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(72) Inventeurs/Inventors:
PENN, MARC S., US;
MAYORGA, MARITZA, US;
KIEDROWSKI, MATTHEW, US

(73) Propriétaires/Owners:
SUMMA HEALTH, US;
NORTHEAST OHIO MEDICAL UNIVERSITY, US

(74) Agent: ROBIC

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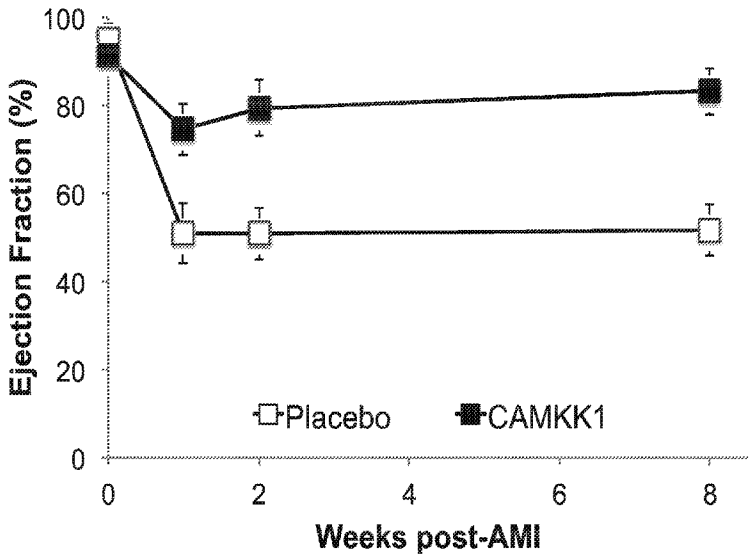

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Disclosed herein are methods of treating an ischemic or inflammatory condition in an organ or tissue of a patient comprising inducing an increase of the level of CAMKK1 in said organ or tissue.

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(71) Applicant: **SUMMA HEALTH** [US/US]; 525 East Market Street, Akron, OH 44304 (US).

(72) Inventor: **PENN, Marc, S.**; 26091 Shaker Boulevard, Beachwood, OH 44122 (US).

(74) Agents: **VALLA, Maurice, S.** et al.; Baker & Hostetler LLP, 2929 Arch Street, Cira Centre, 12th Floor, Philadelphia, PA 19104-2891 (US).

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(54) **Title**: CAMKK1 AS A NOVEL REGENERATIVE THERAPEUTIC

(57) **Abstract**: Disclosed herein are methods of treating an ischemic or inflammatory condition in an organ or tissue of a patient comprising inducing an increase of the level of CAMKK1 in said organ or tissue.

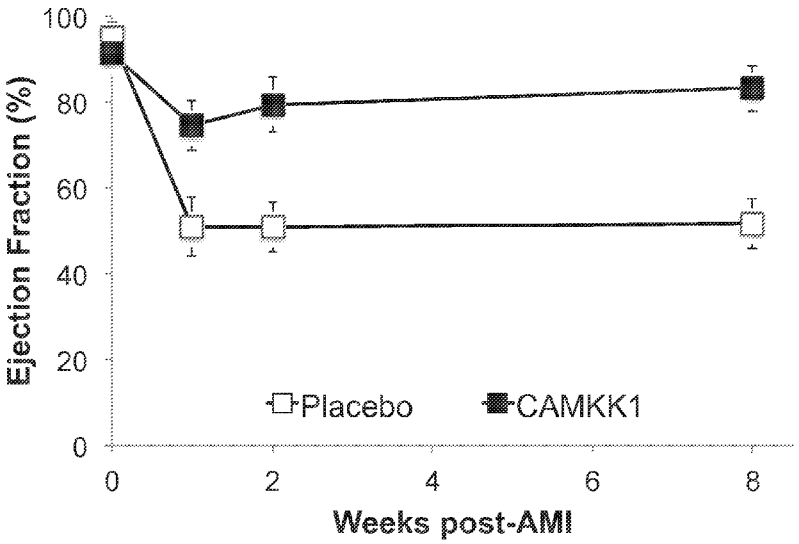

FIG. 7

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CAMKK1 AS A NOVEL REGENERATIVE THERAPEUTIC

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/086,026, filed December 1, 2014.

TECHNICAL FIELD

[0002] The disclosure relates to the use of calcium/calmodulin-dependent protein kinase kinase 1 (CAMKK1) as a novel regenerative therapeutic.

BACKGROUND

[0003] Ischemia is a condition wherein the blood flow is completely obstructed or considerably reduced in localized parts of the body, resulting in anoxia, reduced supply of substrates and accumulation of metabolites. Prolonged ischemia results in atrophy, denaturation, apoptosis, and necrosis of affected tissues.

[0004] Inflammation is a protective response that is intended to eliminate an initial cause of an injury, as well as necrotic cells/tissues resulting from the injury. In some diseases, such as arthritis, however, inflammation occurs in the absence of an injury. Prolonged inflammation can cause tissue destruction, fibrosis, and/or necrosis.

[0005] The goal of regenerative medicine is to induce healing and prevent fibrosis and scar formation following injury through replacement of damaged tissues and induction of endogenous repair pathways.

SUMMARY

[0006] Disclosed herein are methods of treating an ischemic or inflammatory condition in an organ or tissue of a patient, comprising inducing an increase of the level of CAMKK1 in said organ or tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The summary, as well as the following detailed description, is further understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosed methods, there are shown in the drawings exemplary embodiments of the methods; however, the methods are not limited to the specific embodiments disclosed. In the drawings:

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[0008] **FIG. 1A , FIG. 1B, FIG. 1C, and FIG. 1D** illustrate exemplary vector maps for use in the disclosed methods. FIG. 1A) hCamKK1 full vector. FIG. 1B) hCamKK1 truncated constitutive vector. FIG. 1C) myc hCamKK1 full vector. FIG. 1D) myc hCamKK1 truncated constitutive vector.

[0009] **FIG. 2** represents the cardiac function 2 weeks after LAD ligation and injection of conditioned media concentrated from 2 million MSC with the indicated treatment. Data represents individual animals. – represents the mean.

[0010] **FIG. 3** is an exemplary illustration of the CAMKK1 pathway.

[0011] **FIG. 4** represents an exemplary analysis of CAMKK1 expression in cells with decreased DAB2 expression (siRNADab2) compared to controls (control or CDNADAb2).

[0012] **FIG. 5A-FIG. 5C** represent: **FIG. 5A)** Cardiac function 14 days after LAD ligation and injection of conditioned media and cells from the indicated treatment. Data represents mean ± standard deviation (n=4-8 animals per group). **FIG. 5B**) The percent ± standard deviation left ventricular area expressing collagen as measured by Masson's trichrome stain 14 days after transplantation of MSCs pretreated and transfected with either scramble (control siRNA) or siRNA:CAMKK1. Representative photomicrographs of 4 mm sections are pictured from an animal from each treatment group above each data column. Data are presented as mean ± standard deviation. **$P<0.05$ compared with control MSCs. **FIG. 5C**) Vascular density in the border zone 14 day post-AMI. Confocal image of representative immunofluorescent staining. Endothelial cell staining with isolectin (green), corresponding merged image: Wheat germ agglutinin (red) and DAPI (blue) and Number of vascular density. Data represent mean ± SEM (vessels/mm^2, n=3 per group). *$P<0.05$ corresponding saline group.

[0013] **FIG. 6** represents exemplary results from treatment of rats following LAD ligation with a plasmid encoding CAMKK1. Improved cardiac function was observed 1 week and 2 weeks following treatment compared to vehicle (saline or glucose treatment). White bars: saline or glucose treatment; Black bars: CAMKK1 plasmid DNA treatment.

[0014] **FIG. 7** illustrates the effects of CAMKK1 (DNA) over-expression on Ejection Fraction in rodent model of AMI. 30 animals were submitted to anterior wall myocardial infarction via direct LAD ligation. Following ligation of the LAD, the animals were randomized and injected with 0.5 mg of CAMKK1 plasmid or saline in 5 divided 50-100 µl injections. One week, two weeks, and 8 weeks post-LAD ligation the animals underwent echocardiography and ejection fraction was calculated based on a parasternal long-axis view of the heart. All investigators performing LAD ligation, drug injection and echocardiography were blinded to

treatment group of any animal. (n=12 for placebo; n=15 for CAMKK1). Placebo was injected with saline. Mean ± SD.

[0015] **FIG. 8A** and **FIG. 8B** illustrate the effects of CAMKK1 (DNA) over-expression in MSC on MSC and MSC conditioned media on Ejection Fraction in rodent model of AMI. Control was MSC transfected with marker cDNA. Echocardiography was performed at 1, 2 and 5 weeks after AMI. Y-axis is EF (%), mean ± SD.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0016] The disclosed methods may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed methods are not limited to the specific methods described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed methods.

[0017] Similarly, unless specifically otherwise stated, any description as to a possible mechanism or mode of action or reason for improvement is meant to be illustrative only, and the disclosed methods are not to be constrained by the correctness or incorrectness of any such suggested mechanism or mode of action or reason for improvement.

[0018] It is to be appreciated that certain features of the disclosed methods that are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosed methods that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

[0019] As used herein, the singular forms "a," "an," and "the" include the plural.

[0020] The following abbreviations are used throughout the disclosure: AMI (acute myocardial infarction); CAMKK1 (calcium/calmodulin-dependent protein kinase kinase 1); Dab2 (disabled homolog 2); MSC (mesenchymal stem cells).

[0021] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[0022] As used herein, "nucleic acid" refers to a polynucleotide containing at least two covalently linked nucleotide or nucleotide analog subunits. A nucleic acid can be a deoxyribonucleic acid (DNA), a ribonucleic acid (RNA), or an analog of DNA or RNA. Nucleotide analogs are commercially available and methods of preparing polynucleotides

containing such nucleotide analogs are known (Lin et al. (1994) Nucl. Acids Res. 22:5220- 5234; Jellinek et al. (1995) Biochemistry 34: 11363-11372; Pagratis et al. (1997) Nature Biotechnol. 15:68-73). The nucleic acid can be single-stranded, double-stranded, or a mixture thereof. For purposes herein, unless specified otherwise, the nucleic acid is double-stranded, or it is apparent from the context.

[0023] As used herein, "treating" and like terms refer to reducing the severity and/or frequency of symptoms from the ischemic or inflammatory condition, eliminating the ischemic or inflammatory condition symptoms and/or the underlying cause of said symptoms, reducing the frequency or likelihood of the ischemic or inflammatory condition symptoms and/or their underlying cause, and improving or remediating damage caused, directly or indirectly, by theischemic or inflammatory condition.

[0024] The term "patient" as used herein is intended to mean any animal, in particular, mammals. Thus, the methods are applicable to human and nonhuman animals, although preferably used with mice, rats, and humans, and most preferably with humans.

