Proximal occlusion of the posterior cerebral artery causes ischemia of the branches penetrating to calamic and limbic structures. The clinical results are hemisensory disturbances that may chronically change to intractable pain of the defective side (thalamic pain).

[0175] A subject having a stroke is so diagnosed by symptoms experienced and/or by a physical examination including interventional and non-interventional diagnostic tools such as CT and MR imaging. The methods of the invention are advantageous for the treatment of various clinical presentations of stroke subjects. A subject having a stroke may present with one or more of the following symptoms: paralysis, weakness, decreased sensation and/or vision, numbness, tingling, aphasia (e.g., inability to speak or slurred speech, difficulty reading or writing), agnosia (i.e., inability to recognize or identify sensory stimuli), loss of memory, co-ordination difficulties, lethargy, sleepiness or unconsciousness, lack of bladder or bowel control and cognitive decline (e.g., dementia, limited attention span, inability to concentrate). Using medical imaging techniques, it may be possible to identify a subject having a stroke as one having an infarct or one having hemorrhage in the brain.

[0176] An important embodiment of the invention is treatment of a subject with an abnormally elevated risk of an ischemic stroke. As used herein, subjects having an abnormally elevated risk of an ischemic stroke are a category determined according to conventional medical practice (see earlier discussion); such subjects may also be identified in conventional medical practice as having known risk factors for stroke or having increased risk of cerebrovascular events. This category includes, for example, subjects which are having elected vascular surgery. Typically, the risk factors associated with cardiac disease are the same as are associated with stroke. The primary risk factors include hypertension, hypercholesterolemia, and smoking. Atrial fibrillation or recent myocardial infarction are also important risk factors. In addition, modified levels of expression of a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, are also, according to the present invention, important risk factors.

[0177] As used herein, subjects having an abnormally elevated risk of an ischemic stroke also include individuals undergoing surgical or diagnostic procedures which risk release of emboli, lowering of blood pressure or decrease in blood flow to the brain, such as carotid endarterectomy, brain angiography, neurosurgical procedures in which blood vessels are compressed or occluded, cardiac catheterization, angioplasty, including balloon angioplasty, coronary by-pass surgery, or similar procedures. Subjects having an abnormally elevated risk of an ischemic stroke also include individuals having any cardiac condition that may lead to decreased blood flow to the brain, such as atrial fibrillation, ventrical tachycardia, dilated cardiomyopathy and other cardiac conditions requiring anticoagulation. Subjects having an abnormally elevated risk of an ischemic stroke also include individuals having conditions including arteriopathy or brain vasculitis, such as that caused by lupus, congenital diseases of blood vessels, such as CADASIL syndrome, or migraine, especially prolonged episodes.

[0178] The treatment of stroke can be for patients who have experienced a stroke or can be a prophylactic treatment. Short term prophylactic treatment is indicated for subjects having surgical or diagnostic procedures which risk release of emboli, lowering of blood pressure or decrease in blood flow to the brain, to reduce the injury due to any ischemic event that occurs as a consequence of the procedure. Longer term or chronic prophylactic treatment is indicated for subjects having cardiac conditions that may lead to decreased blood flow to the brain, or conditions directly affecting brain vasculature. If prophylactic, then the treatment is for subjects having an abnormally elevated risk of an ischemic stroke, as described above. If the subject has experienced a stroke, then the treatment can include acute treatment. Acute treatment for stroke subjects means administration of an agent of the invention at the onset of symptoms of the condition or within 48 hours of the onset, preferably within 24 hours, more preferably within 12 hours, more preferably within 6 hours, and even more preferably within 3 hours of the onset of symptoms of the condition.

[0179] Criteria for defining hypercholesterolemic and/or hypertriglyceridemic subjects are well known in the art (see, e.g., Harrison's Principles of Experimental Medicine, 13th Edition, McGraw-Hill, Inc., N.Y.—hereinafter "Harrison's). Hypercholesterolemic subjects and hypertriglyceridemic subjects are associated with increased incidence of premature coronary heart disease. A hypercholesterolemic subject has an LDL level of >160 mg/dL or >130 mg/dL and at least two risk factors selected from the group consisting of male gender, family history of premature coronary heart disease, cigarette smoking (more than 10 per day), hypertension, low HDL (<35 mg/dL), diabetes mellitus, hyperinsulinemia, abdominal obesity, high lipoprotein (a), and personal history of cerebrovascular disease or occlusive peripheral vascular disease. A hypertriglyceridemic subject has a triglyceride (TG) level of >250 mg/dL. Thus, a hyperlipidemic subject is defined as one whose cholesterol and triglyceride levels equal or exceed the limits set as described above for both the hypercholesterolemic and hypertriglyceridemic subjects.

[0180] "Myocardial infarction" is a focus of necrosis resulting from inadequate perfusion of the cardiac tissue. Myocardial infarction generally occurs with the abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by atherosclerosis. Generally, infarction occurs when an atherosclerotic plaque fissures, ruptures, or ulcerates, and a mural thrombus forms leading to coronary artery occlusion.

[0181] The diagnosis of myocardial infarction in a subject determines the need for treating the subject according to the methods of the invention. A number of laboratory tests, well known in the art, are described, for example, in Harrison's: Principles of Internal Medicine (McGraw Hill, Inc., New York). Generally, the tests may be divided into four main categories: (1) nonspecific indexes of tissue necrosis and inflammation, (2) electrocardiograms, (3) serum enzyme changes (e.g., creatine phosphokinase levels), and (4) cardiac imaging. A person of ordinary skill in the art could easily apply any of the foregoing tests to determine when a subject is at risk, is suffering, or has suffered, a myocardial infarction. In addition, decreased levels of expression of a

nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, are also, according to the present invention, important risk factors. A positively identified subject would thus benefit from a method of treatment of the invention.

[0182] According to the invention, the method involves administering to a subject having a myocardial infarction any of the foregoing molecules (MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d) in an amount effective to treat the cardiovascular disorder in the subject. By "having a myocardial infarction" it is meant that the subject is at risk of developing, is currently having, or has suffered a myocardial infarction. It is believed that immediate administration of the molecule would greatly benefit the subject by inhibiting apoptotic cell-death of cardiomyocytes (the cells mostly affected by the infarct) prior to, or following the infarct. By "immediate" it is meant that administration occurs before (if it is diagnosed in time), or within 48 hours from the myocardial infarct, although administration up to 14 days after the episode may also be beneficial to the subject.

[0183] Another important embodiment of the invention is the treatment of ischemic injury resulting from arteriosclerosis. Arteriosclerosis is a term used to describe a thickening and hardening of the arterial wall. It is believed to be responsible for the majority of deaths in the United States and in most westernized societies. Atherosclerosis is one type of arteriosclerosis that is believed to be the cause of most coronary artery disease, aortic aneurysm and arterial disease of the lower extremities (including peripheral vascular arteriopathy), as well as contributing to cerebrovascular disease. Atherosclerosis is the leading cause of death in the United States.

[0184] A normal artery typically is lined on its inner-side only by a single layer of endothelial cells, the intima. The intima overlays the media, which contains only a single cell type, the smooth muscle cell. The outer-most layer of the artery is the adventitia. With aging, there is a continuous increase in the thickness of the intima, believed to result in part from migration and proliferation of smooth muscle cells from the media. A similar increase in the thickness of the intima also occurs as a result of various traumatic events or interventions, such as occurs when, for example, a balloon dilatation procedure causes injury to the vessel wall. The invention is used in connection with treating ischemic injury resulting from arteriosclerotic conditions. An arteriosclerotic condition as used herein means classical atherosclerosis, accelerated atherosclerosis, atherosclerosis lesions and any other arteriosclerotic conditions characterized by undesirable endothelial and/or vascular smooth muscle cell proliferation, including vascular complications of diabetes.

[0185] Another important embodiment of the invention is the treatment of heart failure. Heart failure is a clinical syndrome of diverse etiologies linked by the common denominator of impaired heart pumping and is characterized by the failure of the heart to pump blood commensurate with the requirements of the metabolizing tissues, or to do so only from an elevating filling pressure.

[0186] Another important embodiment of the invention is the treatment of cardiac hypertrophy. This condition is typically characterized by left ventricular hypertrophy, usually of a nondilated chamber, without obvious antecedent cause. Current methods of diagnosis include the electrocardiogram and the echocardiogram. Many patients, however, are asymptomatic and may be relatives of patients with known disease. Unfortunately, the first manifestation of the disease may be sudden death, frequently occurring in children and young adults, often during or after physical exertion.

[0187] Agents for reducing the risk of or treating a cardiovascular disorder include those selected from the group consisting of anti-inflammatory agents, anti-thrombotic agents, anti-platelet agents, fibrinolytic agents, lipid reducing agents, direct thrombin inhibitors, glycoprotein IIb/IIIa receptor inhibitors, agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules (e.g. anti-cellular adhesion molecule antibodies), calcium channel blockers, beta-adrenergic receptor blockers, cyclooxygenase-2 inhibitors, angiotensin system inhibitors, and/or any combinations thereof.

[0188] One preferred agent is aspirin.

[0189] The mode of administration and dosage of a therapeutic agent of the invention will vary with the particular stage of the condition being treated, the age and physical condition of the subject being treated, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route of administration, and the like factors within the knowledge and expertise of the health practitioner.

[0190] As described herein, the agents of the invention are administered in effective amounts to treat any of the foregoing cardiovascular disorders. In general, an effective amount is any amount that can cause a beneficial change in a desired tissue of a subject. Preferably, an effective amount is that amount sufficient to cause a favorable phenotypic change in a particular condition such as a lessening, alleviation or elimination of a symptom or of a condition as a whole.

[0191] In general, an effective amount is that amount of a pharmaceutical preparation that alone, or together with further doses, produces the desired response. This may involve only slowing the progression of the condition temporarily, although more preferably, it involves halting the progression of the condition permanently or delaying the onset of or preventing the condition from occurring. This can be monitored by routine methods. Generally, doses of active compounds would be from about 0.01 mg/kg per day to 1000 mg/kg per day. It is expected that doses ranging from 50-500 mg/kg will be suitable, preferably orally and in one or several administrations per day.

[0192] Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. Lower doses will result from certain forms of administration, such as intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds. It is

preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

[0193] The agents of the invention may be combined, optionally, with a pharmaceutically-acceptable carrier to form a pharmaceutical preparation. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy. In some aspects, the pharmaceutical preparations comprise an agent of the invention in an amount effective to treat a disorder.

[0194] The pharmaceutical preparations may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; or phosphoric acid in a salt. The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens or thimerosal.

[0195] A variety of administration routes are available. The particular mode selected will depend, of course, upon the particular drug selected, the severity of the condition being treated and the dosage required for therapeutic efficacy. The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, nasal, intradermal, transdermal, or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion. Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis. As an example, pharmaceutical compositions for the acute treatment of subjects having a migraine headache may be formulated in a variety of different ways and for a variety of administration modes including tablets, capsules, powders, suppositories, injections and nasal sprays.

[0196] The pharmaceutical preparations may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

[0197] Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

[0198] Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of an agent of the invention, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in *Remington's Pharmaceutical Sciences,* Mack Publishing Co., Easton, Pa.

[0199] According to one aspect of the invention, a method for inhibiting apoptotic cell-death of a cell, is provided. The method involves contacting an isolated nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, fragments thereof, with a cell under conditions that permit entry of the nucleic acid molecule (or of an expression product thereof) into the cell, in an amount effective to inhibit apoptotic cell-death of the cell.

[0200] The term "permit entry" of a molecule into a cell according to the invention has the following meanings depending upon the nature of the molecule. For an isolated nucleic acid it is meant to describe entry of the nucleic acid through the cell membrane and into the cell nucleus, where upon the "nucleic acid transgene" can utilize the cell machinery to produce functional polypeptides encoded by the nucleic acid. By "nucleic acid transgene" it is meant to describe all of the nucleic acids of the invention with or without the associated vectors. For a polypeptide, it is meant to describe entry of the polypeptide through the cell membrane and into the cell cytoplasm, and if necessary, utilization of the cell cytoplasmic machinery to functionally modify the polypeptide (e.g., to an active form).

[0201] Various techniques may be employed for introducing nucleic acids of the invention into cells, depending on whether the nucleic acids are introduced in vitro or in vivo in a host. Such techniques include transfection of nucleic acid-$CaPO_4$ precipitates, transfection of nucleic acids associated with DEAE, transfection with a retrovirus including the nucleic acid of interest, liposome mediated transfection, and the like. For certain uses, it is preferred to target the nucleic acid to particular cells. In such instances, a vehicle used for delivering a nucleic acid of the invention into a cell (e.g., a retrovirus, or other virus; a liposome) can have a targeting molecule attached thereto. For example, a molecule such as an antibody specific for a surface membrane protein on the target cell or a ligand for a receptor on the target cell can be bound to or incorporated within the nucleic acid delivery vehicle. For example, where liposomes are employed to deliver the nucleic acids of the invention, proteins which bind to a surface membrane protein associated with endocytosis may be incorporated into the liposome formulation for targeting and/or to facilitate uptake. Such

proteins include capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half life, and the like. Polymeric delivery systems also have been used successfully to deliver nucleic acids into cells, as is known by those skilled in the art. Such systems even permit oral delivery of nucleic acids.

[0202] Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of an agent of the present invention, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the invention is contained in a form within a matrix such as those described in U.S. Pat. Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. Nos. 3,854, 480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[0203] Use of a long-term sustained release implant may be desirable. Long-term release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above. Specific examples include, but are not limited to, long-term sustained release implants described in U.S. Pat. No. 4,748, 024, and Canadian Patent No. 1330939.

[0204] The invention also involves the administration, and in some embodiments co-administration, of agents other than the molecules of the invention (MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, nucleic acids and polypeptides, and/or fragments thereof) that when administered in effective amounts can act cooperatively, additively or synergistically with a molecule of the invention to: (i) modulate cardiac cell anti-apoptotic activity, and (ii) treat any of the conditions in which cardiac cell anti-apoptotic activity of a molecule of the invention is involved. Agents other than the molecules of the invention include anti-inflammatory agents, anti-thrombotic agents, anti-coagulants, anti-platelet agents, fibrinolytic agents, lipid reducing agents, direct thrombin inhibitors, glycoprotein IIb/IIIa receptor inhibitors, agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules, calcium channel blockers, beta-adrenergic receptor blockers, cyclooxygenase-2 inhibitors, angiotensin system inhibitors, anti-hypertensive agents, and/or combinations thereof.

[0205] "Anti-inflammatory" agents include Alclofenac; Alclometasone Dipropionate; Algestone Acetonide; Alpha Amylase; Amcinafal; Amcinafide; Amfenac Sodium; Amiprilose Hydrochloride; Anakinra; Anirolac; Anitrazafen; Apazone; Balsalazide Disodium; Bendazac; Benoxaprofen; Benzydamine Hydrochloride; Bromelains; Broperamole; Budesonide; Carprofen; Cicloprofen; Cintazone; Cliprofen; Clobetasol Propionate; Clobetasone Butyrate; Clopirac; Cloticasone Propionate; Cormethasone Acetate; Cortodoxone; Deflazacort; Desonide; Desoximetasone; Dexamethasone Dipropionate; Diclofenac Potassium; Diclofenac Sodium; Diflorasone Diacetate; Diflumidone Sodium; Diflunisal; Difluprednate; Diftalone; Dimethyl Sulfoxide; Drocinonide; Endrysone; Enlimomab; Enolicam Sodium; Epirizole; Etodolac; Etofenamate; Felbinac; Fenamole; Fenbufen; Fenclofenac; Fenclorac; Fendosal; Fenpipalone; Fentiazac; Flazalone; Fluazacort; Flufenamic Acid; Flumizole; Flunisolide Acetate; Flunixin; Flunixin Meglumine; Fluocortin Butyl; Fluorometholone Acetate; Fluquazone; Flurbiprofen; Fluretofen; Fluticasone Propionate; Furaprofen; Furobufen; Halcinonide; Halobetasol Propionate; Halopredone Acetate; Ibufenac; Ibuprofen; Ibuprofen Aluminum; Ibuprofen Piconol; Ilonidap; Indomethacin; Indomethacin Sodium; Indoprofen; Indoxole; Intrazole; Isoflupredone Acetate; Isoxepac; Isoxicam; Ketoprofen; Lofemizole Hydrochloride; Lornoxicam; Loteprednol Etabonate; Meclofenamate Sodium; Meclofenamic Acid; Meclorisone Dibutyrate; Mefenamic Acid; Mesalamine; Meseclazone; Methylprednisolone Suleptanate; Morniflumate; Nabumetone; Naproxen; Naproxen Sodium; Naproxol; Nimazone; Olsalazine Sodium; Orgotein; Orpanoxin; Oxaprozin; Oxyphenbutazone; Paranyline Hydrochloride; Pentosan Polysulfate Sodium; Phenbutazone Sodium Glycerate; Pirfenidone; Piroxicam; Piroxicam Cinnamate; Piroxicam Olamine; Pirprofen; Prednazate; Prifelone; Prodolic Acid; Proquazone; Proxazole; Proxazole Citrate; Rimexolone; Romazarit; Salcolex; Salnacedin; Salsalate; Salycilates; Sanguinarium Chloride; Seclazone; Sermetacin; Sudoxicam; Sulindac; Suprofen; Talmetacin; Talniflumate; Talosalate; Tebufelone; Tenidap; Tenidap Sodium; Tenoxicam; Tesicam; Tesimide; Tetrydamine; Tiopinac; Tixocortol Pivalate; Tolmetin; Tolmetin Sodium; Triclonide; Triflumidate; Zidometacin; Glucocorticoids; Zomepirac Sodium. One preferred anti-inflammatory agent is aspirin.

[0206] "Anti-thrombotic" and/or "fibrinolytic" agents include Plasminogen (to plasmin via interactions of prekallikrein, kininogens, Factors XII, XIIIa, plasminogen proactivator, and tissue plasminogen activator[TPA]) Streptokinase; Urokinase: Anisoylated Plasminogen-Streptokinase Activator Complex; Pro-Urokinase; (Pro-UK); rTPA (alteplase or activase; r denotes recombinant); rPro-UK; Abbokinase; Eminase; Sreptase Anagrelide Hydrochloride; Bivalirudin; Dalteparin Sodium; Danaparoid Sodium; Dazoxiben Hydrochloride; Efegatran Sulfate; Enoxaparin Sodium; Ifetroban; Ifetroban Sodium; Tinzaparin Sodium; retaplase; Trifenagrel; Warfarin; Dextrans.

[0207] "Anti-platelet" agents include Clopridogrel; Sulfinpyrazone; Aspirin; Dipyridamole; Clofibrate; Pyridinol Carbamate; PGE; Glucagon; Antiserotonin drugs; Caffeine; Theophyllin Pentoxifyllin; Ticlopidine; Anagrelide.

[0208] "Lipid reducing" agents include gemfibrozil, cholystyramine, colestipol, nicotinic acid, probucol lovastatin, fluvastatin, simvastatin, atorvastatin, pravastatin, cirivastatin.

[0209] "Direct thrombin inhibitors" include hirudin, hirugen, hirulog, agatroban, PPACK, thrombin aptamers.

[0210] "Glycoprotein IIb/IIIa receptor inhibitors" are both antibodies and non-antibodies, and include but are not limited to ReoPro (abcixamab), lamifiban, tirofiban.

[0211] "Calcium channel blockers" are a chemically diverse class of compounds having important therapeutic value in the control of a variety of diseases including several cardiovascular disorders, such as hypertension, angina, and cardiac arrhythmias (Fleckenstein, *Cir. Res.* v. 52, (suppl. 1), p.13-16 (1983); Fleckenstein, *Experimental Facts and Therapeutic Prospects,* John Wiley, New York (1983); McCall, D., *Curr Pract Cardiol,* v. 10, p. 1-11 (1985)). Calcium channel blockers are a heterogeneous group of drugs that prevent or slow the entry of calcium into cells by regulating cellular calcium channels. (Remington, *The Science and Practice of Pharmacy,* Nineteenth Edition, Mack Publishing Company, Eaton, Pa, p.963 (1995)). Most of the currently available calcium channel blockers, and useful according to the present invention, belong to one of three major chemical groups of drugs, the dihydropyridines, such as nifedipine, the phenyl alkyl amines, such as verapamil, and the benzothiazepines, such as diltiazem. Other calcium channel blockers useful according to the invention, include, but are not limited to, amrinone, amlodipine, bencyclane, felodipine, fendiline, flunarizine, isradipine, nicardipine, nimodipine, perhexilene, gallopamil, tiapamil and tiapamil analogues (such as 1993RO-11-2933), phenytoin, barbiturates, and the peptides dynorphin, omega-conotoxin, and omega-agatoxin, and the like and/or pharmaceutically acceptable salts thereof.

[0212] "Beta-adrenergic receptor blocking agents" are a class of drugs that antagonize the cardiovascular effects of catecholamines in angina pectoris, hypertension, and cardiac arrhythmias. Beta-adrenergic receptor blockers include, but are not limited to, atenolol, acebutolol, alprenolol, befunolol, betaxolol, bunitrolol, carteolol, celiprolol, hedroxalol, indenolol, labetalol, levobunolol, mepindolol, methypranol, metindol, metoprolol, metrizoranolol, oxprenolol, pindolol, propranolol, practolol, practolol, sotalolnadolol, tiprenolol, tomalolol, timolol, bupranolol, penbutolol, trimepranol, 2-(3-(1,1-dimethylethyl)-amino-2-hydroxypropoxy)-3-pyridenecarbonitrilHCl, 1-butylamino-3-(2,5-dichlorophenoxy)-2-propanol, 1-isopropylamino-3-(4-(2-cyclopropylmethoxyethyl)phenoxy)-2-propanol, 3-isopropylamino-1-(7-methylindan-4-yloxy)-2-butanol, 2-(3-t-butylamino-2-hydroxy-propylthio)-4-(5-carbamoyl-2-thienyl)thiazol,7-(2-hydroxy-3-t-butylaminpropoxy)phthalide. The above-identified compounds can be used as isomeric mixtures, or in their respective levorotating or dextrorotating form.

[0213] Cyclooxygenase-2 (COX-2) is a recently identified form of a cyclooxygenase. "Cyclooxygenase" is an enzyme complex present in most tissues that produces various prostaglandins and thromboxanes from arachidonic acid. Nonsteroidal, anti-inflammatory drugs exert most of their anti-inflammatory, analgesic and antipyretic activity and inhibit hormone-induced uterine contractions and certain types of cancer growth through inhibition of the cyclooxygenase (also known as prostaglandin G/H synthase and/or prostaglandin-endoperoxide synthase). Initially, only one form of cyclooxygenase was known, the "constitutive enzyme" or cyclooxygenase-1 (COX-1). It and was originally identified in bovine seminal vesicles.

[0214] Cyclooxygenase-2 (COX-2) has been cloned, sequenced and characterized initially from chicken, murine and human sources (See, e.g., U.S. Pat. No. 5,543,297, issued Aug. 6, 1996 to Cromlish, et al., and assigned to Merck Frosst Canada, Inc., Kirkland, Calif., entitled: "Human cyclooxygenase-2 cDNA and assays for evaluating cyclooxygenase-2 activity"). This enzyme is distinct from the COX-1. COX-2 is rapidly and readily inducible by a number of agents including mitogens, endotoxin, hormones, cytokines and growth factors. As prostaglandins have both physiological and pathological roles, the constitutive enzyme, COX-1, is responsible, in large part, for endogenous basal release of prostaglandins and hence is important in their physiological functions such as the maintenance of gastrointestinal integrity and renal blood flow. By contrast, it is believed that the inducible form, COX-2, is mainly responsible for the pathological effects of prostaglandins where rapid induction of the enzyme would occur in response to such agents as inflammatory agents, hormones, growth factors, and cytokines. Therefore, it is believed that a selective inhibitor of COX-2 has similar anti-inflammatory, antipyretic and analgesic properties to a conventional non-steroidal anti-inflammatory drug, and in addition inhibits hormone-induced uterine contractions and also has potential anti-cancer effects, but with reduced side effects. In particular, such COX-2 inhibitors are believed to have a reduced potential for gastrointestinal toxicity, a reduced potential for renal side effects, a reduced effect on bleeding times and possibly a decreased potential to induce asthma attacks in aspirin-sensitive asthmatic subjects, and are therefore useful according to the present invention.

[0215] A number of selective "COX-2 inhibitors" are known in the art. These include, but are not limited to, COX-2 inhibitors described in U.S. Pat. No. 5,474,995 "Phenyl heterocycles as cox-2 inhibitors"; U.S. Pat. No. 5,521,213 "Diaryl bicyclic heterocycles as inhibitors of cyclooxygenase-2"; U.S. Pat. No. 5,536,752 "Phenyl heterocycles as COX-2 inhibitors"; U.S. Pat. No. 5,550,142 "Phenyl heterocycles as COX-2 inhibitors"; U.S. Pat. No. 5,552,422 "Aryl substituted 5,5 fused aromatic nitrogen compounds as anti-inflammatory agents"; U.S. Patent 5,604,253 "N-benzylindol-3-yl propanoic acid derivatives as cyclooxygenase inhibitors"; U.S. Pat. No. 5,604,260 "5-methanesulfonamido-1-indanones as an inhibitor of cyclooxygenase-2"; U.S. Pat. No. 5,639,780 N-benzyl indol-3-yl butanoic acid derivatives as cyclooxygenase inhibitors"; U.S. Pat. No. 5,677,318 Diphenyl-1,2-3-thiadiazoles as anti-inflammatory agents"; U.S. Pat. No. 5,691,374 "Diaryl-5-oxygenated-2-(5H) -furanones as COX-2 inhibitors"; U.S. Pat. No. 5,698,584 "3,4-diaryl-2-hydroxy-2,5-dihydrofurans as prodrugs to COX-2 inhibitors"; U.S. Pat. No. 5,710,140 "Phenyl heterocycles as COX-2 inhibitors"; U.S. Pat. No. 5,733,909 "Diphenyl stilbenes as prodrugs to COX-2 inhibitors"; U.S. Pat. No. 5,789,413 "Alkylated styrenes as prodrugs to COX-2 inhibitors"; U.S. Pat. No. 5,817,700 "Bisaryl cyclobutenes derivatives as cyclooxygenase inhibitors"; U.S. Pat. No. 5,849,943 "Stilbene derivatives useful as cyclooxygenase-2 inhibitors"; U.S. Pat. No.

5,861,419 "Substituted pyridines as selective cyclooxygenase-2 inhibitors"; U.S. Pat. No. 5,922,742 "Pyridinyl-2-cyclopenten-1-ones as selective cyclooxygenase-2 inhibitors"; U.S. Pat. No. 5,925,631 "Alkylated styrenes as prodrugs to COX-2 inhibitors"; all of which are commonly assigned to *Merck Frosst Canada, Inc.* (Kirkland, Calif.). Additional COX-2 inhibitors are also described in U.S. Pat. No. 5,643,933, assigned to *G.D. Searle & Co.* (Skokie, Ill.), entitled:"Substituted sulfonylphenylheterocycles as cyclooxygenase-2 and 5-lipoxygenase inhibitors."

[**0216**] A number of the above-identified COX-2 inhibitors are prodrugs of selective COX-2 inhibitors, and exert their action by conversion in vivo to the active and selective COX-2 inhibitors. The active and selective COX-2 inhibitors formed from the above-identified COX-2 inhibitor prodrugs are described in detail in WO 95/00501, published Jan. 5, 1995, WO 95/18799, published Jul. 13, 1995 and U.S. Pat. No. 5,474,995, issued Dec. 12, 1995. Given the teachings of U.S. Pat. No. 5,543,297, entitled:"Human cyclooxygenase-2 cDNA and assays for evaluating cyclooxygenase-2 activity," a person of ordinary skill in the art would be able to determine whether an agent is a selective COX-2 inhibitor or a precursor of a COX-2 inhibitor, and therefore part of the present invention.

[**0217**] An "angiotensin system inhibitor" is an agent that interferes with the function, synthesis or catabolism of angiotensin II. These agents include, but are not limited to, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II antagonists, angiotensin II receptor antagonists, agents that activate the catabolism of angiotensin II, and agents that prevent the synthesis of angiotensin I from which angiotensin II is ultimately derived. The renin-angiotensin system is involved in the regulation of hemodynamics and water and electrolyte balance. Factors that lower blood volume, renal perfusion pressure, or the concentration of Na^+ in plasma tend to activate the system, while factors that increase these parameters tend to suppress its function.

[**0218**] Angiotensin I and angiotensin II are synthesized by the enzymatic renin-angiotensin pathway. The synthetic process is initiated when the enzyme renin acts on angiotensinogen, a pseudoglobulin in blood plasma, to produce the decapeptide angiotensin I. Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II (angiotensin-[1-8] octapeptide). The latter is an active pressor substance which has been implicated as a causative agent in several forms of hypertension in various mammalian species, e.g., humans.

[**0219**] Angiotensin (renin-angiotensin) system inhibitors are compounds that act to interfere with the production of angiotensin II from angiotensinogen or angiotensin I or interfere with the activity of angiotensin II. Such inhibitors are well known to those of ordinary skill in the art and include compounds that act to inhibit the enzymes involved in the ultimate production of angiotensin II, including renin and ACE. They also include compounds that interfere with the activity of angiotensin II, once produced. Examples of classes of such compounds include antibodies (e.g., to renin), amino acids and analogs thereof (including those conjugated to larger molecules), peptides (including peptide analogs of angiotensin and angiotensin I), pro-renin related analogs, etc. Among the most potent and useful renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists. In a preferred embodiment of the invention, the renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists.

[**0220**] "Angiotensin II antagonists" are compounds which interfere with the activity of angiotensin II by binding to angiotensin II receptors and interfering with its activity. Angiotensin II antagonists are well known and include peptide compounds and non-peptide compounds. Most angiotensin II antagonists are slightly modified congeners in which agonist activity is attenuated by replacement of phenylalanine in position 8 with some other amino acid; stability can be enhanced by other replacements that slow degeneration in vivo. Examples of angiotensin II antagonists include: peptidic compounds (e.g., saralasin, [$(San^1)(Val^5)(Ala^8)$] angiotensin -(1-8) octapeptide and related analogs); N-substituted imidazole-2-one (U.S. Pat. No. 5,087,634); imidazole acetate derivatives including 2-N-butyl-4-chloro-1-(2-chlorobenzile) imidazole-5-acetic acid (see Long et al., *J. Pharmacol. Exp. Ther.* 247(1), 1-7 (1988)); 4, 5, 6, 7-tetrahydro-1H-imidazo [4, 5-c] pyridine-6-carboxylic acid and analog derivatives (U.S. Pat. No. 4,816,463); N2-tetrazole beta-glucuronide analogs (U.S. Pat. No. 5,085,992); substituted pyrroles, pyrazoles, and tryazoles (U.S. Pat. No. 5,081,127); phenol and heterocyclic derivatives such as 1, 3-imidazoles (U.S. Pat. No. 5,073,566); imidazo-fused 7-member ring heterocycles (U.S. Pat. No. 5,064,825); peptides (e.g., U.S. Pat. No. 4,772,684); antibodies to angiotensin II (e.g., U.S. Pat. No. 4,302,386); and aralkyl imidazole compounds such as biphenyl-methyl substituted imidazoles (e.g., EP Number 253,310, Jan. 20, 1988); ES8891 (N-morpholinoacetyl-(-1-naphthyl)-L-alanyl-(4, thiazolyl)-L-alanyl (35, 45)-4-amino-3-hydroxy-5-cyclo-hexapentanoyl-N-hexylamide, Sankyo Company, Ltd., Tokyo, Japan); SKF108566 (E-alpha-2-[2-butyl-1-(carboxy phenyl) methyl] 1H-imidazole-5-yl[methylane]-2-thiophenepropanoic acid, Smith Kline Beecham Pharmaceuticals, PA); Losartan (DUP753/MK954, DuPont Merck Pharmaceutical Company); Remikirin (RO42-5892, F. Hoffman LaRoche AG); A_2 agonists (Marion Merrill Dow) and certain non-peptide heterocycles (G.D. Searle and Company).

[**0221**] "Angiotensin converting enzyme (ACE), is an enzyme which catalyzes the conversion of angiotensin I to angiotensin II. ACE inhibitors include amino acids and derivatives thereof, peptides, including di- and tri- peptides and antibodies to ACE which intervene in the renin-angiotensin system by inhibiting the activity of ACE thereby reducing or eliminating the formation of pressor substance angiotensin II. ACE inhibitors have been used medically to treat hypertension, congestive heart failure, myocardial infarction and renal disease. Classes of compounds known to be useful as ACE inhibitors include acylmercapto and mercaptoalkanoyl prolines such as captopril (U.S. Pat. No. 4,105,776) and zofenopril (U.S. Pat. No. 4,316,906), carboxyalkyl dipeptides such as enalapril (U.S. Pat. No. 4,374,829), lisinopril (U.S. Pat. No. 4,374,829), quinapril (U.S. Pat. No. 4,344,949), ramipril (U.S. Pat. No. 4,587,258), and perindopril (U.S. Pat. No. 4,508,729), carboxyalkyl dipeptide mimics such as cilazapril (U.S. Pat. No. 4,512,924) and benazapril (U.S. Pat. No. 4,410,520), phosphinylalkanoyl prolines such as fosinopril (U.S. Pat. No. 4,337,201) and trandolopril.

[0222] "Renin inhibitors" are compounds which interfere with the activity of renin. Renin inhibitors include amino acids and derivatives thereof, peptides and derivatives thereof, and antibodies to renin. Examples of renin inhibitors that are the subject of United States patents are as follows: urea derivatives of peptides (U.S. Pat. No. 5,116,835); amino acids connected by nonpeptide bonds (U.S. Pat. No. 5,114,937); di- and tri- peptide derivatives (U.S. Pat. No. 5,106,835); amino acids and derivatives thereof (U.S. Pat. Nos. 5,104,869 and 5,095,119); diol sulfonamides and sulfinyls (U.S. Pat. No. 5,098,924); modified peptides (U.S. Pat. No. 5,095,006); peptidyl beta-aminoacyl aminodiol carbamates (U.S. Pat. No. 5,089,471); pyrolimidazolones (U.S. Pat. No. 5,075,451); fluorine and chlorine statine or statone containing peptides (U.S. Pat. No. 5,066,643); peptidyl amino diols (U.S. Pat. Nos. 5,063,208 and 4,845,079); N-morpholino derivatives (U.S. Pat. No. 5,055,466); pepstatin derivatives (U.S. Pat. No. 4,980,283); N-heterocyclic alcohols (U.S. Pat. No. 4,885,292); monoclonal antibodies to renin (U.S. Pat. No. 4,780,401); and a variety of other peptides and analogs thereof (U.S. Pat. Nos. 5,071,837, 5,064,965, 5,063,207, 5,036,054, 5,036,053, 5,034,512, and 4,894,437).

[0223] Agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules include polypeptide agents. Such polypeptides include polyclonal and monoclonal antibodies, prepared according to conventional methodology. Such antibodies already are known in the art and include anti-ICAM 1 antibodies as well as other such antibodies (see earlier discussion on antibodies).

[0224] Anticoagulant agents include, but are not limited to, Ancrod; Anticoagulant Citrate Dextrose Solution; Anticoagulant Citrate Phosphate Dextrose Adenine Solution; Anticoagulant Citrate Phosphate Dextrose Solution; Anticoagulant Heparin Solution; Anticoagulant Sodium Citrate Solution; Ardeparin Sodium; Bivalirudin; Bromindione; Dalteparin Sodium; Desirudin; Dicumarol; Heparin Calcium; Heparin Sodium; Lyapolate Sodium; Nafamostat Mesylate; Phenprocoumon; Tinzaparin Sodium; Warfarin Sodium.

[0225] Heparin may stabilize symptoms in evolving stroke, but anticoagulants are useless (and possibly dangerous) in acute completed stroke, and are contraindicated in hypertensives because of the increased possibility of hemorrhage into the brain or other organs. Although the timing is controversial, anticoagulants may be started to prevent recurrent cardiogenic emboli. Clot lysing agents, including tissue plasminogen activator and streptokinase, are being evaluated for the very early treatment of acute stroke. Nimodipine has recently been shown to improve survival and clinical outcome after ischemic stroke.

[0226] Other than aspirin, ticlopidine is another antiplatelet agent that has been shown to be beneficial for stroke treatment. Endarterectomy may be indicated in patients with 70 to 99 percent narrowing of a symptomatic internal carotid artery. However, most authorities agree that carotid endarterectomy is not indicated in patients with TIAs that are referable to the basilar-vertebral system, in patients with significant deficits from prior strokes, or in patients in whom a stroke is evolving.