[0025] As used herein, "an increase of the level of CAMKK1" means an amount of CAMKK1 protein in the organ or tissue of the patient that is greater than what is normally present in the organ or tissue of the patient.

[0026] Disclosed herein are methods of treating an ischemic or inflammatory condition in an organ or tissue of a patient, comprising inducing an increase of the level of CAMKK1 in said organ or tissue.

[0027] CAMKK1 (calcium/calmodulin-dependent protein kinase kinase 1; human CAMKK1 known by UniProtKB number Q8N5S9) belongs to a calcium-triggered signaling cascade and is involved in a number of cellular processes. CAMKK1 is a transferase that belongs to Ser/Thr protein kinase family and CamKK subfamily, and is expressed in heart, pancreas, amygdale, hypothalamus, prostate and lung. CAMKK1 activates CamK1 and CamK4 by phosphorylation of their amino acids Thr(177) and Thr(196), respectively. CAMKK1 activity is itself subjected to regulation by Ca2+/calmodulin; the activity of CAMKK1 is decreased upon phosphorylation by PKA (cAMP-Dependent Protein Kinase) and increased by incubation with PKA in the presence of Ca(2+)/calmodulin but decreased in its absence. This phosphorylation and inhibition of CAMKK1 by PKA is involved in modulating the balance between cAMP- and Ca2+-dependent signal transduction pathways. The nucleic acid sequence and amino acid sequence of CAMKK1 is set forth in SEQ ID NOs:1 and 2, respectively.

[0028] Those skilled in the art know that ischemic conditions and inflammatory conditions can occur in a number of different organs in tissues. For example, ischemic

conditions can occur in the heart, liver, kidney, brain, spine, lungs, small intestine, large intestine, and arteries. Likewise, inflammatory conditions can occur in the heart, liver, kidney, brain, spine, lungs, intestines, arteries, joints, cartilage, and skin. Accordingly, disclosed are methods of treating an ischemic or inflammatory condition in an organ or tissue of a patient, wherein said organ or tissue is heart, liver, kidney, brain, spine, lungs, small intestine, large intestine, arteries, joints, cartilage, skin, or any combination thereof. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the heart. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the myocardium. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the liver. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the kidney. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the brain. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the spine. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the lungs. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the small intestine. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the large intestine. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the arteries. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the joints. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the cartilage. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in the skin. In some embodiments, the methods can be used to treat an ischemic or inflammatory condition in any of the above organs or tissues.

[0029] Suitable techniques for increasing the level of CAMKK1 in an organ or tissue include, but are not limited to, administering CAMKK1 protein, administering a vector comprising a nucleic acid encoding CAMKK1, administering cells that have been modified to produce an increased level of CAMKK1, administering conditioned media from a culture of cells that have been modified to have an increase of the level of CAMKK1, or any combination thereof.

[0030] The increase in the level of CAMKK1 in said organ or tissue can be achieved, for example, by administering CAMKK1 protein to said organ or tissue. Suitable CAMKK1 proteins include wild type CAMKK1 or a constitutively active CAMKK1. In some aspects, the increase in the level of CAMKK1 in said organ or tissue can be achieved by administering wild type CAMKK1 protein to said organ or tissue. For example, the increase in the level of

CAMKK1 in said organ or tissue can be achieved by administering wild type CAMKK1 protein to said organ or tissue, wherein the wild type CAMKK1 protein is set for in SEQ ID NO:2. In other aspects, the increase in the level of CAMKK1 in said organ or tissue can be achieved by administering a constitutively active CAMKK1. The constitutively active CAMKK1 can comprise a CAMKK1 1-413 truncation. The increase in the level of CAMKK1 in said organ or tissue can be achieved by administering a CAMKK1 1-413 truncation to said organ or tissue, wherein the CAMKK1 1-413 truncation is set forth in SEQ ID NO:4. Alternatively, the constitutively active CAMKK1 can comprise a T108A mutant, a S459A mutant, or a T108A/S459A mutant CAMKK1. The increase in the level of CAMKK1 in said organ or tissue can be achieved by administering a T108A mutant CAMKK1 to said organ or tissue, wherein the T108A mutant CAMKK1 is set forth in SEQ ID NO:6. The increase in the level of CAMKK1 in said organ or tissue can be achieved by administering a S459A mutant CAMKK1 to said organ or tissue, wherein the S459A mutant CAMKK1 is set forth in SEQ ID NO:8. The increase in the level of CAMKK1 in said organ or tissue can be achieved by administering a T108A/S459A mutant CAMKK1 to said organ or tissue, wherein the T108A/S459A mutant CAMKK1 is set forth in SEQ ID NO:10. Phosphorylation of CAMKK1 by protein kinase A inhibits CAMKK1 activity. Therefore, construction of a constitutively active and non phosphorylatable CAMKK1 by removing one or more of these residues (through, for example, truncation) or mutating Thr108 and Ser459 to alanine, for example, may result in a more potent therapeutic.

[0031] The increase in the level of CAMKK1 in said organ or tissue can be achieved by administering a vector comprising a nucleic acid encoding CAMKK1 to said organ or tissue. The vector can comprise a plasmid or a viral vector. In some aspects, the increase in the level of CAMKK1 in the organ or tissue can be achieved by administering a plasmid comprising a nucleic acid encoding CAMKK1. In other aspects, the increase in the level of CAMKK1 in the organ or tissue can be achieved by administering a viral vector comprising a nucleic acid encoding CAMKK1. The nucleic acid can encode wild type CAMKK1 or a constitutively active CAMKK1. In some aspects, the increase in the level of CAMKK1 in the organ or tissue can be achieved by administering a plasmid or viral vector comprising a nucleic acid encoding wild type CAMKK1. For example, the increase in the level of CAMKK1 in the organ or tissue can be achieved by administering a plasmid or viral vector comprising CAMKK1 as set forth in SEQ ID NO:1. In other aspects, the increase in the level of CAMKK1 in the organ or tissue can be achieved by administering a plasmid or viral vector comprising a nucleic acid encoding a constitutively active CAMKK1. The constitutively active CAMKK1 can comprise a CAMKK1 1-413 truncation. For example, the increase in the level of CAMKK1 in the organ or tissue can

be achieved by administering a plasmid or viral vector comprising a CAMKK1 1-413 truncation as set forth in SEQ ID NO:3. Alternatively, the constitutively active CAMKK1 can comprise a T108A mutant, a S459A mutant, or a T108A/S459A mutant CAMKK1. For example, the increase in the level of CAMKK1 in the organ or tissue can be achieved by administering a plasmid or viral vector comprising a T108A mutant CAMKK1 as set forth in SEQ ID NO:5. The increase in the level of CAMKK1 in the organ or tissue can be achieved by administering a plasmid or viral vector comprising a S459A mutant CAMKK1 as set forth in SEQ ID NO:7. The increase in the level of CAMKK1 in the organ or tissue can be achieved by administering a plasmid or viral vector comprising a T108A/S459A mutant CAMKK1 as set forth in SEQ ID NO:9.

[0032] Suitable vectors for use in the disclosed methods can comprise components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. Such other components include, for example: components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector by the cell; components that influence localization of the nucleic acid within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of the nucleic acid. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities. Such components also include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Selectable markers include, for example, ampicillin and kanamycin. In some aspects, the selectable marker can be removed from the vector.

[0033] Suitable viral vectors include, but are not limited to, those derived from adenovirus (Ad), adeno-associated virus (AAV), herpes simplex virus (HSV), retrovirus, lentivirus, and alphavirus. Both human and non-human viral vectors can be used. In embodiments wherein human viral vectors are used, the viral vector can be modified to be replication-defective in humans.

[0034] The vector comprising a nucleic acid encoding CAMKK1 can be under a constitutively active promoter, a tissue specific promoter, or a drug inducible promoter. For example, a tissue-specific promoter can be fused to the nucleic acid encoding CAMKK1, limiting its expression to a particular tissue.

[0035] The nucleic acid encoding CAMKK1 can be introduced into the cells by techniques known in the art including, but not limited to, transfection and electroporation. The vector may be modified to improve transfection or electroporation efficiency.

[0036] Exemplary vector maps are illustrated in **FIG. 1A-FIG. 1D.**

[0037] The increase in the level of CAMKK1 in said organ or tissue can be achieved by administering cells that have been modified to produce an increased level of CAMKK1. Cells can be modified to have an increase in the level of CAMKK1 by, for example, modifying the cells with a vector comprising a nucleic acid encoding CAMKK1, modifying the cells with an agent that induces the expression of CAMKK1, or a combination thereof. As discussed above, the CAMKK1 protein can be wild type or a constitutively active CAMKK1. The cells can be modified to express wild type CAMKK1 protein as set forth in SEQ ID NO:2. The cells can be modified to express CAMKK1 1-413 protein as set forth in SEQ ID NO:4. The cells can be modified to express a T108A mutant CAMKK1 as set forth in SEQ ID NO:6. The cells can be modified to express a S459A mutant CAMKK1 as set forth in SEQ ID NO:8. The cells can be modified to express a T108A/S459A mutant CAMKK1 as set forth in SEQ ID NO:10.

[0038] In some embodiments, the increase in the level of CAMKK1 in said organ or tissue can be achieved by administering cells that have been modified with a vector comprising a nucleic acid encoding CAMKK1. The vector can comprise a plasmid or a viral vector. In some aspects, the cells can be modified with a plasmid comprising a nucleic acid encoding CAMKK1. In other aspects, the cells can be modified with a viral vector comprising a nucleic acid encoding CAMKK1. As discussed above, the plasmid or viral vector can comprise a nucleic acid encoding wild type or a constitutively active CAMKK1. The cells can be modified with a plasmid or viral vector comprising a nucleic acid encoding wild type CAMKK1 protein, wherein the nucleic acid is set forth in SEQ ID NO:1. The cells can be modified with a plasmid or viral vector comprising a nucleic acid encoding CAMKK1 1-413 protein, wherein the nucleic acid is set forth in SEQ ID NO:3. The cells can be modified with a plasmid or viral vector comprising a nucleic acid encoding a T108A mutant CAMKK1 protein, wherein the nucleic acid is set forth in SEQ ID NO:5. The cells can be modified with a plasmid or viral vector comprising a nucleic acid encoding a S459A mutant CAMKK1 protein, wherein the nucleic acid is set forth in SEQ ID NO:7. The cells can be modified with a plasmid or viral vector comprising a nucleic acid encoding a T108A/S459A mutant CAMKK1 protein, wherein the nucleic acid is set forth in SEQ ID NO:9.