[0227] HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase is the microsomal enzyme that catalyzes the rate limiting reaction in cholesterol biosynthesis (HMG-CoA6Mevalonate). An HMG-CoA reductase inhibitor inhibits HMG-CoA reductase, and as a result inhibits the synthesis of cholesterol. A number of HMG-CoA reductase inhibitors has been used to treat individuals with hypercholesterolemia. More recently, HMG-CoA reductase inhibitors have been shown to be beneficial in the treatment of stroke (Endres M, et al., *Proc Nat Acad Sci USA,* 1998,95:8880-5).

[0228] HMG-CoA reductase inhibitors useful for co-administration with the agents of the invention include, but are not limited to, simvastatin (U.S. Pat. No. 4, 444,784), lovastatin (U.S. Pat. No. 4,231,938), pravastatin sodium (U.S. Pat. No. 4,346,227), fluvastatin (U.S. Pat. No. 4,739,073), atorvastatin (U.S. Pat. No. 5,273,995), cerivastatin, and numerous others described in U.S. Pat. No. 5,622,985, U.S. Pat. No. 5,135,935, U.S. Pat. No. 5,356,896, U.S. Pat. No. 4,920,109, U.S. Pat. No. 5,286,895, U.S. Pat. No. 5,262,435, U.S. Pat. No. 5,260,332, U.S. Pat. No. 5,317,031, U.S. Pat. No. 5,283,256, U.S. Pat. No. 5,256,689, U.S. Pat. No. 5,182,298, U.S. Pat. No. 5,369,125, U.S. Pat. No. 5,302,604, U.S. Pat. No. 5,166,171, U.S. Pat. No. 5,202,327, U.S. Pat. No. 5,276,021, U.S. Pat. No. 5,196,440, U.S. Pat. No. 5,091,386, U.S. Pat. No. 5,091,378, U.S. Pat. No. 4,904,646, U.S. Pat. No. 5,385,932, U.S. Pat. No. 5,250,435, U.S. Pat. No. 5,132,312, U.S. Pat. No. 5,130,306, U.S. Pat. No. 5,116,870, U.S. Pat. No. 5,112,857, U.S. Pat. No. 5,102,911, U.S. Pat. No. 5,098,931, U.S. Pat. No. 5,081,136, U.S. Pat. No. 5,025,000, U.S. Pat. No. 5,021,453, U.S. Pat. No. 5,017,716, U.S. Pat. No. 5,001,144, U.S. Pat. No. 5,001,128, U.S. Pat. No. 4,997,837, U.S. Pat. No. 4,996,234, U.S. Pat. No. 4,994,494, U.S. Pat. No. 4,992,429, U.S. Pat. No. 4,970,231, U.S. Pat. No. 4,968,693, U.S. Pat. No. 4,963,538, U.S. Pat. No. 4,957,940, U.S. Pat. No. 4,950,675, U.S. Pat. No. 4,946,864, U.S. Pat. No. 4,946,860, U.S. Pat. No. 4,940,800, U.S. Pat. No. 4,940,727, U.S. Pat. No. 4,939,143, U.S. Pat. No. 4,929,620, U.S. Pat. No. 4,923,861, U.S. Pat. No. 4,906,657, U.S. Pat. No. 4,906,624 and U.S. Pat. No. 4,897,402, the disclosures of which patents are incorporated herein by reference.

[0229] Nitric oxide (NO) has been recognized as a messenger molecule with many physiologic roles, in the cardiovascular, neurologic and immune systems (Griffith, T M et al., *J Am Coll Cardiol,* 1988, 12:797-806). It mediates blood vessel relaxation, neurotransmission and pathogen suppression. NO is produced from the guanidino nitrogen of L-arginine by NO Synthase (Moncada, S and Higgs, E A, *Eur J Clin Invest,* 1991, 21:361-374). Agents that upregulate endothelial cell Nitric Oxide Synthase include, but are not limited to, L-arginine, rho GTPase function inhibitors (see International Application WO 99/47153, the disclosure of which is incorporated herein by reference), and agents that disrupt actin cytoskeletal organization (see International Application WO 00/03746, the disclosure of which is incorporated herein by reference).

[0230] "Co-administering," as used herein, refers to administering simultaneously two or more compounds of the invention (e.g., a MIVR-1, IEX-1, VDUP-1, BTG-2, and/or TIS-11d, nucleic acid and/or polypeptide, and an agent known to be beneficial in the treatment of, for example, a cardiovascular condition -e.g., an anticoagulant-), as an admixture in a single composition, or sequentially, close enough in time so that the compounds

may exert an additive or even synergistic effect, i.e., on reducing cardiomyocyte cell-death in a cardiovascular condition.

[0231] The invention also embraces solid-phase nucleic acid molecule arrays. The array consists essentially of a set of nucleic acid molecules, expression products thereof, or fragments (of either the nucleic acid or the polypeptide molecule) thereof, each nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, fixed to a solid substrate. In some embodiments, the solid-phase array further comprises at least one control nucleic acid molecule. In certain embodiments, the set of nucleic acid molecules comprises at least one, at least two, at least three, at least four, or even at least five nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d. In preferred embodiments, the set of nucleic acid molecules comprises a maximum number of 100 different nucleic acid molecules. In important embodiments, the set of nucleic acid molecules comprises a maximum number of 10 different nucleic acid molecules.

[0232] According to the invention, standard hybridization techniques of microarray technology are utilized to assess patterns of nucleic acid expression and identify nucleic acid expression. Microarray technology, which is also known by other names including: DNA chip technology, gene chip technology, and solid-phase nucleic acid array technology, is well known to those of ordinary skill in the art and is based on, but not limited to, obtaining an array of identified nucleic acid probes (e.g., molecules described elsewhere herein such as of MIVR-1, IEX-1, VDUP-1, BTG-2, and/or TIS-11d) on a fixed substrate, labeling target molecules with reporter molecules (e.g., radioactive, chemiluminescent, or fluorescent tags such as fluorescein, Cye3-dUTP, or Cye5-dUTP), hybridizing target nucleic acids to the probes, and evaluating target-probe hybridization. A probe with a nucleic acid sequence that perfectly matches the target sequence will, in general, result in detection of a stronger reporter-molecule signal than will probes with less perfect matches. Many components and techniques utilized in nucleic acid microarray technology are presented in *The Chipping Forecast,* Nature Genetics, Vol.21, January 1999, the entire contents of which is incorporated by reference herein.

[0233] According to the present invention, microarray substrates may include but are not limited to glass, silica, aluminosilicates, borosilicates, metal oxides such as alumina and nickel oxide, various clays, nitrocellulose, or nylon. In all embodiments a glass substrate is preferred. According to the invention, probes are selected from the group of nucleic acids including, but not limited to: DNA, genomic DNA, cDNA, and oligonucleotides; and may be natural or synthetic. Oligonucleotide probes preferably are 20 to 25-mer oligonucleotides and DNA/cDNA probes preferably are 500 to 5000 bases in length, although other lengths may be used. Appropriate probe length may be determined by one of ordinary skill in the art by following art-known procedures. In one embodiment, preferred probes are sets of two or more of the nucleic acid molecules set forth as SEQ ID NOs:1, 3, 4, 6, 8, 10, and/or 12. Probes may be purified to remove contaminants using standard methods known to those of ordinary skill in the art such as gel filtration or precipitation.

[0234] In one embodiment, the microarray substrate may be coated with a compound to enhance synthesis of the probe on the substrate. Such compounds include, but are not limited to, oligoethylene glycols. In another embodiment, coupling agents or groups on the substrate can be used to covalently link the first nucleotide or olignucleotide to the substrate. These agents or groups may include, but are not limited to: amino, hydroxy, bromo, and carboxy groups. These reactive groups are preferably attached to the substrate through a hydrocarbyl radical such as an alkylene or phenylene divalent radical, one valence position occupied by the chain bonding and the remaining attached to the reactive groups. These hydrocarbyl groups may contain up to about ten carbon atoms, preferably up to about six carbon atoms. Alkylene radicals are usually preferred containing two to four carbon atoms in the principal chain. These and additional details of the process are disclosed, for example, in U.S. Pat. No. 4,458,066, which is incorporated by reference in its entirety.

[0235] In one embodiment, probes are synthesized directly on the substrate in a predetermined grid pattern using methods such as light-directed chemical synthesis, photochemical deprotection, or delivery of nucleotide precursors to the substrate and subsequent probe production.

[0236] In another embodiment, the substrate may be coated with a compound to enhance binding of the probe to the substrate. Such compounds include, but are not limited to: polylysine, amino silanes, amino-reactive silanes (Chipping Forecast, 1999) or chromium (Gwynne and Page, 2000). In this embodiment, presynthesized probes are applied to the substrate in a precise, predetermined volume and grid pattern, utilizing a computer-controlled robot to apply probe to the substrate in a contact-printing manner or in a non-contact manner such as ink jet or piezo-electric delivery. Probes may be covalently linked to the substrate with methods that include, but are not limited to, UV-irradiation. In another embodiment probes are linked to the substrate with heat.

[0237] Targets are nucleic acids selected from the group, including but not limited to: DNA, genomic DNA, cDNA, RNA, mRNA and may be natural or synthetic. In all embodiments, nucleic acid molecules from subjects suspected of developing or having a cardiovascular condition, are preferred. In certain embodiments of the invention, one or more control nucleic acid molecules are attached to the substrate. Preferably, control nucleic acid molecules allow determination of factors including but not limited to: nucleic acid quality and binding characteristics; reagent quality and effectiveness; hybridization success; and analysis thresholds and success. Control nucleic acids may include, but are not limited to, expression products of genes such as housekeeping genes or fragments thereof.

[0238] To select a set of cardiovascular disease markers, the expression data generated by, for example, microarray analysis of gene expression, is preferably analyzed to determine which genes in different categories of patients (each category of patients being a different cardiovascular disorder), are significantly differentially expressed. The significance of gene expression can be determined using Permax computer software, although any standard statistical package that can discriminate significant differences is expression may be used. Permax performs permutation 2-sample t-tests on large arrays of data. For high dimensional vectors of observations, the Permax software computes t-statistics

for each attribute, and assesses significance using the permutation distribution of the maximum and minimum overall attributes. The main use is to determine the attributes (genes) that are the most different between two groups (e.g., control healthy subject and a subject with a particular cardiovascular disorder), measuring "most different" using the value of the t-statistics, and their significance levels.

[0239] Expression of cardiovascular disease nucleic acid molecules can also be determined using protein measurement methods to determine expression of SEQ ID NOs:2, 5, 7, 9, and/or 11, e.g., by determining the expression of polypeptides encoded by SEQ ID NOs:1, 4, 6, 8, and/or 10, respectively. Preferred methods of specifically and quantitatively measuring proteins include, but are not limited to: mass spectroscopy-based methods such as surface enhanced laser desorption ionization (SELDI; e.g., Ciphergen ProteinChip System), non-mass spectroscopy-based methods, and immunohistochemistry-based methods such as 2-dimensional gel electrophoresis.

[0240] SELDI methodology may, through procedures known to those of ordinary skill in the art, be used to vaporize microscopic amounts of tumor protein and to create a "fingerprint" of individual proteins, thereby allowing simultaneous measurement of the abundance of many proteins in a single sample. Preferably SELDI-based assays may be utilized to characterize cardiovascular conditions as well as stages of such conditions. Such assays preferably include, but are not limited to the following examples. Gene products discovered by RNA microarrays may be selectively measured by specific (antibody mediated) capture to the SELDI protein disc (e.g., selective SELDI). Gene products discovered by protein screening (e.g., with 2-D gels), may be resolved by "total protein SELDI" optimized to visualize those particular markers of interest from among SEQ ID NOs:1, 3, 4, 6, 8, and/or 10. Predictive models of cardiovascular disease from SELDI measurement of multiple markers from among SEQ ID NOs:1, 3, 4, 6, 8, and/or 10, may be utilized for the SELDI strategies.

[0241] The use of any of the foregoing microarray methods to determine expression of cardiovascular disease nucleic acids can be done with routine methods known to those of ordinary skill in the art and the expression determined by protein measurement methods may be correlated to predetermined levels of a marker used as a prognostic method for selecting treatment strategies for cardiovascular disease patients.

[0242] The invention will be more fully understood by reference to the following examples. These examples, however, are merely intended to illustrate the embodiments of the invention and are not to be construed to limit the scope of the invention.

EXAMPLES

Experimental Procedures

Materials and Methods

[0243] DMEM and Ham's F-12 were obtained from Biowhittaker. Dulbecco's PBS solution, Hanks' balanced salt solution, fibronectin, and other materials required for tissue culture were purchased from Gibco-BRL. [α-^{32}P] dCTP (3000 Ci/mmol) was purchased from Dupont NEN.

[0244] Culture of VSMCs

[0245] Cells were prepared from explants from excess aortic tissue from the donor at the time of organ harvest for orthotopic cardiac transplantation at Brigham and Women's Hospital. Vascular smooth muscle cells (VSMCs) were maintained in DMEM, 10% FCS, and 1% penicillin/streptomycin sulfate. These conditions are selective for growth of VSMCs over endothelial cells. VSMCs were maintained at 37° C., 5% CO_2 up to passage 6 to 7 for experiments. Under these conditions, 50% to 60% of human VSMCs routinely stain positive for a smooth muscle actin. The protocol was approved by the Brigham and Women's Hospital Committee for Human Research.

[0246] Culture of Cardiomyocytes

[0247] Cardiomycytes were cultured essentially as described in Arstall M A, et al., *J Mol Cell Cardiol,* 1998, 30(5):1019-25.

[0248] Mechanical Strain Device

[0249] A mechanical strain device that provides controlled mechanical deformation to cells in culture was utilized. Such a device is well known in the art and has been previously described in detail as the subject matter of U.S. Pat. No. 5,217,899 to Shapiro et al., issued Jun. 8, 1993. Mechanical deformation in this device is applied to a thin and transparent membrane on which cells are cultured, an approach that produces controlled cellular strain and allows visualization of cells. In addition, a nearly homogeneous biaxial strain profile (strains that are equal at all locations on the membrane and in all directions) is generated (Cheng, et al., *Circ. Res.,* 1997, 80:28-36). An advantage of this device over some commonly used systems is that it eliminates locations on the substrate that have very high strains (20% to 30%) in one direction. The membrane undergoes cyclic deformation as the platen assembly moves sinusoidally with a frequency and amplitude derived by the motor speed and the cam size, respectively. It has been previously measured membrane strains with a high-resolution video device (Cheng, et al., *Circ. Res.,* 1997, 80:28-36); the cams used for this study gave strains of 1%, 4%, and 9%.

[0250] The cell culture silicone membrane itself supports negligible adhesion of VSMCs. For the preparation of VSMCs to be subjected to mechanical strain, autoclaved membrane dishes were coated with 2 μg/ml of fibronectin in Hanks' solution for 6 to 12 hours at 4° C. and then washed twice with 10 ml PBS. VSMCs were plated on the coated membrane dish at a density of 700,000 cells/dish in 13 ml of DMEM containing 10% Fetal Bovine Serum (FBS) and incubated 16 to 24 hours. Cells were then washed with 10 ml of Hanks' solution four times to remove residual serum and incubated with 10 ml of serum-free IT medium (equal volumes of DMEM and Ham's F-12 supplemented with 1 μmol/L insulin and 5 μg/ml transferrin) for 48 hours. Before mechanical strain, 10 ml of fresh IT medium was exchanged. Mechanical strain was then applied at a specified constant frequency and amplitude, and control dishes received no mechanical strain.

[0251] Transcriptional Profiling

[0252] The DNA microarray experiment was performed with human aortic smooth muscle cells cultured on fibronectin-coated membranes with serum-free medium for 48

hours. Cells from a single patient donor were then exposed to 12 hours or 24 hours of cyclic deformation (1 Hz, 4% amplitude) or no deformation, and RNA was prepared. The choice of these time points was based on previous observations indicating that small strains regulated gene induction at these time points (Yang, et al., *J Biol. Chem.,* 1998, 273:6550-6555). The DNA microarray hybridization experiment was performed using the UniGem 1.0 array (Incyte Genomics, Inc., Palo Alto, Calif.) using methods previously described. (Iyer, et al., *Science,* 1999, 283:83-87; Lockhart, et al., *Nat. Biotechnol.,* 1996, 14:1675-1680). The UniGem 1.0 array has 5000 well-characterized genes with putative functions. Data were analyzed using the GemTools software package (Incyte Genomics, Inc.). The sensitivity of the assay was detection of one transcript in 75,000.

[**0253**] Microarray reproducibility was determined using two independent assays. First, 200 ng of human RNA was labeled with either Cy3 or Cy5dCTP, mixed, and hybridized to an array. Fluorescent ratios were calculated for all called elements. These data demonstrated that when the same RNA is used for both fluorescent channels, 99% of elements of the UniGem 1.0 microarray give differential expression values within 2-fold. A second series of experiments used RNA isolated from two unrelated cell lines. Comparison of these two RNAs over three separate hybridizations yielded an average correlation coefficient of r=0.97. For the present study, we used a threshold value of 2.5-fold to define differential gene induction to minimize false positive elements. Furthermore, we compared the results for >1000 genes to hybridizations with the GeneChip HU6800 (Affymetrix, Inc., Santa Clara, Calif.), using a different cell donor and methods previously described. (Lockhart, et al., *Nat. Biotechnol.,* 1996, 14:1675-1680).

[**0254**] Northern Blot Hybridization Analyses

[**0255**] The cDNA clones for differentially expressed genes were ordered from the IMAGE consortium. Each clone was sequenced from both 5' and 3' ends to confirm identity. Positive elements in the DNA microarray were confirmed by Northern blot hybridization analysis in at least three independent experiments using three different patient sources of human aortic smooth muscle cells. Total RNA was isolated by the guanidium thiocyanate and phenol chloroform method (Chomcyznski, et al., *Anal. Biochem.,* 1987, 162:156-159). For Northern blotting, 15 μg RNA was loaded on a 1.0% agarose-formaldehyde gel (2.0 mol/l), transferred to a nylon membrane (Amersham Pharmacia Biotech AB, Piscataway, N.J.), and UV cross-linked with a UV Stratalinker (Stratagene, Inc., La Jolla, Calif.). The probe was hybridized with ExpressHyb solution (Clontech Labs., Inc., Palo Alto, Calif.) at 68° C. for 1 hour. The membrane was washed with 2×SSC, 0.05% SDS solution for 30 to 40 minutes and three times at room temperature and 0.1×SSC, 0.1 % SDS solution with continuous shaking at 50° C. for 40 minutes. The membrane was exposed to film at −80° C., and radiographs were scanned and analyzed with Optimas 5.0 software (Optimas Co./Media Cybernetics, Silver Springs, Md.). Densitometric units were normalized to the ethidium-stained 28S ribosomal subunit on the membrane.