[0039] In other embodiments, the increase in the level of CAMKK1 in said organ or tissue can be achieved by administering cells that have been modified with an agent that induces

the expression of CAMKK1. Suitable agents for inducing the expression of CAMKK1 include, but are not limited to, TGF-β, miR145, a Dab2 inhibitor, or any combination thereof. In some aspects, the cells can be modified with TGF-β and administered to said organ or tissue. In other aspects, the cells can be modified with miR145 and administered to said organ or tissue. For example, the cells can be modified with a miR145 as set forth in SEQ ID NO:17 and administered to said organ or tissue. In other aspects, the cells can be modified with a Dab2 inhibitor such as, for example, Dab2 siRNA, and administered to said organ or tissue. Suitable Dab2 siRNA include Dab2 siRNA comprising the sense and antisense stands set forth as SEQ ID NO:11 and 12, SEQ ID NO:13 and 14, or SEQ ID NO:15 and 16. For example, the cells can be modified with a Dab2 siRNA as set forth as SEQ ID NO:11 and 12 and administered to said organ or tissue. The cells can be modified with a Dab2 siRNA as set forth as SEQ ID NO:13 and 14 and administered to said organ or tissue. For example, the cells can be modified with a Dab2 siRNA as set forth as SEQ ID NO:15 and 16 and administered to said organ or tissue.

[0040] The increase in the level of CAMKK1 in said organ or tissue can be achieved by administering conditioned media from a culture of cells that have been modified to have an increase of the level of CAMKK1. A culture of cells can be modified to have an increase in the level of CAMKK1 by, for example, modifying the cells with a vector comprising a nucleic acid encoding CAMKK1, modifying the cells with an agent that induces the expression of CAMKK1, or a combination thereof. In some embodiments, the increase in the level of CAMKK1 in said organ or tissue can be achieved by administering conditioned media from a culture of cells that have been modified with a vector comprising a nucleic acid encoding CAMKK1. The vector can comprise a plasmid or a viral vector. In some aspects, the culture of cells can be modified with a plasmid comprising a nucleic acid encoding CAMKK1. In other aspects, the culture of cells can be modified with a viral vector comprising a nucleic acid encoding CAMKK1. The methods can comprise administering conditioned media from a culture of cells that have been modified with a plasmid or viral vector comprising a nucleic acid encoding wild type CAMKK1 as set forth in SEQ ID NO:1. The methods can comprise administering conditioned media from a culture of cells that have been modified with a plasmid or viral vector comprising a nucleic acid encoding CAMKK1 1-413 as set forth in SEQ ID NO:3. The methods can comprise administering conditioned media from a culture of cells that have been modified with a plasmid or viral vector comprising a nucleic acid encoding a T108A mutant CAMKK1 as set forth in SEQ ID NO:5. The methods can comprise administering conditioned media from a culture of cells that have been modified with a plasmid or viral vector comprising a nucleic acid encoding a S459A mutant CAMKK1 as set forth in SEQ ID NO:7. The methods can comprise administering conditioned

media from a culture of cells that have been modified with a plasmid or viral vector comprising a nucleic acid encoding a T108A/S459A mutant CAMKK1 as set forth in SEQ ID NO:9.

[0041] In other embodiments, the increase in the level of CAMKK1 in said organ or tissue can be achieved by administering conditioned media from a culture of cells that have been modified with an agent that induces the expression of CAMKK1. Suitable agents for inducing the expression of CAMKK1 include, but are not limited to, TGF-β, miR145, a Dab2 inhibitor, or any combination thereof. As shown in **FIG. 3**, TGF-β leads to up regulation of miR-145, miR-145 up regulation leads to down regulation of DAB2, and down regulation of DAB2 leads to up regulation of CAMKK1. In some aspects, the culture of cells can be modified with TGF-β. For example, a the culture of cells can be incubated with TGF-β for a suitable amount of time, the conditioned media or a portion thereof can be harvested, and the conditioned media can be administered to said organ or tissue. In other aspects, the culture of cells can be modified with miR145. For example, the culture of cells can be transfected with miR145, the conditioned media or a portion thereof can be harvested, and the conditioned media can be administered to said organ or tissue. The culture of cells can be transfected with miR145 as set forth in SEQ ID NO:17, the conditioned media or a portion thereof can be harvested, and the conditioned media can be administered to said organ or tissue. In other aspects, the culture of cells can be modified with a Dab2 inhibitor such as, for example, Dab2 siRNA. The culture of cells can be transfected with Dab2 siRNA, the conditioned media or a portion thereof can be harvested, and the conditioned media can be administered to said organ or tissue. The culture of cells can be transfected with Dab2 siRNA as set forth as SEQ ID NO:11 and 12, the conditioned media or a portion thereof can be harvested, and the conditioned media can be administered to said organ or tissue. The culture of cells can be transfected with Dab2 siRNA as set forth as SEQ ID NO:13 and 14, the conditioned media or a portion thereof can be harvested, and the conditioned media can be administered to said organ or tissue. The culture of cells can be transfected with Dab2 siRNA as set forth as SEQ ID NO:15 and 16, the conditioned media or a portion thereof can be harvested, and the conditioned media can be administered to said organ or tissue.

[0042] The cells that have been modified to produce an increased level of CAMKK1 or the culture of cells from which the conditioned media is obtained can be mesenchymal stem cells.

[0043] The protein, vector, cells, or conditioned media can be administered systemically, directly into the ischemic or inflamed tissue or about the periphery of the ischemic or inflamed tissue. Suitable techniques for systemic administration include enteral administration or parenteral administration (injection, infusion, or implantation). The protein,

vector, cells, or conditioned media can be administered, for example, orally, epidurally, intracerebrally, intracerebroventricularly, intraarterially, intraarticularly, intracardially, intramuscularly, intralesionally, intraperitoneally, intrathecally, intravenously, subcutaneously, or any combination thereof.

[0044] The ischemic or inflammatory condition can be acute myocardial infarction, heart failure, peripheral artery disease, stroke, liver disease, ischemic kidney disease, multiple sclerosis, traumatic brain injury, spinal cord injury, graft versus host disease (GVHD), diabetes, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, an injury from a solid organ transplant, an orthopedic injury, a cartilage disorder, a wound, or any combination thereof. Thus, the disclosed methods can be used to treat an ischemic or inflammatory condition in any of the above listed organs or tissues of a patient by inducing an increase of the level of CAMKK1 in said organ or tissue.

[0045] The disclosed methods can further comprise administering one or more additional regenerative therapies. Suitable additional regenerative therapies include, but are not limited to, mesenchymal stem cells derived from bone marrow, adipose tissue, placental tissue, umbilical cord, Wharton's Jelly, menstrual blood, stem cells, M2 macrophages, monocytes, or any combination thereof. The stem cells can be neural progenitor cells, endothelial progenitor cells, organ specific endogenous stem cells, or any combination thereof. In some aspects, the methods can further comprise administering neural progenitor cells. In some aspects, the methods can further comprise administering endothelial progenitor cells. In some aspects, the methods can further comprise administering endogenous stem cells, such as cardiac ckit+ cells. In some aspects, the methods can further comprise administering any combination of the above stem cells.

EXAMPLES

[0046] The following examples are provided to further describe some of the embodiments disclosed herein. The examples are intended to illustrate, not to limit, the disclosed embodiments.

[0047] Pretreatment of MSCs with the growth factor transforming growth factor beta (TGF-β) increased the immune suppressive and proangiogenic activity of the cells and resulted in enhanced regenerative capacity (**FIG. 2**). In a rodent model of AMI, conditioned media collected from TGF-β treated MSCs was delivered to the heart. Improved cardiac function was noted as compared to delivery of conditioned media from untreated MSCs.

[0048] Following receptor binding, TGF-β treatment of MSCs cells resulted in a decrease in the expression of disabled homolog 2 (Dab2), a TGF-β1 receptor adaptor protein, that is mediated by an increase in microRNA miR145 (**FIG. 3**). Decreasing Dab2 expression in MSCs using siRNA also resulted in improved benefit in cardiac function from conditioned media collected from transfected cells (**FIG. 2**) compared to conditioned media from untreated MSCs when delivered to the heart in a rodent model of AMI. The levels of specific chemokines and growth factors in control and Dab2:siRNA transfected MSC were compared and no differences in FGF-2, PDGF-b, IGF-1, SDF-1 or SFRP-2 were observed (data not shown).

[0049] In order to identify the molecular mechanisms which lead to the enhanced secretome of MSCs, an Illumina gene array screen was performed to identify gene changes in response to three different mechanisms of Dab-2 down-regulation: TGF-β treatment, transfection with miR145 and transfection with siRNA against Dab2 compared with untreated cells. In this experiment, cRNA samples from treated cells (TGF-β, mir145, or siRNA Dab2) or untreated cells were hybridized to the Illumina Rat-Ref12 expression BeadChip.

[0050] Briefly, the labeled cRNA samples were hybridized to the Illumina RatRef-12 expression BeadChip, which was scanned using Illumina Beadstation GX (Illumina, San Diego, CA, USA). Each BeadChip contains 22,523 probes that were selected from the National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) database. The microarray image was analyzed and intensity data were normalized using Illumina Beadstudio software (Illumina, San Diego, CA, USA).

[0051] To select differentially expressed genes, Welch two sample t-test was performed. The results were combined with fold change and detection p-values to identify differentially expressed genes. Specifically, the selection criterions include the sum of the detection p-values for the three repeats being less than or equal to 0.1, average p-values less than or equal to 0.05, and fold change between control and treated samples greater than or equal to 1.5. In addition, set operations were performed to identify commonly deregulated molecules.

[0052] Following the identification of differentially expressing genes, the dataset containing these genes and the corresponding expression values was uploaded into the Ingenuity Systems Pathway Analysis (IPA) software (Ingenuity Systems, Redwood City, CA). TGFβ1, miR-145 and Dab2 as well as these differentially expressed genes were marked as focus molecules in IPA. The focus molecules served as seeds and their relationships with other molecules in the Ingenuity Knowledge Base were identified and presented in a set of networks (directed graphs) in which the biological relationship between two molecules (nodes) is represented as a directed edge. In addition, functional and canonical pathway analyses were

carried out using IPA. The focus molecules and their closely related genes were analyzed and over-represented functional groups and canonical pathways were identified. Significance of association between these genes and a functional group or a canonical pathway was measured using the p value obtained using Fisher's exact test determining the probability that the association between the focus molecules and the group/pathway is explained by chance alone. A cutoff threshold of 0.05 was used in this study.

[0053] Twenty-three (23) common significant ($p<0.05$) differentially expressed genes (>1.5 fold difference) were identified from the treated cells compared to the untreated cells (**Table 1**). Pathway analysis identified one gene, calcium/calmodulin-dependent protein kinase kinase 1 (CAMKK1), downstream of miR145, TGF-β signaling and dab-2 downregulation, that is shown herein to modulate the MSC secretome and function. An independent experiment confirmed that downregulation of Dab2 results in upregulation of CAMKK1 expression and associated with an upregulation in CAMKK1 protein (**FIG. 4**).

Table 1 Analysis of Differentially Expressed Genes under Treatment with siRNA:Dab2, miRNA145, or TGFβ

Entrez Gene Name (Symbol)	Avg Fold Change	Avg p_value	Location
annexin A3 (ANXA3)	-22.07	0.03	Cytoplasm
Rho GDP dissociation inhibitor (GDI) beta (ARHGDIB)	-19.47	0.01	Cytoplasm
BCL2-related protein A1 (BCL2A1)	-4.31	0.00	Cytoplasm
ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1 (ATP6V1C1)	-3.83	0.02	Cytoplasm
ATP-binding cassette, sub-family B (MDR/TAP), member 9 (ABCB9)	-2.95	0.02	Cytoplasm
carbonic anhydrase III, muscle specific (CA3)	-2.54	0.01	Cytoplasm
calcium/calmodulin-dependent protein kinase kinase 1, alpha (CAMKK1)	1.72	0.01	Cytoplasm
apolipoprotein E (APOE)	-23.52	0.01	Extracellular
complement component 1, q subcomponent, C chain (C1QC)	-14.50	0.03	Extracellular
complement component 1, q subcomponent, A chain (C1QA)	-5.65	0.01	Extracellular
ADAM metallopeptidase with thrombospondin type 1 motif, 1 (ADAMTS1)	2.51	0.01	Extracellular
allograft inflammatory factor 1 (AIF1)	-15.20	0.02	Nucleus
carbonic anhydrase IX (CA9)	-10.81	0.02	Nucleus
CD68 molecule (CD68)	-20.28	0.01	Membrane
CD83 molecule (CD83)	-6.66	0.02	Membrane
arachidonate 5-lipoxygenase-activating protein (ALOX5AP)	-3.36	0.02	Membrane
arginine vasopressin receptor 1A (AVPR1A)	-2.98	0.02	Membrane
amyloid beta (A4) precursor protein (App)	-2.39	0.02	Membrane
alanine and arginine rich domain containing protein (AARD)	-27.08	0.02	unknown

ADP-ribosylation factor-like 11 (ARL11)	-6.77	0.01	unknown
Cd300 molecule-like family member E, pseudogene 1 (Cd300le-ps1)	-4.08	0.01	unknown
armadillo repeat containing, X-linked 2 (ARMCX2)	-2.78	0.01	unknown
arginine vasopressin-induced 1 (AVPI1)	-1.78	0.01	unknown

[0054] Real-time PCR was performed to confirm that the down-regulation of Dab2 leads to up-regulation of CAMKK1 from MSC in culture (data not shown). Western blot analysis confirmed that this up-regulation was associated with an increase in CAMKK1 protein expression (data not shown).

[0055] CAMKK1 has not previously been demonstrated to be involved in inducing or enhancing endogenous regenerative pathways. To demonstrate the role of CAMKK1 in modulating the MCS secretome, CAMKK1 expression was downregulated in MSCs through transfection with CAMKK1 siRNA. The data in **FIG. 5A** show that the injection of 2 million MSC or the concentrated condition media from 2 million MSC into the infarct border zone at the time of LAD ligation results in inhibition of MSC mediated preservation of left ventricular function, specifically, a 30% and 20% increase in cardiac function, respectively, 14 days after AMI. In the absence of CAMKK1, however, there is no significant increase in cardiac function in response to the injection of MSC or MSC conditioned media (**FIG. 5A**). To begin to understand how the absence of CAMKK1 altered myocardial response to MSC injection, the area of collagen deposition and vascular density in response to MSC and MSC conditioned media injections was quantified. An increase in collagen deposition (**FIG. 5B**) and a blunting in the increase in vascular density (**FIG. 5C**) was observed in response to MSC transfected with CAMKK1:siRNA compared to control MSC. These data demonstrate that modulation of CAMKK1 does alter the MSC secretome and subsequent myocardial repair through potentially multiple mechanisms. Importantly, the down-regulation of CAMKK1 in TGF-β1 pretreated MSC also rendered the conditioned from these cells inactive (data not shown).

[0056] To begin to define paracrine factors that are modulated by CAMKK1 expression in MSC, conditioned media from MSC transfected with scramble or CAMKK1:siRNA was assayed. The media was conditioned for 24 hours, beginning 24 hours after transfection. The media was concentrated as though for intracardiac injection. The concentrated conditioned media was assayed using a nylon based cytokine array. Consistent with the hypothesis that up-regulation of CAMKK1 is pro-healing, down-regulation of CAMKK1 resulted in the up-regulation of 5 cytokines, 4 of which are related to inflammatory cell recruitment.

[0057] To test whether activation of the CAMKK1 pathway can result in enhanced regenerative capacity of other cells types, a plasmid encoding CAMKK1 downstream of the

CMV promoter was developed and utilized to demonstrate that upregulation of CAMKK1 activity can result in improved cardiac function. Following induction of an AMI in rodents by LAD ligation, CAMKK1 encoding plasmid (KCP-CAMKK1) or vehicle was injected directly into the heart into the peri-infarct area. Cardiac function was measured 1 week and 2 weeks later and resulted in improvement in cardiac function as measured by echocardiography in KCP-CAMKK1 treated animals compared to vehicle (saline or glucose) treated animals (**FIG. 6**). Taken together, the data demonstrate that activation of CAMKK1 signaling pathway through overexpression of CAMKK1 results in enhanced regeneration in the heart.

[0058] To determine if the up-regulation of CAMKK1 was sufficient to induce tissue repair, studies with control and CAMKK1 over-expressing MSC and conditioned media from control and CAMKK1 over-expressing MSC were performed. The data in FIG. 8A and FIG. 8B demonstrate that the over-expression of CAMKK1 in MSC enhanced MSC and MSC conditioned media function in AMI.

[0059] Whether CAMKK1 over-expression in the absence of MSC could lead to MSC like effects on the left ventricle in the setting of AMI was evaluated by generating a cDNA expression vector that used the CMV promoter to overexpress CAMKK1. 5 x 100 ug injections of plasmid cDNA were delivered around the infarct border zone at the time of LAD ligation. LV function as a function of time after AMI was quantified. As shown in FIG. 7 CAMKK1 over-expression in the absence of MSC led to significant preservation or recovery of LV function.

[0060] Histological analyses (data not shown) of the tissue at the end of the experiments displayed in FIG. 5B and C demonstrated that CAMKK1 consistently led to:

- a decrease in infarct size and myocardial fibrosis as measured by Mason's trichrome stain;
- an increase in vascular density in the infarct border zone.

[0061] There is no effect of CAMKK1 on SDF-1, or SDF-1 on CAMKK1 expression (data not shown). The CAMKK1 approach appears to have the potential to be completely synergistic and not redundant to SDF-1.

Summary

[0062] As disclosed herein, CAMKK1 was identified as a key regulator of MSC function. The fact that the SDF-1:CXCR4 axis and CAMKK1 have no molecular overlap with respect to induced expression suggests that a combination of CAMKK1 and SDF-1 should have synergistic effects, most obviously in acute injury, and likely in chronic tissue injury.

[0063] The disclosed methods induce the enhancement of the regenerative secretome (secreted molecules) from multiple cell types, including MSCs. Without intending to be bound by theory, it is believed that the increased levels of CAMKK1 result in enhanced functional benefit through alteration of secreted factors from those cells expressing CAMKK1. This secretome induction can be used as a novel regenerative therapeutic.

[0064] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

Date Reçue/Date Received 2021-03-16

Table 2 Sequences

CAMKK1 nucleic acid (NM_032294)	ATGGAGGGGGGGTCCAGCTGTCTGCTGCCAGGATCCTCGGGCAGAGCTGGTAGAACGGGTGGCAGCCA TCGATGTGACTCACTTGGAGGAGGCAGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCC ACCACGGGCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAGCCCGGCCTAGCC TCTCAGCCAGGAAGCTTTCCCTACAGGAGCGGCCAGCAGGAAGCTATCTGGAGGCGCAGGCTGGGCC TTATGCCACGGGGCCTGCCAGCCACATCTCCCCCCGGGCCTGGCGGAGGCCCACCATCGAGTCCCACC ACGTGGCCATCTCAGATGCAGAGGACTGCGTGCAGCTGAACCAGTACAAGCTGCAGAGTGAGATTGG CAAGGGTGCCTACGGTGTGGTGAGGCTGGCCTACAACGAAAGTGAAGACAGACACTATGCAAATGAAA GTCCTTTCCAAAAAGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGGGTCCCA GGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCTGCCCCTGGAGCGGGTGTACCAGGAGATTGCCATC CTGAAGAAGCTGGACCACGTGAATGTGGTCAAACTGATCGAGGTCCTGGATGACCCAGCTGAGGACA ACCTCTATTTGGTGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCTGTGACAAGCCCTTC TCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCATCCTGGGCCTCGAGTACTTGCACTGCCAGAA GATCGTCCACAGGGACATCAAGCCATCCAACCTGCTCCTGGGGGATGATGGGCACGTGAAGATCGCC GACTTTGGCGTCAGCAACCAGTTTGAGGGGAACGACGCTCAGCTGTCCAGCACGGCGGGAACCCCAG CATTCATGGCCCCCGAGGCCATTTCTGATTCCGGCCAGAGCTTCAGTGGGAAGGCCTTGGATGTATGG GCCACTGGCGTCACGTTGTACTGCTTTGTCTATGGGAAGTGCCCATTCATCGACGATTTCATCCTGGCC CTCCACAGGAAGATCAAGAATGAGCCCGTGGTGTTCCTGAGGAGCCAGAAATCAGCGAGGAGCTCA AGGACCTGATCCTGAAGATGTTAGACAAGAATCCCGAGACGAGAATTGGGGTGCCAGACATCAAGTT GCACCCTTGGGTGACCAAGAACGGGGAGGAGCCCCTTCCTTCGGAGGAGCACTGCAGCGTGGTG GAGGTGACAGAGGAGGAGGTTAAGAACTCAGTCAGGCTCATCCCCAGCTGGACCACGGTGATCCTGG TGAAGTCCATGCTGAGGAAGCGTTCCTTTGGGAACCCGTTTGAGCCCCAAGCACGGAGGGAAGAGCG ATCCATGTCTGCTCCAGGAAACCTACTGGTGAAAGAAGGGTTTGGTGAAGGGGGCAAGAGCCCAGAG CTCCCCGGCGTCCAGGAAGACGAGGCTGCATCCTGA **(SEQ ID NO:1)**
CAMKK1 protein	MEGGPAVCCQDPRAELVERVAAIDVTHLEEADGGPEPTRNGVDPPPRARAASVIPGSTSRLLPARPSLSAR KLSLQERPAGSYLEAQAGPYATGPASHISPRAWRRPTIESHHVAISDAEDCVQLNQYKLQSEIGKGAYGVV RLAYNESEDRHYAMKVLSKKKLLKQYGFPRRPPPRGSQAAQGGPAKQLLPLERVYQEIAILKKLDHVNVV KLIEVLDDPAEDNLYLVFDLLRKGPVMEVPCDKPFSEEQARLYLRDVILGLEYLHCQKIVHRDIKPSNLLLG DDGHVKIADFGVSNQFEGNDAQLSSTAGTPAFMAPEAISDSGQSFSGKALDVWATGVTLYCFVYGKCPFI DDFILALHRKIKNEPVVFPEEPEISEELKDLILKMLDKNPETRIGVPDIKLHPWVTKNGEEPLPSEEEHCSVV EVTEEEVKNSVRLIPSWTTVILVKSMLRKRSFGNPFEPQARREERSMSAPGNLLVKEGFGEGGKSPELPGV