[**0256**] Protein Assays

[**0257**] Enzyme-linked immunoassays were performed using commercially available ELISA kits (e.g., Biopool, Ventura, Calif.). For Western analyses, cell lysates (50 μg) were loaded on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane in 25 mmol/L Tris base (pH 8.5), 0.2 mol/L glycine, and 20% methanol. The nitrocellulose membrane was blocked by 5% nonfat dried milk in TBS washing buffer containing 20 mmol/L Tris base (pH 7.6), 137 mmol/L NaCl, and 0.1% Tween 20 for 2 hours.

[**0258**] Strain-Apoptosis Assays

[**0259**] In order to assay the function of the genes of interest in terms of the regulation of strain-dependent cell survival we adapted the TUNEL assay, as described herein, for examining apoptosis in strain-exposed cardiomyocytes. Cardiomyocytes were exposed to strain (as described earlier) or daunorubicine (positive control). After stimulation, the cells were washed with PBS, fixed with 4% paraformaldehyde, parafin embedded, and cut into 5μm sections. The paraformaldehyde fixed sections were deparafinized and rehydrated. TdT enzyme and dUTP conjugated to a fluorescein cocktail were added to the sections according to the manufacturer's specifications (Roche Diagnostics in situ death-detection kit, Roche, Berkeley, Calif.). Nuclei were counterstained with Hoescht 33258 (Sigma), and mounted for examination using mounting media for fluorescence (Kirkegaard & Perry Laboratories, Inc.). Specimens were examined and photographed on a Diaphot microscope (Nikon Inc.) equipped with a phase-contrast and epifluorescence optics (×100) lens. Pictures were recorded on Kodak Gold Plus film (Eastman Kodak Co.). The percentage of apoptotic nuclei were calculated by determining the number of Hoechst stained nuclei that were positive for TUNEL staining. Approximately 100 nuclei were counted for each section.

Results

MIVR-1 in Cardiomyocytes and Smooth Muscle Cells

[**0260**] By applying a highly uniform biaxial cyclic strain to cultured human aorta smooth muscle cells, we found a novel mechanically induced gene, MIVR-1. The message of the human MIVR-1 in smooth muscle cells is ~5kb in length, and appears as a single mRNA species which is heavily expressed in vascular tissues following strain.

[**0261**] The isolated and characterized MIVR-1 cDNA (SEQ ID NO. 1) encodes for a putative MIVR-1 protein sequence (SEQ ID. NO. 2), at a size of ~30 kD, having one transmembrane domain, no secretory sequence, and a long cytoplasmic tail.

[**0262**] The Northern hybridization blot of mouse tissue and mouse embryonic stem cells showed hybridization to two mRNA species ~8kb and ~5kb in left ventricle (L) and aorta (Ao) mRNA.

IEX-1 in Cardiomyocytes and Smooth Muscle Cells

[**0263**] IEX-1 (SEQ ID NOs. 4 and 5) was originally identified as a radiation induced gene in human squamous cell carcinoma cells (Cancer Research;1996:56:1498). The rat homologue was identified as prg-1 (proliferation-related gene-1). A third name for this gene is p22, a gene known to be downregulated during monocyte differentiation. Pro-

moter deletion studies indicate that IEX-1 is induced by NF-kB and p53. A dominant negative mutant of IEX-1 called IEX-1L has been accidentally cloned through a differential display approach in Jurkat cells by a group searching for NF-kB responsive genes that prevent apoptosis (Science 1998;281:998). Similar to the other mechanically regulated gene that regulates apoptosis (VDUP-1:SEQ ID NOs. 6 and 7), nothing was previously reported about this gene and its involvement in the cardiovascular system.

[0264] We initially identified IEX-1 in an SMC strain experiment with HU-6800 arrays. Because we were pursuing a redox and NF-kB regulated mechanical response in cardiac myocytes, IEX-1 was evaluated in both cardiomyocytes (CMs) and VSMCs. We confirmed robust upregulation of IEX-1 in human CMs but explored the homologue prg-1 in rat neonatal CM due to availability. A Northern blot hybridization indicated that in rat neonatal CM, prg-1 is rapidly and robustly induced. An amplitude response Northern blot hybridization in rat neonatal CM confirmed that prg-l/IEX-1 is induced by strain in a dose-dependent manner:

[0265] We hypothesized that IEX-1/prg-1 induction would not be related to angiotensin II (AngII), since it has been previously determined that Ang II is not required for mechanical induction of NF-kB. However, in two experiments performed partial inhibition by an Ang II inhibitor was observed . Thus, IEX-1/prg-1 is a gene that is mechanically induced and has an apoptosis regulatory function.

BTG-2 and TIS-11d in Cardiomyocytes and Smooth Muscle Cells

[0266] Using Northern blot hybridization analyses, two additional hits from the microarray screen were assayed. Rat cDNA probes were generated by PCR using a cardiomyocyte cDNA template. The two new hits included (i) BTG-2 (NGF-inducible anti-proliferative protein PC3, SEQ ID NOs. 8 and 9), a gene downstream of p53 that has anti-proliferative effects, and (ii) TIS-11d (zinc finger transcription factor ERF-2:SEQ ID NOs. 10 and 11), an immediate early gene induced by extracellular hormones and growth factor signals that causes apoptosis when continuously expressed at physiological levels.

Strain-Apoptosis Assays

[0267] Strain-apoptosis assays were performed as described elsewhere herein. These assays showed that both in serum-rich and serum-free conditions, exposure of cardiovascular cells to strain diminishes the degree of apoptosis.

Equivalents

[0268] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[0269] All references disclosed herein are incorporated by reference in their entirety. What is claimed is presented below and is followed by a Sequence Listing.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 17

<210> SEQ ID NO 1
<211> LENGTH: 1321
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (413)...(1273)

<400> SEQUENCE: 1

cgaccgcggt ctcggagcga aacccgatct ccttggactt gaatgaggag gaggaggcgg 60

cggcggcggc ggcggcggag gcgctcggct ggggaaagct agcggcagag gctcagcccc 120

ggcggcagcg cgcgccccgc tgccagccca ttttccggac gccacccgcg ggcactgccg 180

acgcccccgg ggctgccgag gggaggccgg gggggcgcag cggagcgcgg tcccgcgcac 240

tgagccccgc ggcgccccgg gaacttggcg gcgacccgag cccggcgagc cggggcgcgc 300

ctcccccgcc gcgcgcctcc tgcatgcggg gccccagctc cgggcgccgg ccggagcccc 360

ccccggccgc ccccgagccc cccgcgcccc gcgccgcgcc gccgcgccgt cc atg cac 418
Met His
1

cgc ttg atg ggg gtc aac agc acc gcc gcc gcc gcc gcc ggg cag ccc 466
Arg Leu Met Gly Val Asn Ser Thr Ala Ala Ala Ala Ala Gly Gln Pro
5 10 15

-continued

```
aat gtc tcc tgc acg tgc aac tgc aaa cgc tct ttg ttc cag agc atg      514
Asn Val Ser Cys Thr Cys Asn Cys Lys Arg Ser Leu Phe Gln Ser Met
     20                  25                  30

gag atc acg gag ctg gag ttt gtt cag atc atc atc atc gtg gtg gtg      562
Glu Ile Thr Glu Leu Glu Phe Val Gln Ile Ile Ile Ile Val Val Val
 35                  40                  45                  50

atg atg gtg atg gtg gtg gtg atc acg tgc ctg ctg agc cac tac aag      610
Met Met Val Met Val Val Val Ile Thr Cys Leu Leu Ser His Tyr Lys
                 55                  60                  65

ctg tct gca cgg tcc ttc atc agc cgg cac agc cag ggg cgg agg aga      658
Leu Ser Ala Arg Ser Phe Ile Ser Arg His Ser Gln Gly Arg Arg Arg
             70                  75                  80

gaa gat gcc ctg tcc tca gaa gga tgc ctg tgg ccc tcg gag agc aca      706
Glu Asp Ala Leu Ser Ser Glu Gly Cys Leu Trp Pro Ser Glu Ser Thr
         85                  90                  95

gtg tca ggc aac gga atc cca gag ccg cag gtc tac gcc ccg cct cgg      754
Val Ser Gly Asn Gly Ile Pro Glu Pro Gln Val Tyr Ala Pro Pro Arg
    100                 105                 110

ccc acc gac cgc ctg gcc gtg ccg ccc ttc gcc cag cgg gag cgc ttc      802
Pro Thr Asp Arg Leu Ala Val Pro Pro Phe Ala Gln Arg Glu Arg Phe
115                 120                 125                 130

cac cgc ttc cag ccc acc tat ccg tac ctg cag cac gag atc gac ctg      850
His Arg Phe Gln Pro Thr Tyr Pro Tyr Leu Gln His Glu Ile Asp Leu
                135                 140                 145

cca ccc acc atc tcg ctg tca gac ggg gag gag ccc cca ccc tac cag      898
Pro Pro Thr Ile Ser Leu Ser Asp Gly Glu Glu Pro Pro Pro Tyr Gln
            150                 155                 160

ggc ccc tgc acc ctc cag ctt cgg gac ccc gag cag cag ctg gaa ctg      946
Gly Pro Cys Thr Leu Gln Leu Arg Asp Pro Glu Gln Gln Leu Glu Leu
        165                 170                 175

aac cgg gag tcg gtg cgc gca ccc cca aac aga acc atc ttc gac agt      994
Asn Arg Glu Ser Val Arg Ala Pro Pro Asn Arg Thr Ile Phe Asp Ser
    180                 185                 190

gac ctg atg gat agt gcc agg ctg ggc ggc ccc tgc ccc ccc agc agt     1042
Asp Leu Met Asp Ser Ala Arg Leu Gly Gly Pro Cys Pro Pro Ser Ser
195                 200                 205                 210

aac tcg ggc atc agc gcc acg tgc tac ggc agc ggc ggg cgc atg gag     1090
Asn Ser Gly Ile Ser Ala Thr Cys Tyr Gly Ser Gly Gly Arg Met Glu
                215                 220                 225

ggg ccg ccg ccc acc tac agc gag gtc atc ggc cac tac ccg ggg tcc     1138
Gly Pro Pro Pro Thr Tyr Ser Glu Val Ile Gly His Tyr Pro Gly Ser
            230                 235                 240

tcc ttc cag cac cag cag agc agt ggg ccg ccc tcc ttg ctg gag ggg     1186
Ser Phe Gln His Gln Gln Ser Ser Gly Pro Pro Ser Leu Leu Glu Gly
        245                 250                 255

acc cgg ctc cac cac aca cac atc gcg ccc cta gag agc gca gcc atc     1234
Thr Arg Leu His His Thr His Ile Ala Pro Leu Glu Ser Ala Ala Ile
    260                 265                 270

tgg agc aaa gag aag gat aaa cag aaa gga cac cct ctc tagggtcccc      1283
Trp Ser Lys Glu Lys Asp Lys Gln Lys Gly His Pro Leu
275                 280                 285

agggggggccg ggctggggct gcgtaggtga aaaggcag                         1321


<210> SEQ ID NO 2
<211> LENGTH: 287
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens
```

-continued

<400> SEQUENCE: 2

Met His Arg Leu Met Gly Val Asn Ser Thr Ala Ala Ala Ala Ala Gly
 1   5   10   15

Gln Pro Asn Val Ser Cys Thr Cys Asn Cys Lys Arg Ser Leu Phe Gln
            20   25   30

Ser Met Glu Ile Thr Glu Leu Glu Phe Val Gln Ile Ile Ile Ile Val
        35   40   45

Val Val Met Met Val Met Val Val Val Ile Thr Cys Leu Leu Ser His
    50   55   60

Tyr Lys Leu Ser Ala Arg Ser Phe Ile Ser Arg His Ser Gln Gly Arg
65   70   75   80

Arg Arg Glu Asp Ala Leu Ser Ser Glu Gly Cys Leu Trp Pro Ser Glu
                85   90   95

Ser Thr Val Ser Gly Asn Gly Ile Pro Glu Pro Gln Val Tyr Ala Pro
            100   105   110

Pro Arg Pro Thr Asp Arg Leu Ala Val Pro Pro Phe Ala Gln Arg Glu
        115   120   125

Arg Phe His Arg Phe Gln Pro Thr Tyr Pro Tyr Leu Gln His Glu Ile
    130   135   140

Asp Leu Pro Pro Thr Ile Ser Leu Ser Asp Gly Glu Glu Pro Pro Pro
145   150   155   160

Tyr Gln Gly Pro Cys Thr Leu Gln Leu Arg Asp Pro Glu Gln Gln Leu
                165   170   175

Glu Leu Asn Arg Glu Ser Val Arg Ala Pro Pro Asn Arg Thr Ile Phe
            180   185   190

Asp Ser Asp Leu Met Asp Ser Ala Arg Leu Gly Gly Pro Cys Pro Pro
        195   200   205

Ser Ser Asn Ser Gly Ile Ser Ala Thr Cys Tyr Gly Ser Gly Gly Arg
    210   215   220

Met Glu Gly Pro Pro Pro Thr Tyr Ser Glu Val Ile Gly His Tyr Pro
225   230   235   240

Gly Ser Ser Phe Gln His Gln Gln Ser Ser Gly Pro Pro Ser Leu Leu
                245   250   255

Glu Gly Thr Arg Leu His His Thr His Ile Ala Pro Leu Glu Ser Ala
            260   265   270

Ala Ile Trp Ser Lys Glu Lys Asp Lys Gln Lys Gly His Pro Leu
        275   280   285

<210> SEQ ID NO 3
<211> LENGTH: 861
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(861)

<400> SEQUENCE: 3

atgcaccgct tgatgggggt caacagcacc gccgccgccg ccgccgggca gcccaatgtc 60

tcctgcacgt gcaactgcaa acgctctttg ttccagagca tggagatcac ggagctggag 120

tttgttcaga tcatcatcat cgtggtggtg atgatggtga tggtggtggt gatcacgtgc 180

ctgctgagcc actacaagct gtctgcacgg tccttcatca gccggcacag ccaggggcgg 240

-continued

```
aggagagaag atgccctgtc ctcagaagga tgcctgtggc cctcggagag cacagtgtca    300

ggcaacggaa tcccagagcc gcaggtctac gccccgcctc ggcccaccga ccgcctggcc    360

gtgccgccct tcgcccagcg ggagcgcttc caccgcttcc agcccaccta tccgtacctg    420

cagcacgaga tcgacctgcc acccaccatc tcgctgtcag acggggagga gccccaccc     480

taccagggcc cctgcaccct ccagcttcgg gaccccgagc agcagctgga actgaaccgg    540

gagtcggtgc gcgcaccccc aaacagaacc atcttcgaca gtgacctgat ggatagtgcc    600

aggctgggcg gcccctgccc ccccagcagt aactcgggca tcagcgccac gtgctacggc    660

agcggcgggc gcatggaggg gccgccgccc acctacagcg aggtcatcgg ccactacccg    720

gggtcctcct tccagcacca gcagagcagt gggccgccct ccttgctgga ggggacccgg    780

ctccaccaca cacacatcgc gcccctagag agcgcagcca tctggagcaa agagaaggat    840

aaacagaaag gacaccctct c                                              861


<210> SEQ ID NO 4
<211> LENGTH: 477
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (7)...(474)
<223> OTHER INFORMATION: IEX1

<400> SEQUENCE: 4

ctcacc atg tgt cac tct cgc agc tgc cac ccg acc atg acc atc ctg        48
       Met Cys His Ser Arg Ser Cys His Pro Thr Met Thr Ile Leu
        1               5                  10

cag gcc ccg acc ccg gcc ccc tcc acc atc ccg gga ccc cgg cgg ggc       96
Gln Ala Pro Thr Pro Ala Pro Ser Thr Ile Pro Gly Pro Arg Arg Gly
 15                  20                  25                  30

tcc ggt cct gag atc ttc acc ttc gac cct ctc ccg gag ccc gca gcg      144
Ser Gly Pro Glu Ile Phe Thr Phe Asp Pro Leu Pro Glu Pro Ala Ala
                 35                  40                  45

gcc cct gcc ggg cgc ccc agc gcc tct cgc ggg cac cga aag cgc agc      192
Ala Pro Ala Gly Arg Pro Ser Ala Ser Arg Gly His Arg Lys Arg Ser
             50                  55                  60

cgc agg gtt ctc tac cct cga gtg gtc cgg cgc cag ctg cca gtc gag      240
Arg Arg Val Leu Tyr Pro Arg Val Val Arg Arg Gln Leu Pro Val Glu
         65                  70                  75

gaa ccg aac cca gcc aaa agg ctt ctc ttt ctg ctg ctc acc atc gtc      288
Glu Pro Asn Pro Ala Lys Arg Leu Leu Phe Leu Leu Leu Thr Ile Val
     80                  85                  90

ttc tgc cag atc ctg atg gct gaa gag ggt gtg ccg gcg ccc ctg cct      336
Phe Cys Gln Ile Leu Met Ala Glu Glu Gly Val Pro Ala Pro Leu Pro
 95                 100                 105                 110

cca gag gac gcc cct aac gcc gca tcc ctg gcg ccc acc cct gtg tcc      384
Pro Glu Asp Ala Pro Asn Ala Ala Ser Leu Ala Pro Thr Pro Val Ser
                115                 120                 125

ccc gtc ctc gag ccc ttt aat ctg act tcg gag ccc tcg gac tac gct      432
Pro Val Leu Glu Pro Phe Asn Leu Thr Ser Glu Pro Ser Asp Tyr Ala
            130                 135                 140

ctg gac ctc agc act ttc ctc cag caa cac ccg gcc gcc ttc              474
Leu Asp Leu Ser Thr Phe Leu Gln Gln His Pro Ala Ala Phe
        145                 150                 155

taa                                                                  477
```

-continued

<210> SEQ ID NO 5
<211> LENGTH: 156
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 5

Met Cys His Ser Arg Ser Cys His Pro Thr Met Thr Ile Leu Gln Ala
 1   5   10   15

Pro Thr Pro Ala Pro Ser Thr Ile Pro Gly Pro Arg Arg Gly Ser Gly
            20   25   30

Pro Glu Ile Phe Thr Phe Asp Pro Leu Pro Glu Pro Ala Ala Ala Pro
        35   40   45

Ala Gly Arg Pro Ser Ala Ser Arg Gly His Arg Lys Arg Ser Arg Arg
    50   55   60

Val Leu Tyr Pro Arg Val Val Arg Arg Gln Leu Pro Val Glu Glu Pro
65   70   75   80

Asn Pro Ala Lys Arg Leu Leu Phe Leu Leu Leu Thr Ile Val Phe Cys
                85   90   95

Gln Ile Leu Met Ala Glu Glu Gly Val Pro Ala Pro Leu Pro Pro Glu
            100   105   110

Asp Ala Pro Asn Ala Ala Ser Leu Ala Pro Thr Pro Val Ser Pro Val
        115   120   125

Leu Glu Pro Phe Asn Leu Thr Ser Glu Pro Ser Asp Tyr Ala Leu Asp
    130   135   140

Leu Ser Thr Phe Leu Gln Gln His Pro Ala Ala Phe
145   150   155

<210> SEQ ID NO 6
<211> LENGTH: 2704
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (222)...(1394)
<223> OTHER INFORMATION: VDUP1

<400> SEQUENCE: 6

gcttagtgta accagcggcg tatatttttt aggcgccttt tcgaaaacct agtagttaat   60

attcatttgt ttaaatctta ttttatttttt aagctcaaac tgcttaagaa taccttaatt   120

ccttaaagtg aaataatttt ttgcaaaggg gtttcctcga tttggagctt ttttttctt   180

ccaccgtcat ttctaactct taaaaccaac tcagttccat c atg gtg atg ttc aag   236
                                                Met Val Met Phe Lys
                                                  1   5

aag atc aag tct ttt gag gtg gtc ttt aac gac cct gaa aag gtg tac   284
Lys Ile Lys Ser Phe Glu Val Val Phe Asn Asp Pro Glu Lys Val Tyr
                 10   15   20

ggc agt ggc gag agg gtg gct ggc cgg gtg ata gtg gag gtg tgt gaa   332
Gly Ser Gly Glu Arg Val Ala Gly Arg Val Ile Val Glu Val Cys Glu
             25   30   35

gtt act cgt gtc aaa gcc gtt agg atc ctg gct tgc gga gtg gct aaa   380
Val Thr Arg Val Lys Ala Val Arg Ile Leu Ala Cys Gly Val Ala Lys
         40   45   50

gtg ctt tgg atg cag gga tcc cag cag tgc aaa cag act tcg gag tac   428
Val Leu Trp Met Gln Gly Ser Gln Gln Cys Lys Gln Thr Ser Glu Tyr
     55   60   65

-continued

```
ctg cgc tat gaa gac acg ctt ctt ctg gaa gac cag cca aca ggt gag      476
Leu Arg Tyr Glu Asp Thr Leu Leu Leu Glu Asp Gln Pro Thr Gly Glu
 70                  75                  80                  85

aat gag atg gtg atc atg aga cct gga aac aaa tat gag tac aag ttc      524
Asn Glu Met Val Ile Met Arg Pro Gly Asn Lys Tyr Glu Tyr Lys Phe
                 90                  95                 100

ggc ttt gag ctt cct cag ggg cct ctg gga aca tcc ttc aaa gga aaa      572
Gly Phe Glu Leu Pro Gln Gly Pro Leu Gly Thr Ser Phe Lys Gly Lys
            105                 110                 115

tat ggg tgt gta gac tac tgg gtg aag gct ttt ctt gac cgc ccg agc      620
Tyr Gly Cys Val Asp Tyr Trp Val Lys Ala Phe Leu Asp Arg Pro Ser
        120                 125                 130

cag cca act caa gag aca aag aaa aac ttt gaa gta gtg gat ctg gtg      668
Gln Pro Thr Gln Glu Thr Lys Lys Asn Phe Glu Val Val Asp Leu Val
    135                 140                 145

gat gtc aat acc cct gat tta atg gca cct gtg tct gct aaa aaa gaa      716
Asp Val Asn Thr Pro Asp Leu Met Ala Pro Val Ser Ala Lys Lys Glu
150                 155                 160                 165

aag aaa gtt tcc tgc atg ttc att cct gat ggg cgg gtg tct gtc tct      764
Lys Lys Val Ser Cys Met Phe Ile Pro Asp Gly Arg Val Ser Val Ser
                170                 175                 180

gct cga att gac aga aaa gga ttc tgt gaa ggt gat gag att tcc atc      812
Ala Arg Ile Asp Arg Lys Gly Phe Cys Glu Gly Asp Glu Ile Ser Ile
            185                 190                 195

cat gct gac ttt gag aat aca tgt tcc cga att gtg gtc ccc aaa gct      860
His Ala Asp Phe Glu Asn Thr Cys Ser Arg Ile Val Val Pro Lys Ala
        200                 205                 210

gcc att gtg gcc cgc cac act tac ctt gcc aat ggc cag acc aag gtg      908
Ala Ile Val Ala Arg His Thr Tyr Leu Ala Asn Gly Gln Thr Lys Val
    215                 220                 225

ctg act cag aag ttg tca tca gtc aga ggc aat cat att atc tca ggg      956
Leu Thr Gln Lys Leu Ser Ser Val Arg Gly Asn His Ile Ile Ser Gly
230                 235                 240                 245

aca tgc gca tca tgg cgt ggc aag agc ctt cgg gtt cag aag atc agg     1004
Thr Cys Ala Ser Trp Arg Gly Lys Ser Leu Arg Val Gln Lys Ile Arg
                250                 255                 260

cct tct atc ctg ggc tgc aac atc ctt cga gtt gaa tat tcc tta ctg     1052
Pro Ser Ile Leu Gly Cys Asn Ile Leu Arg Val Glu Tyr Ser Leu Leu
            265                 270                 275

atc tat gtt agc gtt cct gga tcc aag aag gtc atc ctt gac ctg ccc     1100
Ile Tyr Val Ser Val Pro Gly Ser Lys Lys Val Ile Leu Asp Leu Pro
        280                 285                 290

ctg gta att ggc agc aga tca ggt cta agc agc aga aca tcc agc atg     1148
Leu Val Ile Gly Ser Arg Ser Gly Leu Ser Ser Arg Thr Ser Ser Met
    295                 300                 305

gcc agc cga acc agc tct gag atg agt tgg gta gat ctg aac atc cct     1196
Ala Ser Arg Thr Ser Ser Glu Met Ser Trp Val Asp Leu Asn Ile Pro
310                 315                 320                 325

gat acc cca gaa gct cct ccc tgc tat atg gat gtc att cct gaa gat     1244
Asp Thr Pro Glu Ala Pro Pro Cys Tyr Met Asp Val Ile Pro Glu Asp
                330                 335                 340

cac cga ttg gag agc cca aca act cct ctg cta gat gac atg gat ggc     1292
His Arg Leu Glu Ser Pro Thr Thr Pro Leu Leu Asp Asp Met Asp Gly
            345                 350                 355

tct caa gac agc cct atc ttt atg tat gcc cct gag ttc aag ttc atg     1340
Ser Gln Asp Ser Pro Ile Phe Met Tyr Ala Pro Glu Phe Lys Phe Met
        360                 365                 370
```

-continued

```
cca cca ccg act tat act gag gtg gat ccc tgc atc ctc aac aac aat     1388
Pro Pro Pro Thr Tyr Thr Glu Val Asp Pro Cys Ile Leu Asn Asn Asn
    375                 380                 385

gtg cag tgagcatgtg gaagaaaaga agcagcttta cctacttgtt tctttttgtc      1444
Val Gln
390

tctcttcctg gacactcact ttttcagaga ctcaacagtc tcgtcaatgg agtgtgggtc   1504

caccttagcc tctgacttcc taatgtagga ggtggtcagc aggcaatctc ctgggcctta   1564

aaggatgcgg actcatcctc agccagcgcc catgttgtga tacaggggtg tttgttggat   1624

gggtttaaaa ataactagaa aaactcaggc ccatccattt tctcagatct ccttgaaaat   1684

tgaggccttt tcgatagttt cgggtcaggt aaaaatggcc tcctggcgta agcttttcaa   1744

ggtttttgg aggctttttg taaattgtga taggaacttt ggaccttgaa cttacgtatc   1804

atgtggagaa gagccaattt aacaaactag gaagatgaaa agggaaattg tggccaaaac   1864

tttgggaaaa ggaggttctt aaaatcagtg tttccccttt gtgcacttgt agaaaaaaaa   1924

gaaaaacctt ctagagctga tttgatggac aatggagaga gctttccctg tgattataaa   1984

aaaggaagct agctgctcta cggtcatctt tgcttagagt atactttaac ctggctttta   2044

aagcagtagt aactgcccca ccaaaggtct taaaagccat ttttggagcc tattgcactg   2104

tgttctccta ctgcaaatat tttcatatgg gaggatggtt ttctcttcat gtaagtcctt   2164

ggaattgatt ctaaggtgat gttcttagca ctttaattcc tgtcaaattt tttgttctcc   2224

ccttctgcca tcttaaatgt aagctgaaac tggtctactg tgtctctagg gttaagccaa   2284

aagacaaaaa aaattttact acttttgaga ttgccccaat gtacagaatt atataattct   2344

aacgcttaaa tcatgtgaaa gggttgctgc tgtcagcctt gcccactgtg acttcaaacc   2404

caaggaggaa ctcttgatca agatgcccaa ccctgtgatc agaacctcca aatactgcca   2464

tgagaaacta gagggcaggt gttcataaaa gccctttgaa ccccccttcct gccctgtgtt   2524

aggagatagg gatattggcc cctcactgca gctgccagca cttggtcagt cactctcagc   2584

catagcactt tgttcactgt cctgtgtcag agcactgagc tccacccttt tctgagagtt   2644

attacagcca gaaagtgtgg gctgaagatg gttggtttca tgtgggggta ttatgtaccc   2704


<210> SEQ ID NO 7
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 7

Met Val Met Phe Lys Lys Ile Lys Ser Phe Glu Val Val Phe Asn Asp
 1               5                  10                  15

Pro Glu Lys Val Tyr Gly Ser Gly Glu Arg Val Ala Gly Arg Val Ile
            20                  25                  30

Val Glu Val Cys Glu Val Thr Arg Val Lys Ala Val Arg Ile Leu Ala
        35                  40                  45

Cys Gly Val Ala Lys Val Leu Trp Met Gln Gly Ser Gln Gln Cys Lys
    50                  55                  60

Gln Thr Ser Glu Tyr Leu Arg Tyr Glu Asp Thr Leu Leu Leu Glu Asp
65                  70                  75                  80

Gln Pro Thr Gly Glu Asn Glu Met Val Ile Met Arg Pro Gly Asn Lys
                85                  90                  95
```

-continued

```
Tyr Glu Tyr Lys Phe Gly Phe Glu Leu Pro Gln Gly Pro Leu Gly Thr
            100                 105                 110

Ser Phe Lys Gly Lys Tyr Gly Cys Val Asp Tyr Trp Val Lys Ala Phe
        115                 120                 125

Leu Asp Arg Pro Ser Gln Pro Thr Gln Glu Thr Lys Lys Asn Phe Glu
    130                 135                 140

Val Val Asp Leu Val Asp Val Asn Thr Pro Asp Leu Met Ala Pro Val
145                 150                 155                 160

Ser Ala Lys Lys Glu Lys Lys Val Ser Cys Met Phe Ile Pro Asp Gly
                165                 170                 175

Arg Val Ser Val Ser Ala Arg Ile Asp Arg Lys Gly Phe Cys Glu Gly
            180                 185                 190

Asp Glu Ile Ser Ile His Ala Asp Phe Glu Asn Thr Cys Ser Arg Ile
        195                 200                 205

Val Val Pro Lys Ala Ala Ile Val Ala Arg His Thr Tyr Leu Ala Asn
    210                 215                 220

Gly Gln Thr Lys Val Leu Thr Gln Lys Leu Ser Ser Val Arg Gly Asn
225                 230                 235                 240

His Ile Ile Ser Gly Thr Cys Ala Ser Trp Arg Gly Lys Ser Leu Arg
                245                 250                 255

Val Gln Lys Ile Arg Pro Ser Ile Leu Gly Cys Asn Ile Leu Arg Val
            260                 265                 270

Glu Tyr Ser Leu Leu Ile Tyr Val Ser Val Pro Gly Ser Lys Lys Val
        275                 280                 285

Ile Leu Asp Leu Pro Leu Val Ile Gly Ser Arg Ser Gly Leu Ser Ser
    290                 295                 300

Arg Thr Ser Ser Met Ala Ser Arg Thr Ser Ser Glu Met Ser Trp Val
305                 310                 315                 320

Asp Leu Asn Ile Pro Asp Thr Pro Glu Ala Pro Pro Cys Tyr Met Asp
                325                 330                 335

Val Ile Pro Glu Asp His Arg Leu Glu Ser Pro Thr Thr Pro Leu Leu
            340                 345                 350

Asp Asp Met Asp Gly Ser Gln Asp Ser Pro Ile Phe Met Tyr Ala Pro
        355                 360                 365

Glu Phe Lys Phe Met Pro Pro Pro Thr Tyr Thr Glu Val Asp Pro Cys
    370                 375                 380

Ile Leu Asn Asn Asn Val Gln
385                 390


<210> SEQ ID NO 8
<211> LENGTH: 2717
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (72)...(545)
<223> OTHER INFORMATION: BTG2

<400> SEQUENCE: 8

cagggtaacg ctgtcttgtg gacccgcact tcccacccga gacctctcac tgagcccgag     60

ccgcgcgcga c atg agc cac ggg aag gga acc gac atg ctc ccg gag atc     110
             Met Ser His Gly Lys Gly Thr Asp Met Leu Pro Glu Ile
              1               5                  10
```

-continued

```
gcc gcc gcc gtg ggc ttc ctc tcc agc ctc ctg agg acc cgg ggc tgc      158
Ala Ala Ala Val Gly Phe Leu Ser Ser Leu Leu Arg Thr Arg Gly Cys
     15                  20                  25

gtg agc gag cag agg ctt aag gtc ttc agc ggg gcg ctc cag gag gca      206
Val Ser Glu Gln Arg Leu Lys Val Phe Ser Gly Ala Leu Gln Glu Ala
 30                  35                  40                  45

ctc aca gag cac tac aaa cac cac tgg ttt ccc gaa aag ccg tcc aag      254
Leu Thr Glu His Tyr Lys His His Trp Phe Pro Glu Lys Pro Ser Lys
                 50                  55                  60

ggc tcc ggc tac cgc tgc att cgc atc aac cac aag atg gac ccc atc      302
Gly Ser Gly Tyr Arg Cys Ile Arg Ile Asn His Lys Met Asp Pro Ile
             65                  70                  75

atc agc agg gtg gcc agc cag atc gga ctc agc cag ccc cag ctg cac      350
Ile Ser Arg Val Ala Ser Gln Ile Gly Leu Ser Gln Pro Gln Leu His
         80                  85                  90

cag ctg ctg ccc agc gag ctg acc ctg tgg gtg gac ccc tat gag gtg      398
Gln Leu Leu Pro Ser Glu Leu Thr Leu Trp Val Asp Pro Tyr Glu Val
     95                 100                 105

tcc tac cgc att ggg gag gac ggc tcc atc tgc gtc ttg tac gag gag      446
Ser Tyr Arg Ile Gly Glu Asp Gly Ser Ile Cys Val Leu Tyr Glu Glu
110                 115                 120                 125

gcc cca ctg gcc gcc tcc tgt ggg ctc ctc acc tgc aag aac caa gtg      494
Ala Pro Leu Ala Ala Ser Cys Gly Leu Leu Thr Cys Lys Asn Gln Val
                130                 135                 140

ctg ctg ggc cgg agc agc ccc tcc aag aac tac gtg atg gca gtc tcc      542
Leu Leu Gly Arg Ser Ser Pro Ser Lys Asn Tyr Val Met Ala Val Ser
            145                 150                 155

agc taggcccttc cgcccccgcc ctgggcgccg ccgtgctcat gctgccgtga           595

caacaggcca ccacatacct caacctgggg aactgtattt ttaaatgaag agctatttat    655

atatattatt ttttttaag aaaggaggaa aagaaaccaa aagttttttt taagaaaaaa    715

aatccttcaa gggagctgct tggaagtggc ctccccaggt gcctttggag agaactgttg    775

cgtgcttgag tctgtgagcc agtgtctgcc tataggaggg ggagctgtta gggggtagac    835

ctagccaagg agaagtggga gacgtttggc tagcacccca ggaagatgtg agagggagca    895

agcaaggtta gcaactgtga acagagaggt cgggatttgc cctgggggag gaagagaggc    955

caagttcaga gctctctgtc tcccccagcc agacacctgc atccctggct cctctattac   1015

tcaggggcat tcatgcctgg acttaaacaa tactatgtta tcttttcttt tatttttcta   1075

atgaggtcct gggcagagag tgaaaaggcc tctcctgatt cctactgtcc taagctgctt   1135

ttcttgaaat catgacttgt ttctaattct accctcaggg gcctgtagat gttgctttcc   1195

agccaggaat ctaaagcttt gggttttctg agggggggag gagggaactg gaggttattg   1255

gggttaggat ggaagggaac tctgcacaaa acctttgctt tgctagtgct gctttgtgtg   1315

tatgtgtggc aaataatttg ggggtgattt gcaatgaaat tttgggaccc aaagagtatc   1375

cactggggat gtttttggc caaaactctt ccttttggaa ccacatgaaa gtcttgatgc   1435

tgctgccatg atccctttga gaggtggctc aaaagctaca gggaactcca ggtcctttat   1495

tactgccttc ttttcaaaag cacaactctc ctctaaccct cccctccccc ttcccttctg   1555

gtcgggtcat agagctaccg tattttctag gacaagagtt ctcagtcact gtgcaatatg   1615

ccccctgggt cccaggaggg tctggaggaa aactggctat cagaacctcc tgatgccctg   1675

gtgggcttag ggaaccatct ctcctgctct ccttgggatg atggctggct agtcagcctt   1735
```