	QEDEAAS **(SEQ ID NO:2)**
CAMKK1 1-413 nucleic acid	ATGGAGGGGGGTCCAGCTGTCTGCTGCCAGGATCCTCGGGCAGAGCTGGTAGAACGGGTGGCAGCCA TCGATGTGACTCACTTGGAGGAGGCAGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCC ACCACGGGCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAGCCCGGCCTAGCC TCTCAGCCAGGAAGCTTTCCCTACAGGAGCGGCCAGCAGGAAGCTATCTGGAGGCGCAGGCTGGGCC TTATGCCACGGGGCCTGCCAGCCACACATCTCCCCCCGGGCCTGGCGGAGGCCCACCATCGAGTCCCACC ACGTGGCCATCTCAGATGCAGAGGACTGCGTGCAGCTGAACCAGTACAAGCTGCAGAGTGAGATTGG CAAGGGTGCCTACGGTGTGGTGAGGCTGGCCTACAACGAAAGTGAAGACAGACACTATGCAATGAAA GTCCTTTCCAAAAAGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGGGTCCCA GGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCTGCCCCTGGAGCGGGTGTACCAGGAGATTGCCATC CTGAAGAAGCTGGACCACCGTGAATGTGGTCAAACTGATCGAGGTCCTGGATGACCCAGCTGAGGACA ACCTCTATTTGGTGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCTGTGACAAGCCCTTC TCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCATCCTGGGCCTCGAGTACTTGCACTGCCAGAA GATCGTCCACAGGGACATCAAGCCATCCAACCTGCTCCTGGGGGATGATGGCACGTGAAGATCGCC GACTTTGGCGTCAGCAACCAGTTTGAGGGGGAACGACGCTCAGCTGTCCAGCACGGCGGGAACCCCAG CATTCATGGCCCCCGAGGCCATTTCTGATTCCGGCCAGAGCTTCAGTGGGAAGGCCTTGGATGTATGG GCCACTGGCGTCACGTTGTACTGCTTTGTCTATGGGAAGTGCCCATTCATCGACGATTTCATCCTGGCC CTCCACAGGAAGATCAAGAATGAGCCCGTGGTGTTTCCTGAGGAGCCAGAAATCAGCGAGGAGCTCA AGGACCTGATCCTGAAGATGTTAGACAAGAATCCCGAGACGAGAATTGGGGTGCCAGACATCAAGTT GCACCCTTGGGTGACCAAGAACGGGTGA **(SEQ ID NO:3)**
CAMKK1 1-413 protein	MEGGPAVCCQDPRAELVERVAAIDVTHLEEADGGPEPTRNGVDPPPRARAASVIPGSTSRLLPARPSLSAR KLSLQERPAGSYLEAQAGPYATGPASHISPRAWRRPTIESHHVAISDAEDCVQLNQYKLQSEIGKGAYGVV RLAYNESEDRHYAMKVLSKKKLLKQYGFPRRPPPRGSQAAQGGPAKQLLPLERVYQEIAILKKLDHVNVV KLIEVLDDPAEDNLYLVFDLLRKGPVMEVPCDKPFSEEQARLYLRDVILGLEYLHCQKIVHRDIKPSNLLLG DDGHVKIADFGVSNQFEGNDAQLSSTAGTPAFMAPEAISDSGQSFSGKALDVWATGVTLYCFVYGKCPFI DDFILALHRKIKNEPVVFPEEPEISEELKDLILKMLDKNPETRIGVPDIKLHPWVTKNG **(SEQ ID NO:4)**
CAMKK1 T108A nucleic acid	ATGGAGGGGGGGTCCAGCTGTCTGCTGCCAGGATCCTCGGGCAGAGCTGGTAGAACGGGTGGCAGCCA TCGATGTGACTCACTTGGAGGAGGCAGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCC ACCACGGGCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAGCCCGGCCTAGCC TCTCAGCCAGGAAGCTTTCCCTACAGGAGCGGCCAGCAGGAAGCTATCTGGAGGCGCAGGCTGGGCC

	TTATGCCACGGGGCCTGCCAGCCACATCTCCCCCCGGGCCTGGCGGAGGCCCGACATCGAGTCCCACC ACGTGGCCATCTCAGATGCAGAGGACTGCGTGCAGCTGAACCAGTACAAGCTGCAGAGTGAGATTGG CAAGGGTGCCTACGGTGTGGTGAGGCTGGCCTACAACGAAAGTGAAGACAGACACTATGCAATGAAA GTCCTTTCCAAAAAGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGGGTCCCA GGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCTGCCCCTGGAGCGGGTGTACCAGGAGATTGCCATC CTGAAGAAGCTGGACCACCGTGAATGTGGTCAAACTGATCGAGGTCCTGGATGACCCAGCTGAGGACA ACCTCTATTTGGTGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCTGTGACAAGCCCTTC TCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCATCCTGGGCCTCGAGTACTTGCACTGCCAGAA GATCGTCCACAGGGACATCAAGCCATCCAACCTGCTCCTGGGGGATGATGGGCACGTGAAGATCGCC GACTTTGGCGTCAGCAACCAGTTTGAGGGGAACGACGCTCAGCTGTCCAGCACGGCGGGAACCCCAG CATTCATGGCCCCCGAGGCCATTTCTGATTCCGGCCAGAGCTTCAGTGGGAAGGCCTTGGATGTATGG GCCACTGGCGTCACGTTGTACTGCTTTGTCTATGGGAAGTGCCCATTCATCGACGATTTCATCCTGGCC CTCCACAGGAAGATCAAGAATGAGCCCGTGGTGTTTCCTGAGGAGCCAGAAATCAGCGAGGAGCTCA AGGACCTGATCCTGAAGATGTTAGACAAGAATCCCGAGACGAGAATTGGGGTGCCAGACATCAAGTT GCACCCTTGGGTGACCAAGAACGGGGAGGAGCCCCCTTCCTTCGGAGGAGCACTGCAGCGTGGTG GAGGTGACAGAGGAGGAGGTTAAGAACTCAGTCAGGCTCATCCCCAGCTGGACCACGGTGATCCTGG TGAAGTCCATGCTGAGGAAGCGTTCCTTTGGGAACCCGTTTGAGCCCCAAGCACGGAGGGAAGAGCG ATCCATGTCTGCTCCAGGAAACCTACTGGTGAAAGAAGGGTTTGGTGAAGGGGGCAAGAGCCCAGAG CTCCCCGGCGTCCAGGAAGACGAGGCTGCATCCTGA **(SEQ ID NO:5)**
CAMKK1 T108A protein	MEGGPAVCCQDPRAELVERVAAIDVTHLEEADGGPEPTRNGVDPPPRARAASVIPGSTSRLLPARPSLSAR KLSLQERPAGSYLEAQAGPYATGPASHISPRAWRRPDIESHHVAISDAEDCVQLNQYKLQSEIGKGAYGVV RLAYNESEDRHYAMKVLSKKKLLKQYGFPRRPPPRGSQAAQGGPAKQLLPLERVYQEIAILKKLDHVNVV KLIEVLDDPAEDNLYLVFDLLRKGPVMEVPCDKPFSEEQARLYLRDVILGLEYLHCQKIVHRDIKPSNLLLG DDGHVKIADFGVSNQFEGNDAQLSSTAGTPAFMAPEAISDSGQSFSGKALDVWATGVTLYCFVYGKCPFI DDFILALHRKIKNEPVVFPEEPEISEELKDLILKMLDKNPETRIGVPDIKLHPWVTKNGEEPLPSEEEHCSVV EVTEEEVKNSVRLIPSWTTVILVKSMLRKRSFGNPFEPQARREERSMSAPGNLLVKEGFGEGGKSPELPGV QEDEAAS **(SEQ ID NO:6)**
CAMKK1 S459A nucleic acid	ATGGAGGGGGGTCCAGCTGTCTGCTGCCAGGATCCTCGGGCAGAGCTGGTAGAACGGGTGGCAGCCA TCGATGTGACTCACTTGGAGGAGGCAGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCCC ACCACGGGCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAGCCCGGCCTAGCC TCTCAGCCAGGAAGCTTTCCCTACAGGAGCGGCCAGCAGGAAGCTATCTGGAGGCGCAGGCTGGGCC