-continued

```
gcatgtattc cttggctgaa tgggagagtg ccccatgttc tgcaagacta cttggtattc   1795

ttgtagggcc gacactaaat aaaagccaaa ccttgggcac tgtttttttct ccctggtgct   1855

cagagcacct gtgggaaagg ttgctgtctg tctcagtaca atccaaattt gtcgtagact   1915

tgtgcaatat atactgttgt gggttggaga aaagtggaaa gctacactgg gaagaaactc   1975

ccttccttca atttctcagt gacattgatg aggggtcctc aaaagacctc gagtttccca   2035

aaccgaatca ccttaagaag gacagggcta gggcatttgg ccaggatggc caccctcctg   2095

ctgttgcccc ttagtgagga atcttcaccc cacttcctct acccccaggt tctcctcccc   2155

acagccagtc ccctttcctg gatttctaaa ctgctcaatt ttgactcaaa ggtgctattt   2215

accaaacact ctccctaccc attcctgcca gctctgcctc cttttcaact ctccacattt   2275

tgtattgcct tcccagacct gcttccagtc tttattgctt taaagttcac tttgggccca   2335

cagacccaag agctaatttt ctggtttgtg ggttgaaaca aagctgtgaa tcactgcagg   2395

ctgtgttctt gcatcttgtc tgcaaacagg tccctgcctt tttagaagca gcctcatggt   2455

ctcatgctta atcttgtctc tcttctcttc tttatgatgt tcactttaaa aacaacaaaa   2515

cccctgagct ggactgttga gcaggcctgt ctctcctatt aagtaaaaat aaatagtagt   2575

agtatgtttg taagctattc tgacagaaaa gacaaaggtt actaattgta tgatagtgtt   2635

tttatatgga agaatgtaca gcttatggac aaatgtacac ctttttgtta ctttaataaa   2695

aatgtagtag gataaaaaaa aa                                            2717
```

<210> SEQ ID NO 9
<211> LENGTH: 157
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 9

```
Met Ser His Gly Lys Gly Thr Asp Met Leu Pro Glu Ile Ala Ala Ala
 1               5                  10                  15

Val Gly Phe Leu Ser Ser Leu Leu Arg Thr Arg Gly Cys Val Ser Glu
            20                  25                  30

Gln Arg Leu Lys Val Phe Ser Gly Ala Leu Gln Glu Ala Leu Thr Glu
        35                  40                  45

His Tyr Lys His His Trp Phe Pro Glu Lys Pro Ser Lys Gly Ser Gly
    50                  55                  60

Tyr Arg Cys Ile Arg Ile Asn His Lys Met Asp Pro Ile Ile Ser Arg
65                  70                  75                  80

Val Ala Ser Gln Ile Gly Leu Ser Gln Pro Gln Leu His Gln Leu Leu
                85                  90                  95

Pro Ser Glu Leu Thr Leu Trp Val Asp Pro Tyr Glu Val Ser Tyr Arg
            100                 105                 110

Ile Gly Glu Asp Gly Ser Ile Cys Val Leu Tyr Glu Glu Ala Pro Leu
        115                 120                 125

Ala Ala Ser Cys Gly Leu Leu Thr Cys Lys Asn Gln Val Leu Leu Gly
    130                 135                 140

Arg Ser Ser Pro Ser Lys Asn Tyr Val Met Ala Val Ser
145                 150                 155
```

<210> SEQ ID NO 10
<211> LENGTH: 1746

-continued

<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (60)...(1037)
<223> OTHER INFORMATION: TIS11d

<400> SEQUENCE: 10

gagcctgact tcagcgctcc cactctcggc cgacacccct catggccaac cgttacacc 59

atg gat ctg act gcc atc tac gag agc ctc ctg tcg ctg agc cct gac 107
Met Asp Leu Thr Ala Ile Tyr Glu Ser Leu Leu Ser Leu Ser Pro Asp
1 5 10 15

gtg ccc gtg cca tcc gac cat gga ggg act gag tcc agc cca ggc tgg 155
Val Pro Val Pro Ser Asp His Gly Gly Thr Glu Ser Ser Pro Gly Trp
20 25 30

ggc tcc tcg gga ccc tgg agc ctg agc ccc tcc gac tcc agc ccg tct 203
Gly Ser Ser Gly Pro Trp Ser Leu Ser Pro Ser Asp Ser Ser Pro Ser
35 40 45

ggg gtc acc tcc cgc ctg cct ggc cgc tcc acc agc cta gtg gag ggc 251
Gly Val Thr Ser Arg Leu Pro Gly Arg Ser Thr Ser Leu Val Glu Gly
50 55 60

cgc agc tgt ggc tgg gtg ccc cca ccc cct ggc ttc gca ccg ctg gct 299
Arg Ser Cys Gly Trp Val Pro Pro Pro Pro Gly Phe Ala Pro Leu Ala
65 70 75 80

ccc cgc ctg ggc cct gag ctg tca ccc tca ccc act tcg ccc act gca 347
Pro Arg Leu Gly Pro Glu Leu Ser Pro Ser Pro Thr Ser Pro Thr Ala
85 90 95

acc tcc acc acc ccc tcg cgc tac aag act gag cta tgt cgg acc ttc 395
Thr Ser Thr Thr Pro Ser Arg Tyr Lys Thr Glu Leu Cys Arg Thr Phe
100 105 110

tca gag agt ggg cgc tgc cgc tac ggg gcc aag tgc cag ttt gcc cat 443
Ser Glu Ser Gly Arg Cys Arg Tyr Gly Ala Lys Cys Gln Phe Ala His
115 120 125

ggc ctg ggc gag ctg cgc cag gcc aat cgc cac ccc aaa tac aag acg 491
Gly Leu Gly Glu Leu Arg Gln Ala Asn Arg His Pro Lys Tyr Lys Thr
130 135 140

gaa ctc tgt cac aag ttc tac ctc cag ggc cgc tgc ccc tac ggc tct 539
Glu Leu Cys His Lys Phe Tyr Leu Gln Gly Arg Cys Pro Tyr Gly Ser
145 150 155 160

cgc tgc cac ttc atc cac aac cct agc gaa gac ctg gcg gcc ccg ggc 587
Arg Cys His Phe Ile His Asn Pro Ser Glu Asp Leu Ala Ala Pro Gly
165 170 175

cac cct cct gtg ctt cgc cag agc atc agc ttc tcc ggc ctg ccc tct 635
His Pro Pro Val Leu Arg Gln Ser Ile Ser Phe Ser Gly Leu Pro Ser
180 185 190

ggc cgc cgg acc tca cca cca cca cca ggc ctg gcc ggc cct tcc ctg 683
Gly Arg Arg Thr Ser Pro Pro Pro Pro Gly Leu Ala Gly Pro Ser Leu
195 200 205

tcc tcc agc tcc ttc tcg ccc tcc agc tcc cca cca cca cct ggg gac 731
Ser Ser Ser Ser Phe Ser Pro Ser Ser Ser Pro Pro Pro Pro Gly Asp
210 215 220

ctt cca ctg tca ccc tct gcc ttc tct gct gcc cct ggc acc ccc ctg 779
Leu Pro Leu Ser Pro Ser Ala Phe Ser Ala Ala Pro Gly Thr Pro Leu
225 230 235 240

gct cga aga gac ccc acc cca gtc tgt tgc ccc tcc tgc cga agg gcc 827
Ala Arg Arg Asp Pro Thr Pro Val Cys Cys Pro Ser Cys Arg Arg Ala
245 250 255

-continued

```
act cct atc agc gtc tgg ggg ccc ttg ggt ggc ctg gtt cgg acc ccc      875
Thr Pro Ile Ser Val Trp Gly Pro Leu Gly Gly Leu Val Arg Thr Pro
            260                 265                 270

tct gta cag tcc ctg gga tcc gac cct gat gaa tat gcc agc agc ggc      923
Ser Val Gln Ser Leu Gly Ser Asp Pro Asp Glu Tyr Ala Ser Ser Gly
        275                 280                 285

agc agc ctg ggg ggc tct gac tct ccc gtc ttc gag gcg gga gtt ttt      971
Ser Ser Leu Gly Gly Ser Asp Ser Pro Val Phe Glu Ala Gly Val Phe
    290                 295                 300

gca cca ccc cag ccc gtg gca gcc ccc cgg cga ctc ccc atc ttc aat     1019
Ala Pro Pro Gln Pro Val Ala Ala Pro Arg Arg Leu Pro Ile Phe Asn
305                 310                 315                 320

cgc atc tct gtt tct gag tgacaaagtg actgcccggt cagatcagct            1067
Arg Ile Ser Val Ser Glu
                325

ggatctcagc ggggagccac gtctcttgca ctgtggtctc tgcatggacc ccagggctgt   1127

ggggacttgg gggacagtaa tcaagtaatc ccctttttcca gaatgcatta acccactccc   1187

ctgacctcac gctggggcag gtccccaagt gtgcaagctc agtattcatg atggtggggg   1247

atggagtgtc ttccgaggtt cttgggggaa aaaaaattgt agcatattta agggaggcaa   1307

tgaaccctct cccccacctc ttccctgccc aaatctgtct cctagaatct tatgtgctgt   1367

gaataatagg ccttcactgc ccctccagtt tttatagacc tgaggttcca gtgtctcctg   1427

gtaactggaa cctctcctga gggggaatcc tggtgctcaa attaccctcc aaaagcaagt   1487

agccaaagcc gttgccaaac cccacccata aatcaatggg ccctttattt atgacgactt   1547

tatttattct aatatgattt tatagtattt atatatattg ggtcgtctgc ttcccttgta   1607

tttttcttcc tttttttgta atattgaaaa cgacgatata attattataa gtagactata   1667

atatatttag taatatatat tattacctta aaagtctatt tttgtgtttt gggcattttt   1727

aaataaacaa tctgagtgt                                               1746
```

<210> SEQ ID NO 11
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 11

```
Met Asp Leu Thr Ala Ile Tyr Glu Ser Leu Leu Ser Leu Ser Pro Asp
 1               5                  10                  15

Val Pro Val Pro Ser Asp His Gly Gly Thr Glu Ser Ser Pro Gly Trp
            20                  25                  30

Gly Ser Ser Gly Pro Trp Ser Leu Ser Pro Ser Asp Ser Ser Pro Ser
        35                  40                  45

Gly Val Thr Ser Arg Leu Pro Gly Arg Ser Thr Ser Leu Val Glu Gly
    50                  55                  60

Arg Ser Cys Gly Trp Val Pro Pro Pro Pro Gly Phe Ala Pro Leu Ala
65                  70                  75                  80

Pro Arg Leu Gly Pro Glu Leu Ser Pro Ser Pro Thr Ser Pro Thr Ala
                85                  90                  95

Thr Ser Thr Thr Pro Ser Arg Tyr Lys Thr Glu Leu Cys Arg Thr Phe
            100                 105                 110

Ser Glu Ser Gly Arg Cys Arg Tyr Gly Ala Lys Cys Gln Phe Ala His
        115                 120                 125
```

-continued

```
Gly Leu Gly Glu Leu Arg Gln Ala Asn Arg His Pro Lys Tyr Lys Thr
    130                 135                 140

Glu Leu Cys His Lys Phe Tyr Leu Gln Gly Arg Cys Pro Tyr Gly Ser
145                 150                 155                 160

Arg Cys His Phe Ile His Asn Pro Ser Glu Asp Leu Ala Ala Pro Gly
                165                 170                 175

His Pro Pro Val Leu Arg Gln Ser Ile Ser Phe Ser Gly Leu Pro Ser
            180                 185                 190

Gly Arg Arg Thr Ser Pro Pro Pro Pro Gly Leu Ala Gly Pro Ser Leu
        195                 200                 205

Ser Ser Ser Ser Phe Ser Pro Ser Ser Ser Pro Pro Pro Pro Gly Asp
    210                 215                 220

Leu Pro Leu Ser Pro Ser Ala Phe Ser Ala Ala Pro Gly Thr Pro Leu
225                 230                 235                 240

Ala Arg Arg Asp Pro Thr Pro Val Cys Cys Pro Ser Cys Arg Arg Ala
                245                 250                 255

Thr Pro Ile Ser Val Trp Gly Pro Leu Gly Gly Leu Val Arg Thr Pro
            260                 265                 270

Ser Val Gln Ser Leu Gly Ser Asp Pro Asp Glu Tyr Ala Ser Ser Gly
        275                 280                 285

Ser Ser Leu Gly Gly Ser Asp Ser Pro Val Phe Glu Ala Gly Val Phe
    290                 295                 300

Ala Pro Pro Gln Pro Val Ala Ala Pro Arg Arg Leu Pro Ile Phe Asn
305                 310                 315                 320

Arg Ile Ser Val Ser Glu
                325
```

<210> SEQ ID NO 12
<211> LENGTH: 878
<212> TYPE: DNA
<213> ORGANISM: Mus Musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (20)...(841)

<400> SEQUENCE: 12

```
aattcgtcga catggcttg atg ggg gtc aac ggc acc gcc gcc gcc gcc gcc      52
                    Met Gly Val Asn Gly Thr Ala Ala Ala Ala Ala
                     1               5                  10

ggg cag ccc aat gtc tcc tgc gcg tgc aac tgc cag cgc tct ttg ttc      100
Gly Gln Pro Asn Val Ser Cys Ala Cys Asn Cys Gln Arg Ser Leu Phe
            15                  20                  25

ccc agc atg gag atc acg gag ctg gag ttc gtg caa atc gtg gtc atc      148
Pro Ser Met Glu Ile Thr Glu Leu Glu Phe Val Gln Ile Val Val Ile
        30                  35                  40

gtg gta gtg atg atg gtg atg gtg gtt atg att acg tgc ctg ctg agc      196
Val Val Val Met Met Val Met Val Val Met Ile Thr Cys Leu Leu Ser
    45                  50                  55

cac tac aag ctg tca gcc cgc tcc ttc atc agc cga cac agc cag gcc      244
His Tyr Lys Leu Ser Ala Arg Ser Phe Ile Ser Arg His Ser Gln Ala
60                  65                  70                  75

agg agg aga gac gat gga ctg tcc tcg gaa gga tgc ctc tgg ccc tca      292
Arg Arg Arg Asp Asp Gly Leu Ser Ser Glu Gly Cys Leu Trp Pro Ser
                80                  85                  90
```

-continued

```
gag agt acg gtg tca ggt gga atg ccg gag cca cag gtc tat gcc ccg      340
Glu Ser Thr Val Ser Gly Gly Met Pro Glu Pro Gln Val Tyr Ala Pro
             95                  100                 105

cct cgg ccc act gac cga ctc gct gtg ccc ccc ttc atc cag cgg agc      388
Pro Arg Pro Thr Asp Arg Leu Ala Val Pro Pro Phe Ile Gln Arg Ser
        110                 115                 120

cga ttc caa ccc acc tac ccc tac ctg cag cac gaa att gcc ctg cca      436
Arg Phe Gln Pro Thr Tyr Pro Tyr Leu Gln His Glu Ile Ala Leu Pro
    125                 130                 135

ccc acc atc tca ctg tct gat ggg gag gag ccc cca ccc tac cag ggc      484
Pro Thr Ile Ser Leu Ser Asp Gly Glu Glu Pro Pro Pro Tyr Gln Gly
140                 145                 150                 155

ccc tgc acc ctc cag cta cgg gac cct gag caa cag ctg gag ctg aac      532
Pro Cys Thr Leu Gln Leu Arg Asp Pro Glu Gln Gln Leu Glu Leu Asn
                160                 165                 170

cgg gaa tct gtg cgc gca ccc cct aac cgg acc atc ttc gac agt gac      580
Arg Glu Ser Val Arg Ala Pro Pro Asn Arg Thr Ile Phe Asp Ser Asp
            175                 180                 185

ctt ata gac agc acc atg ctg ggg ggc ccc tgt ccc ccc agc agt aac      628
Leu Ile Asp Ser Thr Met Leu Gly Gly Pro Cys Pro Pro Ser Ser Asn
        190                 195                 200

tcg ggc atc agc gcc acc tgc tac agc agc ggt ggg cgc atg gag ggg      676
Ser Gly Ile Ser Ala Thr Cys Tyr Ser Ser Gly Gly Arg Met Glu Gly
    205                 210                 215

ccg ccc ccc acc tac agc gag gtc att ggc cac tac cct ggc tcc tcc      724
Pro Pro Pro Thr Tyr Ser Glu Val Ile Gly His Tyr Pro Gly Ser Ser
220                 225                 230                 235

ttc cag cac cag caa agt aac ggg cca tcc tcc ctg cta gag ggg acc      772
Phe Gln His Gln Gln Ser Asn Gly Pro Ser Ser Leu Leu Glu Gly Thr
                240                 245                 250

cgg ctc cat cac tcg cac att gcc cca ctg gag aac aag gag aag gag      820
Arg Leu His His Ser His Ile Ala Pro Leu Glu Asn Lys Glu Lys Glu
            255                 260                 265

aaa cag aaa ggt cac ccc ctc taggagtggg ggccggggcg cctgtaggca        871
Lys Gln Lys Gly His Pro Leu
        270

aaaccgc                                                            878


<210> SEQ ID NO 13
<211> LENGTH: 274
<212> TYPE: PRT
<213> ORGANISM: Mus Musculus

<400> SEQUENCE: 13

Met Gly Val Asn Gly Thr Ala Ala Ala Ala Ala Gly Gln Pro Asn Val
 1               5                  10                  15

Ser Cys Ala Cys Asn Cys Gln Arg Ser Leu Phe Pro Ser Met Glu Ile
            20                  25                  30

Thr Glu Leu Glu Phe Val Gln Ile Val Val Ile Val Val Val Met Met
        35                  40                  45

Val Met Val Val Met Ile Thr Cys Leu Leu Ser His Tyr Lys Leu Ser
    50                  55                  60

Ala Arg Ser Phe Ile Ser Arg His Ser Gln Ala Arg Arg Arg Asp Asp
65                  70                  75                  80

Gly Leu Ser Ser Glu Gly Cys Leu Trp Pro Ser Glu Ser Thr Val Ser
                85                  90                  95
```