	TTATGCCACGGGGCCTGCCAGCCACATCTCCCCCCGGGCCTGGCGGAGGCCCACCATCGAGTCCCACC ACGTGGCCATCTCAGATGCAGAGGACTGCGTGCAGCTGAACCAGTACAAGCTGCAGAGTGAGATTGG CAAGGGTGCCTACGGTGTGGTGAGGCTGGCCTACAACGAAAGTGAAGACAGACACTATGCAATGAAA GTCCTTTCCAAAAAGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGGGTCCCA GGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCTGCCCCTGGAGCGGGTGTACCAGGAGATTGCCATC CTGAAGAAGCTGGACCACCGTGAATGTGGTCAAACTGATCGAGGTCCTGGATGACCCAGCTGAGGACA ACCTCTATTTGGTGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCTGTGACAAGCCCTTC TCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCATCCTGGGCCTCGAGTACTTGCACTGCCAGAA GATCGTCCACAGGGACATCAAGCCATCCAACCTGCTCCTGGGGGATGATGGGCACGTGAAGATCGCC GACTTTGGCGTCAGCAACCAGTTTGAGGGGAACGACGCTCAGCTGTCCAGCACGGCGGGAACCCCAG CATTCATGGCCCCCGAGGCCATTTCTGATTCCGGCCAGAGCTTCAGTGGGAAGGCCTTGGATGTATGG GCCACTGGCGTCACGTTGTACTGCTTTGTCTATGGGAAGTGCCCATTCATCGACGATTTCATCCTGGCC CTCCACAGGAAGATCAAGAATGAGCCCGTGGTGTTTCCTGAGGAGCCAGAAATCAGCGAGGAGCTCA AGGACCTGATCCTGAAGATGTTAGACAAGAATCCCGAGACGAGAATTGGGGTGCCAGACATCAAGTT GCACCCTTGGGTGACCAAGAACGGGGAGGAGCCCCTTCCTTCGGAGGAGCACTGCAGCGTGGTG GAGGTGACAGAGGAGGAGGTTAAGAACTCAGTCAGGCTCATCCCCAGCTGGACCACGGTGATCCTGG TGAAGTCCATGCTGAGGAAGCGTGACTTTGGGAACCCGTTTGAGCCCCAAGCACGGAGGGAAGAGCG ATCCATGTCTGCTCCAGGAAACCTACTGGTGAAAGAAGGGTTTGGTGAAGGGGGCAAGAGCCCAGAG CTCCCCGGCGTCCAGGAAGACGAGGCTGCATCCTGA **(SEQ ID NO:7)**
CAMKK1 S459A protein	MEGGPAVCCQDPRAELVERVAAIDVTHLEEADGGPEPTRNGVDPPPRARAASVIPGSTSRLLPARPSLSAR KLSLQERPAGSYLEAQAGPYATGPASHISPRAWRRPTIESHHVAISDAEDCVQLNQYKLQSEIGKGAYGVV RLAYNESEDRHYAMKVLSKKKLLKQYGFPRRPPPRGSQAAQGGPAKQLLPLERVYQEIAILKKLDHVNVV KLIEVLDDPAEDNLYLVFDLLRKGPVMEVPCDKPFSEEQARLYLRDVILGLEYLHCQKIVHRDIKPSNLLLG DDGHVKIADFGVSNQFEGNDAQLSSTAGTPAFMAPEAISDSGQSFSGKALDVWATGVTLYCFVYGKCPFI DDFILALHRKIKNEPVVFPEEPEISEELKDLILKMLDKNPETRIGVPDIKLHPWVTKNGEEPLPSEEEHCSVV EVTEEEVKNSVRLIPSWTTVILVKSMLRKRDFGNPFEPQARREERSMSAPGNLLVKEGFGEGGKSPELPGV QEDEAAS **(SEQ ID NO:8)**
CAMKK1 T108A/S459A nucleic acid	ATGGAGGGGGGTCCAGCTGTCTGCTGCCAGGATCCTCGGGCAGAGCTGGTAGAACGGGTGGCAGCCA TCGATGTGACTCACTTGGAGGAGGCAGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCCC ACCACGGGCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAGCCCGGCCTAGCC TCTCAGCCAGGAAGCTTTCCCTACAGGAGCGGCCAGCAGGAAGCTATCTGGAGGCGCAGGCTGGGCC

	TTATGCCACGGGGCCTGCCAGCCACATCTCCCCCCGGGCCTGGCGGAGGCCCGACATCGAGTCCCACC ACGTGGCCATCTCAGATGCAGAGGACTGCGTGCAGCTGAACCAGTACAAGCTGCAGAGTGAGATTGG CAAGGGTGCCTACGGTGTGGTGAGGCTGGCCTACAACGAAAGTGAAGACAGACACTATGCAATGAAA GTCCTTTCCAAAAAGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGGGTCCCA GGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCTGCCCCTGGAGCGGGTGTACCAGGAGATTGCCATC CTGAAGAAGCTGGACCACCGTGAATGTGGTCAAACTGATCGAGGTCCTGGATGACCCAGCTGAGGACA ACCTCTATTTGGTGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCTTGTGACAAGCCCTTC TCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCATCCTGGGCCTCGAGTACTTGCACTGCCAGAA GATCGTCCACAGGGACATCAAGCCATCCAACCTGCTCCTGGGGGATGATGGGCACGTGAAGATCGCC GACTTTGGCGTCAGCAACCAGTTTGAGGGGAACGACGCTCAGCTGTCCAGCACGGCGGGAACCCCAG CATTCATGGCCCCCGAGGCCATTTCTGATTCCGGCCAGAGCTTCAGTGGGAAGGCCTTGGATGTATGG GCCACTGGCGTCACGTTGTACTGCTTTGTCTATGGGAAGTGCCCATTCATCGACGATTTCATCCTGGCC CTCCACAGGAAGATCAAGAATGAGCCCGTGGTGTTTCCTGAGGAGCCAGAAATCAGCGAGGAGCTCA AGGACCTGATCCTGAAGATGTTAGACAAGAATCCCGAGACGAGAATTGGGGTGCCAGACATCAAGTT GCACCCTTGGGTGACCAAGAACGGGGAGGAGCCCCTTCCTTCGGAGGAGCACTGCAGCGTGGTG GAGGTGACAGAGGAGGAGGTTAAGAACTCAGTCAGGCTCATCCCCAGCTGGACCACGGTGATCCTGG TGAAGTCCATGCTGAGGAAGCGTGACTTTGGGAACCCGTTTGAGCCCCAAGCACGGAGGGAAGAGCG ATCCATGTCTGCTCCAGGAAACCTACTGGTGAAAGAAGGGTTTGGTGAAGGGGGCAAGAGCCCAGAG CTCCCCGGCGTCCAGGAAGACGAGGCTGCATCCTGA **(SEQ ID NO:9)**
CAMKK1 T108A/S459A protein	MEGGPAVCCQDPRAELVERVAAIDVTHLEEADGGPEPTRNGVDPPPRARAASVIPGSTSRLLPARPSLSAR KLSLQERPAGSYLEAQAGPYATGPASHISPRAWRRPDIESHHVAISDAEDCVQLNQYKLQSEIGKGAYGVV RLAYNESEDRHYAMKVLSKKKLLKQYGFPRRPPPRGSQAAQGGPAKQLLPLERVYQEIAILKKLDHVNVV KLIEVLDDPAEDNLYLVFDLLRKGPVMEVPCDKPFSEEQARLYLRDVILGLEYLHCQKIVHRDIKPSNLLLG DDGHVKIADFGVSNQFEGNDAQLSSTAGTPAFMAPEAISDSGQSFSGKALDVWATGVTLYCFVYGKCPFI DDFILALHRKIKNEPVVFPEEPEISEELKDLILKMLDKNPETRIGVPDIKLHPWVTKNGEEPLPSEEEHCSVV EVTEEEVKNSVRLIPSWTTVILVKSMLRKRDFGNPFEPQARREERSMSAPGNLLVKEGFGEGGKSPELPGV QEDEAAS **(SEQ ID NO:10)**
Dab2 siRNA Sequence 1 (5'->3'): Sense	GGAUUCUAUGAUGAAACUCTT **(SEQ ID NO:11)**

Antisense	GAGUUUCAUCAUAGAAUCCTG **(SEQ ID NO:12)**
Dab2 siRNA Sequence 2 (5'->3'): Sense Antisense	 GCACCAUCAAAGAAGGAAATT **(SEQ ID NO:13)** UUUCCUUCUUUGAUGGUGCTT **(SEQ ID NO:14)**
Dab2 siRNA Sequence 3 (5'->3'): Sense Antisense	 GGUGAUGGUGUAAAAUACATT **(SEQ ID NO:15)** UGUAUUUUACACCAUCACCTT **(SEQ ID NO:16)**
miR145 (NR_029686)	CACCTTGTCCTCACGGTCCAGTTTTCCCAGGAATCCCTTAGATGCTAAGATGGGGATTCCTGGAAATA CTGTTCTTGAGGTCATGGTT **(SEQ ID NO:17)**

EMBODIMENTS

The following list of embodiments is intended to complement, rather than displace or supersede, the previous descriptions.

Embodiment 1. A method of treating an ischemic or inflammatory condition in an organ or tissue of a patient, comprising inducing an increase of the level of CAMKK1 in said organ or tissue.

Embodiment 2. The method of embodiment 1, wherein said organ or tissue is heart, liver, kidney, brain, spine, lungs, small intestine, large intestine, arteries, joints, cartilage, skin, or any combination thereof.

Embodiment 3. The method of embodiment 2, wherein said organ or tissue is the heart or myocardium.

Embodiment 4. The method of any one of the previous embodiments, wherein the increase in the level of CAMKK1 in said organ or tissue is achieved by administering CAMKK1 protein to said organ or tissue.

Embodiment 5. The method of any one of the previous embodiments, wherein the increase in the level of CAMKK1 in said organ or tissue is achieved by administering a vector comprising a nucleic acid encoding CAMKK1 to said organ or tissue.

Embodiment 6. The method of embodiment 5, wherein the vector is a plasmid or a viral vector.

Embodiment 7. The method of any one of the previous embodiments, wherein the increase in the level of CAMKK1 in said organ or tissue is achieved by administering cells that have been modified to produce an increased level of CAMKK1.

Embodiment 8. The method of any one of the previous embodiments, wherein the increase in the level of CAMKK1 in said organ or tissue is achieved by administering conditioned media from a culture of cells that have been modified to have an increase of the level of CAMKK1.

Embodiment 9. The method of embodiment 7 or 8, wherein the cells have been modified with a vector comprising a nucleic acid encoding CAMKK1.

Embodiment 10. The method of embodiment 9, wherein the vector comprises a plasmid or a viral vector.

Embodiment 11. The method of embodiment 7 or 8, wherein the cells have been modified with an agent that induces the expression of CAMKK1.

Embodiment 12. The method of embodiment 11, wherein the agent is TGF-β, miR145, a Dab2 inhibitor, or any combination thereof.

Embodiment 13. The method of embodiment 12, wherein the Dab2 inhibitor is Dab2 siRNA.

Embodiment 14. The method of any one of the previous embodiments, wherein the CAMKK1 is a constitutively active CAMKK1.

Embodiment 15. The method of embodiment 14, wherein the constitutively active CAMKK1 comprises a CAMKK1 1-413 truncation.

Embodiment 16. The method of embodiment 14, wherein the constitutively active CAMKK1 comprises a T108A mutant CAMKK1, a S459A mutant CAMKK1, or a T108A/S459A mutant CAMKK1.

Embodiment 17. The method of any one of the previous embodiments, wherein the protein, vector, cells, or conditioned media are administered systemically, directly into the ischemic or inflamed tissue, or about the periphery of the ischemic or inflamed tissue.

Embodiment 18. The method of any one of the previous embodiments, wherein the cells are mesenchymal stem cells.

Embodiment 19. The method of any one of the previous embodiments, wherein the ischemic or inflammatory condition is acute myocardial infarction, heart failure, peripheral artery disease, stroke, liver disease, ischemic kidney disease, multiple sclerosis, traumatic brain injury, spinal cord injury, graft versus host disease (GVHD), diabetes, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, an injury from a solid organ transplant, an orthopedic injury, a cartilage disorder, a wound, or any combination thereof.

Embodiment 20. The method of any one of the previous embodiments, further comprising administering one or more additional regenerative therapies.

Embodiment 21. The method of embodiment 20, wherein the one or more regenerative therapies are mesenchymal stem cells derived from bone marrow, adipose tissue, placental tissue, umbilical cord, Wharton's Jelly, menstrual blood, stem cells, M2 macrophages, monocytes, or any combination thereof.

Embodiment 22. The method of embodiment 21, wherein the stem cells are neural progenitor cells, endothelial progenitor cells, organ specific endogenous stem cells, or any combination thereof.

Embodiment 23. The method of embodiment 22, wherein the organ specific endogenous stem cells are cardiac ckit+ cells.

In some aspects, embodiments of the present invention as described herein include the following items:

Item 1. Use of a composition for treating an ischemic or inflammatory condition in an organ or tissue of a patient, comprising a human calcium/calmodulin-dependent protein kinase kinase 1 (CAMKK1) protein, a vector comprising a nucleic acid encoding the CAMKK1 protein, cells which have been modified to increase the level of CAMKK1 protein, and/or conditioned media from a culture of the cells, and a carrier, to induce a repair of said organ or tissue and/or to improve said organ or tissue function, by inducing an increase of the level of CAMKK1 in said organ or tissue.

Item 2. The use according to item 1, wherein the organ or tissue is heart, liver, kidney, brain, spine, lungs, small intestine, large intestine, arteries, joints, cartilage, skin, or any combination thereof.

Item 3. The use according to item 1 or 2, wherein the organ or tissue is the heart or myocardium.

Item 4. The use according to any one of items 1-3, wherein the vector is a plasmid or a viral vector.

Item 5. The use according to any one of items 1-4, wherein the cells have been modified with the vector comprising the nucleic acid encoding the CAMKK1 protein.

Item 6. The use according to any one of items 1-5, wherein the cells have been modified to induce the expression of CAMKK1 protein by TGF-β, miR145, a Dab2 inhibitor, or any combination thereof.

Item 7. The use according to item 6, wherein the Dab2 inhibitor is Dab2 siRNA.

Item 8. The use according to any one of items 1-7, wherein the CAMKK1 protein comprises a T108A mutant CAMKK1, a S459A mutant CAMKK1, a T108A/S459A mutant CAMKK1, or the CAMKK1 protein as set forth in SEQ ID NO: 4, wherein said mutants are with reference to the CAMKK1 protein as set forth in SEQ ID NO:4.

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Item 9. The use according to any one of items 1-8, wherein the cells are mesenchymal stem cells (MSC).

Item 10. The use according to any one of items 1-9, wherein the protein, vector, cells, or conditioned media are for systemic administration, for direct administration into the ischemic or inflamed tissue, or for administration about the periphery of the ischemic or inflamed tissue.

Item 11. The use according to any one of items 1-10, wherein the ischemic or inflammatory condition is acute myocardial infarction, heart failure, peripheral artery disease, stroke, liver disease, ischemic kidney disease, multiple sclerosis, traumatic brain injury, spinal cord injury, graft versus host disease (GVHD), diabetes, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, an injury from a solid organ transplant, an orthopedic injury, a cartilage disorder, a wound, or any a combination thereof.

Item 12. The use according to any one of items 1-11, wherein the composition is for administration with one or more additional regenerative therapies.

Item 13. The use according to item 12, wherein the one or more regenerative therapies are mesenchymal stem cells derived from bone marrow, adipose tissue, placental tissue, umbilical cord, Wharton's Jelly, menstrual blood, stem cells, M2 macrophages, monocytes, or any combination thereof.

Item 14. The use according to item 13, wherein the stem cells are neural progenitor cells, endothelial progenitor cells, organ specific endogenous stem cells, or any combination thereof.

Item 15. The use according to item 14, wherein the organ specific endogenous stem cells are cardiac ckit+ cells.

Item 16. The use according to any one of items 1-15, wherein the CAMKK1 protein is a human CAMKK1 protein, human CAMKK1 1-413 truncation, human T108A mutant CAMKK1, human S459A mutant CAMKK1, or human T108A/S459A mutant CAMKK1, wherein said human CAMKK1 protein, human CAMKK1 1-413 truncation, human T108A mutant CAMKK1, human S459A mutant CAMKK1, or human T108A/S459A mutant CAMKK1 are with reference to the CAMKK1 protein as set forth in SEQ ID NO:4.

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Item 17. The use according to item 1, wherein the nucleic acid encodes a human CAMKK1 protein, human CAMKK1 1-413 truncation protein, human T108A mutant CAMKK1 protein, human S459A mutant CAMKK1 protein, or human T108A/S459A mutant CAMKK1 protein, wherein said human CAMKK1 protein, human CAMKK1 1-413 truncation protein, human T108A mutant CAMKK1 protein, human S459A mutant CAMKK1 protein, or human T108A/S459A mutant CAMKK1 protein are with reference to the CAMKK1 protein as set forth in SEQ ID NO:4.

Item 18. Use of a composition for inducing a repair of an organ or tissue or improving said organ or tissue function of a patient by modulating a secretome expression of a cultured cell or a cell of said organ or tissue by human calcium/calmodulin-dependent protein kinase kinase 1 (CAMKK1), said composition comprising:

cultured cells modified with a vector comprising a nucleic acid encoding human CAMKK1;

conditioned media from a culture of cells modified with a vector comprising a nucleic acid encoding human CAMKK1;

a vector comprising a nucleic acid encoding human CAMKK1 administered to the cell of said organ or tissue; and/or

a human CAMKK1 protein for administration to the cell of said organ or tissue and

a carrier

to modulate the secretome expression of the cultured cell or the cell of said organ or tissue to induce the repair of said organ or tissue or to improve said organ or tissue function in the patient, by inducing an increase of the level of CAMKK1 in said organ or tissue.

Item 19. The use according to item 18, wherein the organ or tissue is heart, liver, kidney, brain, spine, lungs, small intestine, large intestine, arteries, joints, cartilage, skin, or any combination thereof.

Item 20. The use according to item 18 or 19, wherein the organ or tissue is the heart or myocardium.

Item 21. The use according to any one of items 18-20, wherein the vector is a plasmid or a viral vector.

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Item 22. The use according to any one of items 18-21, wherein the human CAMKK1 protein comprises a human T108A mutant CAMKK1, a human S459A mutant CAMKK1, a human T108A/S459A mutant CAMKK1, or the CAMKK1 protein as set forth in SEQ ID NO: 4.

Item 23. The use according to any one of items 18-22, wherein the cultured cells are mesenchymal stem cells (MSC).

Item 24. The use according to any one of items 18-23, wherein the protein, vector, cells, or conditioned media are for systemic administration, for direct administration into the tissue, or for administration about the periphery of the tissue.

Item 25. The use according to any one of items 18-24, wherein the composition is for administration with one or more additional regenerative therapies.

Item 26. The use according to item 25, wherein the one or more regenerative therapies are mesenchymal stem cells derived from bone marrow, adipose tissue, placental tissue, umbilical cord, Wharton's Jelly, menstrual blood, stem cells, M2 macrophages, monocytes, or any combination thereof for use in regenerative therapies.

Item 27. The use according to item 26, wherein the stem cells are neural progenitor cells, endothelial progenitor cells, organ specific endogenous stem cells, or any combination thereof.

Item 28. The use according to item 27, wherein the organ specific endogenous stem cells are cardiac ckit+ cells.

Item 29. The use according to any one of items 1-28, wherein the human CAMKK1 protein comprises the protein as set forth in SEQ ID NO: 4.

Item 30. The use according to any one of items 1-28, wherein the human CAMKK1 protein comprises the protein as set forth in SEQ ID NO: 10.

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What is Claimed:

1. Use of a composition for treating an ischemic or inflammatory condition in an organ or tissue of a patient, comprising a human calcium/calmodulin-dependent protein kinase kinase 1 (CAMKK1) protein, a vector comprising a nucleic acid encoding the CAMKK1 protein, cells which have been modified to increase the level of CAMKK1 protein, and/or conditioned media from a culture of the cells, and a carrier, to induce a repair of said organ or tissue and/or to improve said organ or tissue function, by inducing an increase of the level of CAMKK1 in said organ or tissue.
2. The use according to claim 1, wherein the organ or tissue is heart, liver, kidney, brain, spine, lungs, small intestine, large intestine, arteries, joints, cartilage, skin, or any combination thereof.
3. The use according to claim 1 or 2, wherein the organ or tissue is the heart or myocardium.
4. The use according to any one of claims 1-3, wherein the vector is a plasmid or a viral vector.
5. The use according to any one of claims 1-4, wherein the cells have been modified with the vector comprising the nucleic acid encoding the CAMKK1 protein.
6. The use according to any one of claims 1-5, wherein the cells have been modified to induce the expression of CAMKK1 protein by TGF-β, miR145, a Dab2 inhibitor, or any combination thereof.
7. The use according to claim 6, wherein the Dab2 inhibitor is Dab2 siRNA.
8. The use according to any one of claims 1-7, wherein the CAMKK1 protein comprises a T108A mutant CAMKK1, a S459A mutant CAMKK1, a T108A/S459A mutant CAMKK1, or the CAMKK1 protein as set forth in SEQ ID NO: 4, wherein said mutants are with reference to the CAMKK1 protein as set forth in SEQ ID NO:4.
9. The use according to any one of claims 1-8, wherein the cells are mesenchymal stem cells (MSC).
10. The use according to any one of claims 1-9, wherein the protein, vector, cells, or conditioned media are for systemic administration, for direct administration into the ischemic or inflamed tissue, or for administration about the periphery of the ischemic or inflamed tissue.

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11. The use according to any one of claims 1-10, wherein the ischemic or inflammatory condition is acute myocardial infarction, heart failure, peripheral artery disease, stroke, liver disease, ischemic kidney disease, multiple sclerosis, traumatic brain injury, spinal cord injury, graft versus host disease (GVHD), diabetes, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, an injury from a solid organ transplant, an orthopedic injury, a cartilage disorder, a wound, or any a combination thereof.

12. The use according to any one of claims 1-11, wherein the composition is for administration with one or more additional regenerative therapies.

13. The use according to claim 12, wherein the one or more regenerative therapies are mesenchymal stem cells derived from bone marrow, adipose tissue, placental tissue, umbilical cord, Wharton's Jelly, menstrual blood, stem cells, M2 macrophages, monocytes, or any combination thereof.

14. The use according to claim 13, wherein the stem cells are neural progenitor cells, endothelial progenitor cells, organ specific endogenous stem cells, or any combination thereof.

15. The use according to claim 14, wherein the organ specific endogenous stem cells are cardiac ckit+ cells.

16. The use according to any one of claims 1-15, wherein the CAMKK1 protein is a human CAMKK1 protein, human CAMKK1 1-413 truncation, human T108A mutant CAMKK1, human S459A mutant CAMKK1, or human T108A/S459A mutant CAMKK1, wherein said human CAMKK1 protein, human CAMKK1 1-413 truncation, human T108A mutant CAMKK1, human S459A mutant CAMKK1, or human T108A/S459A mutant CAMKK1 are with reference to the CAMKK1 protein as set forth in SEQ ID NO:4.