-continued

```
Gly Gly Met Pro Glu Pro Gln Val Tyr Ala Pro Pro Arg Pro Thr Asp
            100                 105                 110

Arg Leu Ala Val Pro Pro Phe Ile Gln Arg Ser Arg Phe Gln Pro Thr
        115                 120                 125

Tyr Pro Tyr Leu Gln His Glu Ile Ala Leu Pro Pro Thr Ile Ser Leu
    130                 135                 140

Ser Asp Gly Glu Glu Pro Pro Pro Tyr Gln Gly Pro Cys Thr Leu Gln
145                 150                 155                 160

Leu Arg Asp Pro Glu Gln Gln Leu Glu Leu Asn Arg Glu Ser Val Arg
                165                 170                 175

Ala Pro Pro Asn Arg Thr Ile Phe Asp Ser Asp Leu Ile Asp Ser Thr
            180                 185                 190

Met Leu Gly Gly Pro Cys Pro Pro Ser Ser Asn Ser Gly Ile Ser Ala
        195                 200                 205

Thr Cys Tyr Ser Ser Gly Gly Arg Met Glu Gly Pro Pro Pro Thr Tyr
    210                 215                 220

Ser Glu Val Ile Gly His Tyr Pro Gly Ser Ser Phe Gln His Gln Gln
225                 230                 235                 240

Ser Asn Gly Pro Ser Ser Leu Leu Glu Gly Thr Arg Leu His His Ser
                245                 250                 255

His Ile Ala Pro Leu Glu Asn Lys Glu Lys Glu Lys Gln Lys Gly His
            260                 265                 270

Pro Leu
```

<210> SEQ ID NO 14
<211> LENGTH: 693
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (639)...(639)
<223> OTHER INFORMATION: a, c, g, or t/u

<400> SEQUENCE: 14

```
ttttttgca agctctctta gcttgtgcat tcagaccaga catcacatgt aaatatttat     60

acacagggag gtgggagggg agggccacac gatgcgttgc tgcgcccccc gccttcctct    120

cactcctctt ctaagaagcg cggagtgttc tgccttttca cctacgcagc cccagcccgg    180

ccccccctggg gaccctagag agggtgtcct ttctgtttat ccttctcttt gctccagatg    240

gctgcgctct ctaggggcgc gatgtgtgtg tgggggaacc cggtcccccc ccagcaagga    300

gggcggccca ctgctctgct ggtgctggaa ggaggacccc gggtagtggc cgatgacctc    360

gctgtaggtg ggcggcggcc cctccatgcg cccgccgctg ccgtagcacg tggcgctgat    420

gcccgagtta ctgctggggg ggcaggggcc gcccagcctg gcactatcca tcaggtcact    480

gtcgaagatg gttctgtttg ggggtgcgcg caccgactcc cggttcagtt ccagctgctg    540

ctcggggtcc cgaagctgga gggtgcaggg gccctggtag ggtggggctc ctcccgtctg    600

acagcgagat ggtgggcggc agtcgatctc gtgctgcang tacggatagg tgggctggaa    660

gcggtggaag cgctcccgct gggcgaaagg cgg                                 693
```

<210> SEQ ID NO 15
<211> LENGTH: 475

-continued

<212> TYPE: DNA
<213> ORGANISM: Mus Musculus

<400> SEQUENCE: 15

aattcgtcga catggcttga tgggggtcaa cggcaccgcc gccgccgccg ccgggcagcc 60

caatgtctcc tgcgcgtgca actgccagcg ctctttgttc cccagcatgg agatcacgga 120

gctggagttc gtgcaaatcg tggtcatcgt ggtagtgatg atggtgatgg tggttatgat 180

tacgtgcctg ctgagccact acaagctgtc agcccgctcc ttcatcagcc gacacagcca 240

ggccaggagg agagacgatg gactgtcctc ggaaggatgc ctctggccct cagagagtac 300

ggtgtcaggt ggaatgccgg agccacaggt ctatgccccg cctcggccca ctgaccgact 360

cgctgtgccc cccttcatcc agcggagccg attccaaccc acctaccct acctgcagca 420

cgaaattgcc ctgccaccca ccatctcact gtctgatggg gaggagcccc caccc 475

<210> SEQ ID NO 16
<211> LENGTH: 8093
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (6477)...(6477)
<223> OTHER INFORMATION: c or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (6837)...(6837)
<223> OTHER INFORMATION: a or c

<400> SEQUENCE: 16

ctcagctccc tgtggtgggg gatggcagag gcttcccgct ccccgcagct ggtttgcaca 60

gcctcccgac tgtgccgtgg tgtctccttc cctaaagtgg gacagtcgtt tctgtgtcct 120

gctggcctct gaggaacaga cgtgtgtgag aggccttcag ggcctgatgg ctggggtggt 180

gacagtgcct ggagaatggg tggggactgg aggggccagg tggctaacca ctctcctctt 240

ccatggcagc ggagctggag ttcgcccaaa tcatcatcat cgtcgtggtg gtcacggtga 300

tggtggtggt catcgtctgc ctgctgaacc actacaaagt ctccacgcgg tccttcatca 360

accgcccgaa ccagagccgg aggcgggagg acgggctgcc gcaggaaggg tgcctgtggc 420

cttcagacag cgccgcaccg cggctgggcg cctcggagat catgcatgcc ccgcggtcca 480

gggacaggtt cacagcgccg tccttcatcc agagggatcg cttcagccgc ttccagccca 540

cctaccccta tgtgcagcac gagattgatc ttcctcccac catctccctg tccgacggtg 600

aagagccacc tccttaccag gggccctgca ccctgcagct ccgggaccct gaacagcaga 660

tggaactcaa ccgagagtcc gtgagggccc cacccaaccg aaccatattt gacagtgatt 720

taatagacat tgctatgtat agcgggggtc catgcccacc cagcagcaac tcgggcatca 780

gtgcaagcac ctgcagcagt aacgggagga tggaggggcc acccccaca tacagcgagg 840

tgatgggcca ccacccaggc gcctctttcc tccatcacca gcgcagcaac gcacacaggg 900

gcagcagact gcagtttcag cagaacaatg cagagagcac aatagtaccc atcaaaggca 960

aagataggaa gcctgggaac ctggtctgat tccttccaac gtgcacttca gctggagaaa 1020

gaaaccaaga agggaagcgg ccgctgggcc cctcctgcgc acagtgttgt tcagtttcac 1080

atggtacaaa taagtaaaac caaatgagca aacacggtct ttgtttctga ttccttttag 1140

gggaattgca tgcaaactag actgaaatga tacaaacttc catctggtct gaccgcaaac 1200

-continued

agtgtttatt tggggacagg ggttgggatg ggggtgtggg caggggaaaa cagagaacgg 1260

gatgctttga agataccatg aaataaaacc cacagaggta tttgatgtat ttaattgtga 1320

aaggagactt tgcagataaa tgaggccaga atggcatgtt ttataattaa ctgaataaag 1380

aaggaagcat tattatatat tattgtgggg aagaaccagc cagttcgctt tttctcctaa 1440

ggtgtggact tttattttgt tttaaaaata tgaatcaaaa ttcctgtgtt gtgtgccaag 1500

gtataaagtg gagaagttag atgagtgcaa ggagctcctt tgtgttgtga tgatgtgttt 1560

taaaagttgc actatcttaa tgttgaaaat atttacaagg gaactgtttt acgtgaagtt 1620

ctgtatgttg tcttttcacc tgtggattgt aatcaggccc aaggaatatc ctggagtggt 1680

ccccagaagc atccaagaaa agatatttgg ggacgtagcc taacatttta ccaacttacg 1740

taaatcaaaa aagtcattat tgttgcagga gtttgcatca aatagcagtg catcgctgaa 1800

gcttttggag acttttggat ggaagataag atagggaaga ttaagttcca gcatttctga 1860

cttgttattt tgagttactc tgctactctt aggctgcata gtttatgaga aaatgaacac 1920

atgcatttat ggatccagta tcatgcagtg ctgccctcat cctccagcag tgcaatttct 1980

tcagtaattt agatttttt cactatagca tgaaatatat tcaaatacat accttatttt 2040

atgcaataaa ttgtttaaaa tgcaaggtgg ttattctgca tactgttgaa atatgtgact 2100

cctcagtata ttcccattgc ctctcccct ttcctcgaca gcttagttca gttctgcagg 2160

gctgctcagt tcacaggagg ctcccagcag ccaccccaca tccagcctac acagaacttt 2220

cgtgtgggag tggtgtgggt ggtggttttc ttatgctttg gaagccccta gaaataatga 2280

cggaagaatg ccatgttgct gatcgtggta ataagccatt gtgggttatt gtatgtcact 2340

agtattagca tagcattctt aaaggaatgc agtgttcaaa acctacccaa attccccgca 2400

ggattttacc aaacccttcc ccaggccagt tttgtactga aggcaagaac tggacagtca 2460

gagaacagtg gaggggggcaa gtgactgaag agcaccgggt aaaaagcaca acatgcagtt 2520

aaaatgcaaa ctagaaaact aattttaaat attgttagtt ttaatatttc ctgatattta 2580

caaatattca ttcttatata caatgaaaaa aataactttc ttctgcagat gtaagcactg 2640

gcttttataa gagcagcagc caacacgttt agcagacact gcgcgtggag aagggcttat 2700

ctgcagtaca ctctgccatg tggagggtgg gcctctgtgg cctcttcaca taacaagatg 2760

agctggaatg atgattccat gactcccacc tatgcagcct taaagccaaa tccgcgtgtg 2820

tgtgtttgtg tctgtctgtg ggtctcgaag gtgatccgtc ggtgcggtgg ctctgtgctg 2880

taactggaga gactgttcca aaccccaaga gttgtctgat cctagtctgt tcccttctgc 2940

ttcttacctc tgtagatagg tcactggttt ttgtttgttt gttttgagga ttggaatttc 3000

cattacattc atcctttgca cacagtaaca tccacagaac tagtccaact cttaaaagga 3060

gagaggaaaa acacaggcac cagttgtcag ctcatgctta caacctgtgt ggaagtatat 3120

acagttgaga gtcacagtgg aggttctgag actggattca gtcttgttcc agtgacagtt 3180

ggaaggcctc tgctggagag acaccagctc tcagggcaga gattggcttg gggccagaag 3240

gaccctcccc aaccctggag acaccctgaa ggttcactgg ctctccagat tagcctctct 3300

tcctctgtca ggcaaagatg aggagcccgt gttcccatcg ggccctgctg gcagggactt 3360

gcagtggatt cttggtcagg tgtgcccaca gatgcggagg cgaggtgagt gattccatca 3420

tttcagttct cacctgcagt tttggtgaag caggagatgc accccacagc tctagctctc 3480

-continued

aaatggcttc acagtcctta cttctctacc tgcctcaaga aggggctcag agcagagact 3540

tgtgaattcc ttagtaactg tgagtatatg aatgtgttgc acatgtccac agtattggcg 3600

agataattac ataattcaga tacctttaat catctttcaa gaaagaggct cctcccattc 3660

aaccacccta gagaactgcc tttgttaaat agttatttaa agactcatac atatcaaacc 3720

atgactttga aaggtcttcg aggctggggc tctgtaatga attagtttaa aagccaaggt 3780

cataacatga attgatggtc aatttccctt cagcagaagg aaaaggtgat ttagatcagt 3840

agctcttttg aaggttgtgg ctgacctgtt cataccgtgt cgcctcatgg ctagtgtggc 3900

gttgaaagag tagcgactgg gaagatacaa cttacacagt ggggcctatt gttctttcaa 3960

gaacccttt tttagcttat agaacccatg ggtccagttt agtaacgagt gatttaggca 4020

atcaatgata ggtttataat cttagattat tccagcaaag tgtggattgc attgttagga 4080

agaacatttg gtgggaatga acactcctgg gcataccgct gacttttgtc ccttgttccc 4140

ggtgtaggag acccaaggca tcttgaatcc catctataag aacacaatct tccagcatac 4200

gtttgctttt tcagaaactc tagcattctc tttaaatact gacgcaatcc ttaatggaaa 4260

agagatttca tgaagcaaat tatgtatttc aatagttctt ctatttttag tgtccaaaat 4320

ttactaatac agaagcttga caagcatgtc ctcaccctcc ccaccacata aacacatgga 4380

cacacaccca agccacaaga aatcccaaga gagcagaagc gaatttttaa aagatttatc 4440

gtgaggactg catttccatt cactaatttt ggctcaaact tatgaggcag gaaatagggg 4500

ccaacagtaa atgggggagg cctcctgaca ccagcagagg aattttgtac ccaggcgagg 4560

acttcttgaa cttctgcgta tctccgtttg atctctttca cctttatttc atcttcataa 4620

gaatgagaaa ggctcaaaag gaagcacttt tagaaatctt ctctgaccta gaagaatcca 4680

tccaaatccc tgccttcctc tctgaaccaa cagttccctt ctctgacagg gggccatcct 4740

ctatcttcca tccagcggct cttcctttta ggaaggctct ggtgcagagc acttcaaata 4800

tgtcctcagg ccagatactg attgctagta gagagacacc cggcacccag tccgaagccc 4860

tccctcaaag gaccggctta tggcgttggt cactggcagg ctcagagaca ttctactgtg 4920

ggcgcaggga gcccggcccc ccatgcagcc atgactggat gcgcccccat ctcgggggct 4980

tgctgcactg cttgtttatt gaattttgct acttagaatg gcaacattaa ctttgtgtac 5040

cattcatttt ttaaaaattt tccaaagctc ggcagtgtat gaaagaaaaa actgggaaag 5100

atacttggtt tctgttaact tttgtgttgc ttgcttaagt gattaaagcc agtgcttgga 5160

gccaagcctt catgccacga acatgctcca cagcctgccc tttgctctcc tgctcacact 5220

gaccaagaat gccgcgtgct tggcctactg aggtgaaagg acaattgaat gacaggtggg 5280

caaagggaga acttcccctt cttggtgcga ggaaagtcac aaatttaaaa atgttgcttc 5340

cagcccagat cctaaatgct agttctcagc agctgcgtgg cttaccgttc gccatttcca 5400

ccaccgccag ctgccagcac cgctacagat cacagagatg tgaacagaca atggaaagca 5460

ctcttagcct tgcagtggtc tacatttttt aggaaccaat atttcagcat tctttattac 5520

ccggcacgct gtgtcctttg cagagttcaa gtttatgtta ctgccagggt cagacagtca 5580

tttgctgctg ctgctgctgc tgctgctgct tctcgaactg gatgcattag gaagctgctg 5640

tctgagtgta ggaatgtctt gctaagaaag caatgtcttc cttcatcctt ttctttcttc 5700

cctctgcgtg tccttgtttt tgtgtaatgc gggagagggt tagagctata gagattatat 5760

-continued

atacactatc cgtgcacatt atatatatgt agatataccc ctatcatgtc agagatctgc 5820

atgtcagttt ttcagcaact aaggtgcctc atgttctgag ttcagcagat ataggaacca 5880

agccgccccc tcctgcactt gatgctccca cctttgttgt gcctcactta aaatggtgct 5940

tttttcagtt gtctgtcttt tcttatgttt ttatttgtaa ggtgctgtat ataagttgaa 6000

tatattatgc acatatccta cccaatgggt agaacaaaaa gttgttaata ctgtaatata 6060

atgtatagat gataccaatt ttaacagaaa tggcatagaa tttgtgaatg cctatgtgct 6120

ttgtcctctt ttgtaaggaa atttgcaaat ggatgcatac agattaaagt ctatgtagtt 6180

tattttccta ttaaatatca atattataac acaagagaaa gaagtgtgaa caaacaagca 6240

acagtttatg accagcgtat atatagcaat ggaaagttgc atctttgctg tgaaaacact 6300

ttaaagaaaa tacttttttaa aaaatcccac agctttttgg ttgccactag acgcttctta 6360

ttttaatcat tttagtaatg ctcagctgga ccagtgttag ttatatttga gtcagaaaaa 6420

tgttgttttt caacttgctt tataatctcc tgcatctatc tcctgctgta gcatcaygaa 6480

ggtgtcaggc aacagtgaaa agtgcacatt tttgttgttg cagaaactgt gtcagaggaa 6540

taagtaaatc agcctgcagc agaagacttt gttcagctcc agaggcatct gtgaccgtct 6600

gtgtccaagt ctctctgtgc ctttttcttt tacaaactga agctgtggag ccaatgaagt 6660

aacagtagag attgtaggga aagaatacct caggaaaaac aaatacactt acaagaagac 6720

cctgttctta gaaaatgtgt ttagttatgg gttagcacta gaagagactt ggctgtcagc 6780

cagccaagtg aaggacctct catccattcc cattcatgtc ccatcataat acggacmcaa 6840

aaagcaaact cggttttgcc atcagttaga aattacgttt tggattgtat attgttacat 6900

ctctcttcca gcttagtttt tagtgtctga ttgtgacctc tgcatttatc ttcaaatacc 6960

ctaattttaa aacaaaagaa caagaaaagt ttataacacc atgttcacta aaaccacggt 7020

tgaatcttgg gtgtgggcat cctttcgagt gttgtccata agagcagttc gtggaatttt 7080

gcccatctga cccatattat cagcttattc tgccaccaga gtagagtcta ataaattcca 7140

aagtttttat ttgctccatg gtgtatgttc tgactttgaa aatgtcagat tctataatca 7200

taccctaac atccaggaga caaatgacag attatcttta aactgaaatt gactctacaa 7260

tgcaaccctt aatgctgaat ggattaaaaa agtcagccct tttagtatct gtttgaaagg 7320

gccgtaaaaa gttgacactt ttgttgttgt ggatcctgcg tgtctagacc cacgtgttgt 7380

ttccatcgta tactgtaggg tgcacccctt gggattcatc attaagaact gaggctcact 7440

gttgtcagaa acaaagctcc caccccccag gttcaacctt gtgggagaac tgttgagcat 7500

gagaatgttc tagactcaga ggtactaaaa tttgttacca catcattgct tcctttctac 7560

aggacgaatt gaggcttaaa ctttactgtt aatgatactg gttcatttta atgtgcttgt 7620

tggtatgttg ctatttttca tttcatagct ttcaaaaatc atgctaattg tatacttgtc 7680

tagtttaagg ctattttaaa atatgtacaa tactattcac agcatttagt tcgtttaatt 7740

tttattataa agcaatctac taaaaaagta caactgtatt tgaacttttc aatagttgtt 7800

tgtgagctat gataatcaaa agtcattaaa gtctttttta acaaacattc gtgcttactt 7860

ttcaacataa ttcccagtta tatacagaaa aagatttcca cctgtcacgt atctgcctct 7920

tttacctgag caatggtgta gttcttagac ctaaggtctg taattgcaat acttttaaag 7980

-continued

aaagatgttg ctctaagtgc tgtttgttag ttatgaaatc agatttttct gcttgttctt 8040

aatgctgtgg tcaaaccata gcacaaaatc attaaaaata atcagcggca tac 8093

<210> SEQ ID NO 17
<211> LENGTH: 513
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens
<220> FEATURE:
<221> NAME/KEY: unsure
<222> LOCATION: (8)...(9)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (28)...(28)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (34)...(34)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (42)...(42)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (44)...(44)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (46)...(46)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (49)...(49)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (53)...(53)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (55)...(55)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (66)...(66)
<223> OTHER INFORMATION: a, c, g, or t/u
<221> NAME/KEY: unsure
<222> LOCATION: (410)...(410)
<223> OTHER INFORMATION: a, c, g, or t/u

<400> SEQUENCE: 17

aggggganna gagctgatcc ttgtatanct gacnactttg cnananacnc aanangaaga 60

ggaagnagcg ccggtgcaca cacaccagag cgtttcgccc atttgaacct ttacctcctt 120

agcgagatgc tgaatcgctg atttatggca tatcatccgc cttgggcact tttctcgact 180

atcctgttat tcacctgtga actgggacat caataatgat gggctcacta gatcaaggga 240

gagaaagact gcatacaaat aaatgcgtaa aagcactgat taacctatga cacgtgctgg 300

atagggaatc aacacatgac aaggttcacg gtatacgccc ttcatatgct ggtctacata 360

ggtctgcagg cgactctgga cctgaacgag tatcatagcg agccgagcan cctagcctgc 420

cgtgtagagg acgtgtagct acgagcagaa cttgatgaag cccgaagggg ctggtcatca 480

tggatgccag gctgtggacg gacacccaca cac 513

We claim:

1. An isolated nucleic acid molecule selected from the group consisting of:

(a) nucleic acid molecules which hybridize under stringent conditions to a molecule consisting of a nucleotide sequence set forth as SEQ ID NO:1 and which code for a MIVR-1 polypeptide having cardiac cell anti-apoptotic activity,

(b) nucleic acid molecules that differ from the nucleic acid molecules of (a) or (b) in codon sequence due to the degeneracy of the genetic code, and

(c) complements of (a) or (b).

2. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule comprises the nucleotide sequence set forth as SEQ ID NO:1.

3. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:3 or a fragment thereof.

4. An isolated nucleic acid molecule selected from the group consisting of

(a) unique fragments of a nucleotide sequence set forth as SEQ ID NO:1, and

(b) complements of (a),

provided that a unique fragment of (a) includes a sequence of contiguous nucleotides which is not identical to any sequence selected from the sequence group consisting of

(1) sequences selected from the group consisting of SEQ ID NOs. 14-16, and 17,

(2) complements of (1), and

(3) fragments of (1) and (2).

5. The isolated nucleic acid molecule of claim 4, wherein the sequence of contiguous nucleotides is selected from the group consisting of:

(1) at least two contiguous nucleotides nonidentical to the sequence group,

(2) at least three contiguous nucleotides nonidentical to the sequence group,

(3) at least four contiguous nucleotides nonidentical to the sequence group,

(4) at least five contiguous nucleotides nonidentical to the sequence group,

(5) at least six contiguous nucleotides nonidentical to the sequence group, and

(6) at least seven contiguous nucleotides nonidentical to the sequence group.

6. The isolated nucleic acid molecule of claim 4, wherein the unique fragment has a size selected from the group consisting of at least:8 nucleotides, 10 nucleotides, 12 nucleotides, 14 nucleotides, 16 nucleotides, 18 nucleotides, 20, nucleotides, 22 nucleotides, 24 nucleotides, 26 nucleotides, 28 nucleotides, 30 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, and 200 nucleotides.

7. The isolated nucleic acid molecule of claim 4, wherein the molecule encodes a polypeptide which is immunogenic.

8. An expression vector comprising the isolated nucleic acid molecule of claims **1**, **2**, **3**, **4**, **5**, **6**, or **7** operably linked to a promoter.

9. An expression vector comprising the isolated nucleic acid molecule of claim 4 operably linked to a promoter.

10. A host cell transformed or transfected with the expression vector of claim 8.

11. A host cell transformed or transfected with the expression vector of claim 9.

12. An isolated polypeptide encoded by a nucleic acid molecule of claim 1, **2**, **3**, or **4**, wherein the polypeptide, or fragment of the polypeptide, has cardiac cell anti-apoptotic activity.

13. The isolated polypeptide of claim 12, wherein the polypeptide is encoded by the nucleic acid molecule of claim 2.

14. The isolated polypeptide of claim 13, wherein the polypeptide comprises a polypeptide having the sequence of amino acids 1-287 of SEQ ID NO:2.

15. An isolated polypeptide encoded by a nucleic acid molecule of claim 1, **2**, **3**, or **4**, wherein the polypeptide, or fragment of the polypeptide, is immunogenic.

16. The isolated polypeptide of claim 15, wherein the fragment of the polypeptide, or portion of the fragment, binds to a human antibody.

17. An isolated binding polypeptide which binds selectively a polypeptide encoded by an isolated nucleic acid molecule of claim 1, **2**, **3**, or **4**.

18. The isolated binding polypeptide of claim 17, wherein the isolated binding polypeptide binds to a polypeptide having the sequence of amino acids of SEQ ID NO:2.

19. The isolated binding polypeptide of claim 18, wherein the isolated binding polypeptide is an antibody or an antibody fragment selected from the group consisting of a Fab fragment, a $F(ab)_2$ fragment or a fragment including a CDR3 region.

20. A method for determining the level of MIVR-1 expression in a subject, comprising measuring expression of MIVR-1 in a test sample from the subject to determine the level of MIVR-1 expression in the subject.

21. The method of claim 20, wherein the measured MIVR-1 expression in the test sample is compared to MIVR-1 expression in a control containing a known level of expression.

22. The method of claim 20, wherein the expression of MIVR-1 is MIVR-1 mRNA expression.

23. The method of claim 20, wherein the expression of MIVR-1 is MIVR-1 polypeptide expression.

24. The method of claim 20, wherein the test sample is tissue.

25. The method of claim 20, wherein the test sample is a biological fluid.

26. The method of claim 22, wherein MIVR-1 mRNA expression is measured using PCR.

27. The method of claim 22, wherein MIVR-1 mRNA expression is measured using Northern blotting.

28. The method of claim 23, wherein MIVR-1 polypeptide expression is measured using monoclonal antibodies to MIVR-1.

29. The method of claim 23, wherein MIVR-1 polypeptide expression is measured using polyclonal antisera to MIVR-1.

30. The method of claim 23, wherein expression of MIVR-1 is measured using MIVR-1 cardiac cell anti-apoptotic activity.

31. A method for identifying an agent useful in modulating cardiac cell anti-apoptotic activity of a molecule, comprising:

(a) contacting a molecule having cardiac cell anti-apoptotic activity with a candidate agent,

(b) measuring cardiac cell anti-apoptotic activity of the molecule, and

(c) comparing the measured cardiac cell anti-apoptotic activity of the molecule to a control to determine whether the candidate agent modulates cardiac cell anti-apoptotic activity of the molecule,

wherein the molecule is a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof.

32. A method of diagnosing a condition characterized by aberrant expression of a nucleic acid molecule or an expression product thereof, said method comprising:

a) contacting a biological sample from a subject with an agent, wherein said agent specifically binds to said nucleic acid molecule, an expression product thereof, or a fragment of an expression product thereof; and

b) measuring the amount of bound agent and determining therefrom if the expression of said nucleic acid molecule or of an expression product thereof is aberrant, aberrant expression being diagnostic of the condition;

wherein the nucleic acid molecule is at least one nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

33. The method of claim 32, wherein the nucleic acid molecule is at least two nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

34. The method of claim 32, wherein the nucleic acid molecule is at least three nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

35. The method of claim 32, wherein the nucleic acid molecule is at least four nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

36. The method of claim 32, wherein the nucleic acid molecule is at least five nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

37. The method of claim 32, wherein the condition is a cardiovascular condition selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure.

38. The method of claim 32, wherein the condition is cardiac hypertrophy.

39. A method for determining regression, progression or onset of a vascular condition in a subject characterized by aberrant expression of a nucleic acid molecule or an expression product thereof, comprising:

monitoring a sample from a patient, for a parameter selected from the group consisting of

(i) a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, BTG-2, TIS-11d, and VDUP-1,

(ii) a polypeptide encoded by the nucleic acid molecule,

(iii) a peptide derived from the polypeptide, and

(iv) an antibody which selectively binds the polypeptide or peptide,

as a determination of regression, progression or onset of said vascular condition in the subject.

40. The method of claim 39, wherein the sample is a biological fluid or a tissue.

41. The method of claim 39, wherein the step of monitoring comprises contacting the sample with a detectable agent selected from the group consisting of

(a) an isolated nucleic acid molecule which selectively hybridizes under stringent conditions to the nucleic acid molecule of (i),

(b) an antibody which selectively binds the polypeptide of (ii), or the peptide of (iii), and

(c) a polypeptide or peptide which binds the antibody of (iv).

42. The method of claim 41, wherein the antibody, the polypeptide, the peptide or the nucleic acid is labeled with a radioactive label or an enzyme.

43. The method of claim 39, comprising assaying the sample for the peptide.

44. A kit, comprising a package containing:

an agent that selectively binds to the isolated nucleic acid of claim 1 or an expression product thereof, and

a control for comparing to a measured value of binding of said agent to said isolated nucleic acid of claim 1 or expression product thereof.

45. The kit of claim 44, wherein the control is a predetermined value for comparing to the measured value.

46. The kit of claim 44, wherein the control comprises an epitope of the expression product of the nucleic acid of claim 1.

47. The kit of claim 44, further comprising a second agent that selectively binds to an isolated nucleic acid molecule selected from the group consisting of IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, and

a control for comparing to a measured value of binding of said second agent to said nucleic acid molecule or expression product thereof.

48. A method for treating a cardiovascular condition, comprising:

administering to a subject in need of such treatment an agent that modulates expression of a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, in an amount effective to treat the cardiovascular condition.

49. The method of claim 48, further comprising co-administering an agent selected from the group consisting of an anti-inflammatory agent, an anti-thrombotic agent, an anti-platelet agent, a fibrinolytic agent, a lipid reducing agent, a direct thrombin inhibitor, a glycoprotein IIb/IIIa receptor inhibitor, an agent that binds to cellular adhesion molecules and inhibits the ability of white blood cells to attach to such molecules, a calcium channel blocker, a beta-adrenergic receptor blocker, a cyclooxygenase-2 inhibitor, or an angiotensin system inhibitor.

50. A method of treating apoptotic cell-death of a cardiac cell in a subject, comprising:

administering to a subject in need of such treatment an agent that modulates expression of a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11 d, in an effective amount to inhibit apoptotic cell-death of the cardiac cell in the subject.

51. The method of claim 50, wherein the subject has a condition selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure.

52. A method for inhibiting apoptotic cell-death of a cell, comprising:

contacting a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, with a cell under conditions that permit entry of the molecule into the cell, in an amount effective to inhibit apoptotic cell-death of the cell.

53. The method of claim 52, wherein the cell is selected from the group consisting of a cardiomyocyte and a vascular endothelial cell.

54. A method for treating a condition mediated by increased apoptotic cell-death of vascular endothelial cells in a subject, comprising:

administering to a subject in need of such treatment a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, in an amount effective to inhibit increased apoptotic cell-death of vascular endothelial cells.

55. A method for treating cardiac hypertrophy, comprising:

administering to a subject in need of such treatment an agent that increases cardiac cell-death, in an amount effective to treat cardiac hypertrophy in the subject,

wherein the agent that increases cardiac cell-death is an inhibitor of a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof.

56. A method for treating a subject to reduce the risk of a cardiovascular condition developing in the subject, comprising:

administering to a subject who is known to express decreased levels of a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, an agent for reducing the risk of the cardiovascular disorder in an amount effective to lower the risk of the subject developing a future cardiovascular disorder,

wherein the agent is an anti-inflammatory agent, an anti-thrombotic agent, an anti-platelet agent, a fibrinolytic agent, a lipid reducing agent, a direct thrombin inhibitor, a glycoprotein IIb/IIIa receptor inhibitor, an agent that binds to cellular adhesion molecules and inhibits the ability of white blood cells to attach to such molecules, a calcium channel blocker, a beta-adrenergic receptor blocker, a cyclooxygenase-2 inhibitor, or an angiotensin system inhibitor, or an agent that modulates expression of a molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

57. A method for identifying a candidate agent useful in the treatment of a cardiovascular condition, comprising:

determining expression of a set of nucleic acid molecules in a cardiac cell or tissue under conditions which, in the absence of a candidate agent, permit a first amount of expression of the set of nucleic acid molecules, wherein the set of nucleic acid molecules comprises at least one nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d,

contacting the cardiac cell or tissue with the candidate agent, and

detecting a test amount of expression of the set of nucleic acid molecules, wherein an increase in the test amount of expression in the presence of the candidate agent relative to the first amount of expression indicates that the candidate agent is useful in the treatment of the cardiovascular condition.

58. The method of claim 57, wherein the cardiovascular condition is selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure.

59. The method of claim 57, wherein the set of nucleic acid molecules comprises at least two nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

60. The method of claim 57, wherein the set of nucleic acid molecules comprises at least three nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

61. The method of claim 57, wherein the set of nucleic acid molecules comprises at least four nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d .

62. The method of claim 57, wherein the set of nucleic acid molecules comprises at least five nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

63. A method for identifying a candidate agent useful in the treatment of cardiac hypertrophy, comprising:

determining expression of a set of nucleic acid molecules in a cardiac cell or tissue under conditions which, in the absence of a candidate agent, permit a first amount of expression of the set of nucleic acid molecules, wherein the set of nucleic acid molecules comprises at least one nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d,

contacting the cardiac cell or tissue with the candidate agent, and

detecting a test amount of expression of the set of nucleic acid molecules, wherein a decrease in the test amount of expression in the presence of the candidate agent relative to the first amount of expression indicates that the candidate agent is useful in the treatment of cardiac hypertrophy.

64. The method of claim 63, wherein the set of nucleic acid molecules comprises at least two nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d .

65. The method of claim 63, wherein the set of nucleic acid molecules comprises at least three nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

66. The method of claim 63, wherein the set of nucleic acid molecules comprises at least four nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d .

67. The method of claim 63, wherein the set of nucleic acid molecules comprises at least five nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

68. A pharmaceutical composition, comprising:

an agent comprising an isolated nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, in a pharmaceutically effective amount to treat a cardiovascular condition, and

a pharmaceutically acceptable carrier.

69. The pharmaceutical composition of claim 68, wherein the agent is an expression product of the isolated nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

70. The pharmaceutical composition of claim 68, wherein the cardiovascular condition is selected from the group consisting of myocardial infarction, stroke, arteriosclerosis, and heart failure.

71. A pharmaceutical composition, comprising:

an agent that increases cardiac cell-death in a pharmaceutically effective amount to treat cardiac hypertrophy, wherein the agent that increases cardiac cell-death is an inhibitor of a nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, or an expression product thereof, and

a pharmaceutically acceptable carrier.

72. A solid-phase nucleic acid molecule array consisting essentially of a set of nucleic acid molecules, expression products thereof, or fragments thereof, each nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d, fixed to a solid substrate.

73. The solid-phase nucleic acid molecule array of claim 72, further comprising at least one control nucleic acid molecule.

74. The solid-phase nucleic acid molecule array of claim 72, wherein the set of nucleic acid molecules comprises at least one nucleic acid molecule selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

75. The solid-phase nucleic acid molecule array of claim 72, wherein the set of nucleic acid molecules comprises at least two nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

76. The solid-phase nucleic acid molecule array of claim 72, wherein the set of nucleic acid molecules comprises at least three nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

77. The solid-phase nucleic acid molecule array of claim 72, wherein the set of nucleic acid molecules includes at least four nucleic acid molecules, each selected from the group consisting of MIVR-1 , IEX-1, VDUP-1, BTG-2, and TIS-11d.

78. The solid-phase nucleic acid molecule array of claim 72, wherein the set of nucleic acid molecules includes at least 5 nucleic acid molecules, each selected from the group consisting of MIVR-1, IEX-1, VDUP-1, BTG-2, and TIS-11d.

* * * * *

专利名称(译)	心血管疾病的诊断和治疗		
公开(公告)号	US20020115081A1	公开(公告)日	2002-08-22
申请号	US09/934249	申请日	2001-08-21
[标]申请(专利权)人(译)	LEE RICHARDŧ LANDSCHULZ凯瑟琳ŧ KENNEDY SCOTT P THOMPSON约翰·F· TURI THOMAS摹		
申请(专利权)人(译)	LEE RICHARD T. LANDSCHULZ凯瑟琳T. KENNEDY SCOTT P. THOMPSON约翰·F TURI THOMAS G.		
当前申请(专利权)人(译)	LEE RICHARD T. LANDSCHULZ凯瑟琳T. KENNEDY SCOTT P. THOMPSON约翰·F TURI THOMAS G.		
[标]发明人	LEE RICHARD T LANDSCHULZ KATHERINE T KENNEDY SCOTT P THOMPSON JOHN F TURI THOMAS G		
发明人	LEE, RICHARD T. LANDSCHULZ, KATHERINE T. KENNEDY, SCOTT P. THOMPSON, JOHN F. TURI, THOMAS G.		
IPC分类号	G01N33/50 A61K38/00 A61K39/395 A61K45/00 A61K45/06 A61K48/00 A61P9/00 A61P9/04 A61P9/10 A61P43/00 C07K14/00 C07K14/47 C07K14/705 C07K16/18 C12N1/15 C12N1/19 C12N1/21 C12N5/02 C12N5/10 C12N15/09 C12N15/12 C12N15/63 C12Q1/02 C12Q1/68 G01N33/15 G01N33/53 G01N33/577 G01N37/00 G01N33/567 A61K38/17		
CPC分类号	A61K38/00 C07K14/705 A61P9/00 A61P9/04 A61P9/10 A61P35/00 A61P43/00		
优先权	60/227159 2000-08-22 US		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及用于诊断和治疗心血管疾病的方法和组合物。更具体地，本发明涉及涉及可用于抑制心脏凋亡细胞死亡的分离的分子的诊断和治疗。

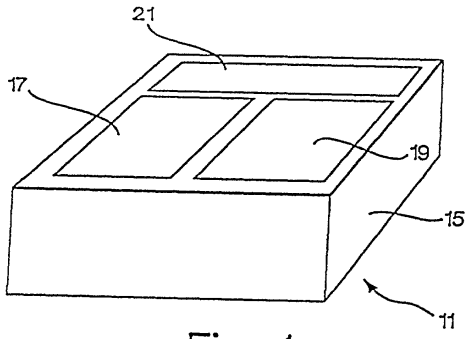

Fig. 1