17. The use according to claim 1, wherein the nucleic acid encodes a human CAMKK1 protein, human CAMKK1 1-413 truncation protein, human T108A mutant CAMKK1 protein, human S459A mutant CAMKK1 protein, or human T108A/S459A mutant CAMKK1 protein, wherein said human CAMKK1 protein, human CAMKK1 1-413 truncation protein, human T108A mutant CAMKK1 protein, human S459A mutant CAMKK1 protein, or human T108A/S459A mutant CAMKK1 protein are with

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reference to the CAMKK1 protein as set forth in SEQ ID NO:4.

18. Use of a composition for inducing a repair of an organ or tissue or improving said organ or tissue function of a patient by modulating a secretome expression of a cultured cell or a cell of said organ or tissue by human calcium/calmodulin-dependent protein kinase kinase 1 (CAMKK1), said composition comprising:

    cultured cells modified with a vector comprising a nucleic acid encoding human CAMKK1;

    conditioned media from a culture of cells modified with a vector comprising a nucleic acid encoding human CAMKK1;

    a vector comprising a nucleic acid encoding human CAMKK1 administered to the cell of said organ or tissue; and/or

    a human CAMKK1 protein for administration to the cell of said organ or tissue and

    a carrier

    to modulate the secretome expression of the cultured cell or the cell of said organ or tissue to induce the repair of said organ or tissue or to improve said organ or tissue function in the patient, by inducing an increase of the level of CAMKK1 in said organ or tissue.

19. The use according to claim 18, wherein the organ or tissue is heart, liver, kidney, brain, spine, lungs, small intestine, large intestine, arteries, joints, cartilage, skin, or any combination thereof.

20. The use according to claim 18 or 19, wherein the organ or tissue is the heart or myocardium.

21. The use according to any one of claims 18-20, wherein the vector is a plasmid or a viral vector.

22. The use according to any one of claims 18-21, wherein the human CAMKK1 protein comprises a human T108A mutant CAMKK1, a human S459A mutant CAMKK1, a human T108A/S459A mutant CAMKK1, or the CAMKK1 protein as set forth in SEQ ID NO: 4.

23. The use according to any one of claims 18-22, wherein the cultured cells are mesenchymal stem cells (MSC).

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24. The use according to any one of claims 18-23, wherein the protein, vector, cells, or conditioned media are for systemic administration, for direct administration into the tissue, or for administration about the periphery of the tissue.

25. The use according to any one of claims 18-24, wherein the composition is for administration with one or more additional regenerative therapies.

26. The use according to claim 25, wherein the one or more regenerative therapies are mesenchymal stem cells derived from bone marrow, adipose tissue, placental tissue, umbilical cord, Wharton's Jelly, menstrual blood, stem cells, M2 macrophages, monocytes, or any combination thereof for use in regenerative therapies.

27. The use according to claim 26, wherein the stem cells are neural progenitor cells, endothelial progenitor cells, organ specific endogenous stem cells, or any combination thereof.

28. The use according to claim 27, wherein the organ specific endogenous stem cells are cardiac ckit+ cells.

29. The use according to any one of claims 1-28, wherein the human CAMKK1 protein comprises the protein as set forth in SEQ ID NO: 4.

30. The use according to any one of claims 1-28, wherein the human CAMKK1 protein comprises the protein as set forth in SEQ ID NO: 10.

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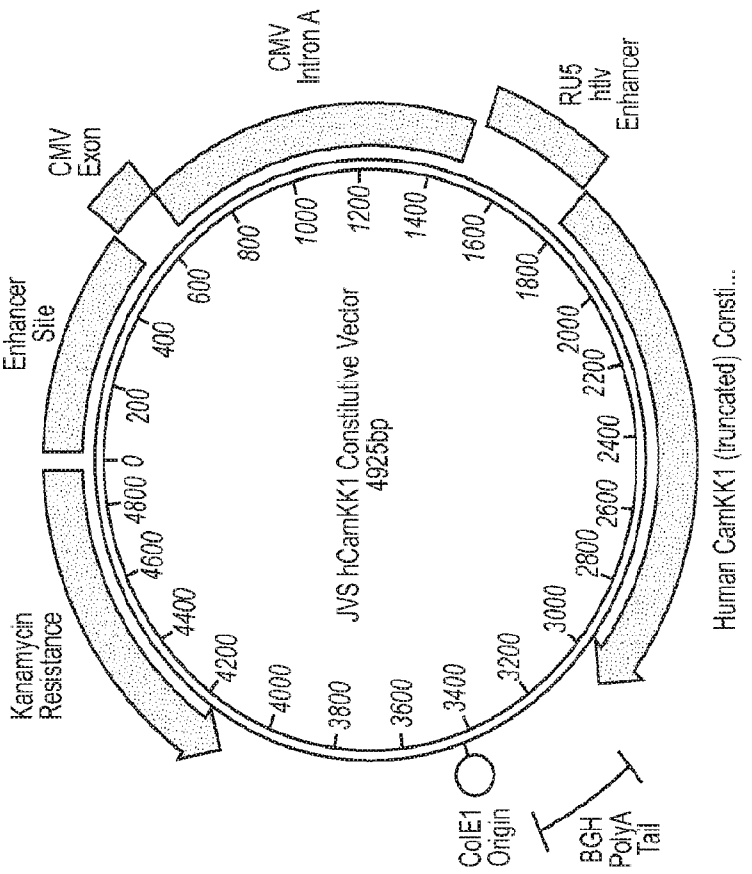

CMV Intron A
CMV Exon
CMV Enhancer Site
RU5 hllv Enhancer
Kanamycin Resistance
JVS hCamKK1 Constitutive Vector 4925bp
Human CamKK1 (truncated) Consti...
ColE1 Origin
BGH PolyA Tail
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
4000
4200
4400
4600
4800

FIG. 1B

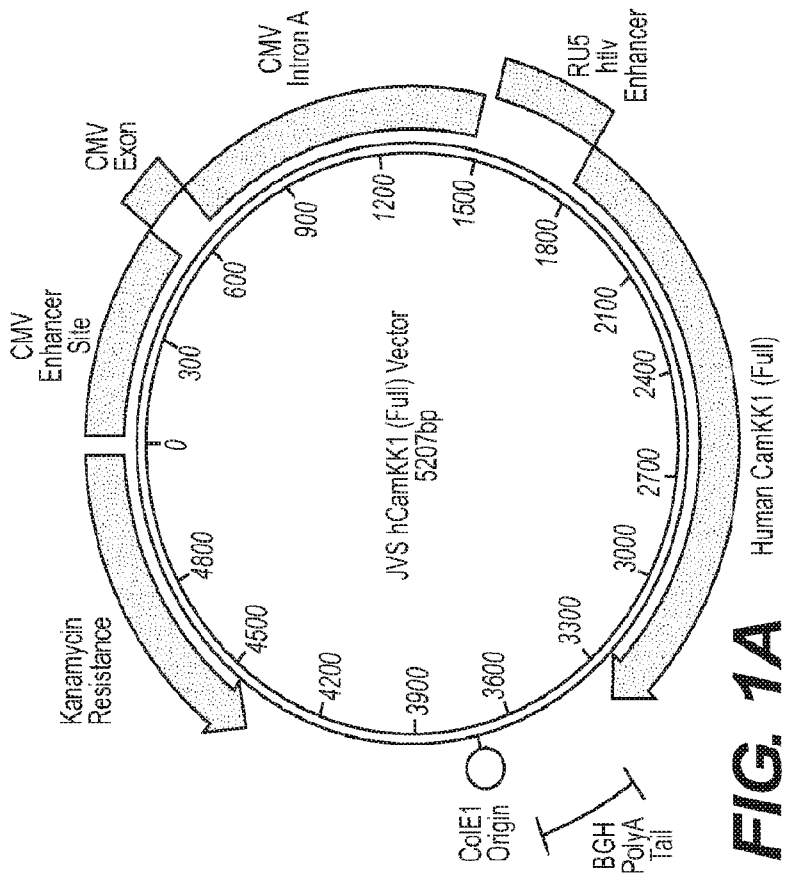

CMV Intron A
CMV Exon
CMV Enhancer Site
RU5 hllv Enhancer
Kanamycin Resistance
JVS hCamKK1 (Full) Vector 5207bp
Human CamKK1 (Full)
ColE1 Origin
BGH PolyA Tail
0
300
600
900
1200
1500
1800
2100
2400
2700
3000
3300
3600
3900
4200
4500
4800

FIG. 1A

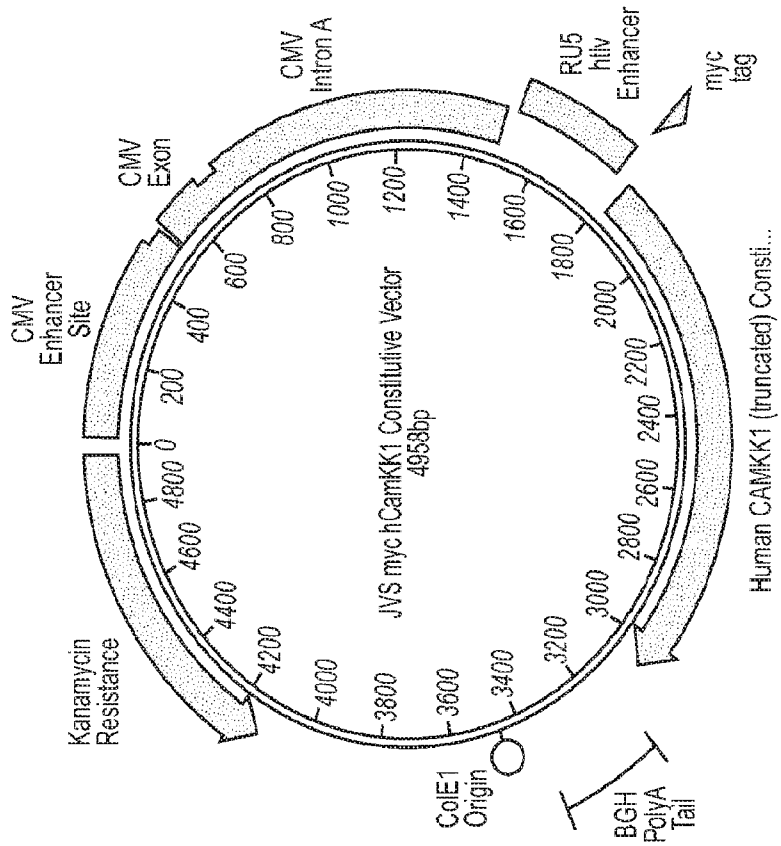

JVS myc hCamKK1 Constitutive Vector
4959bp
CMV Enhancer Site
CMV Exon
CMV Intron A
RU5 htlv Enhancer
myc tag
Human CAMKK1 (truncated) Consti...
Kanamycin Resistance
ColE1 Origin
BGH PolyA Tail

FIG. 1D

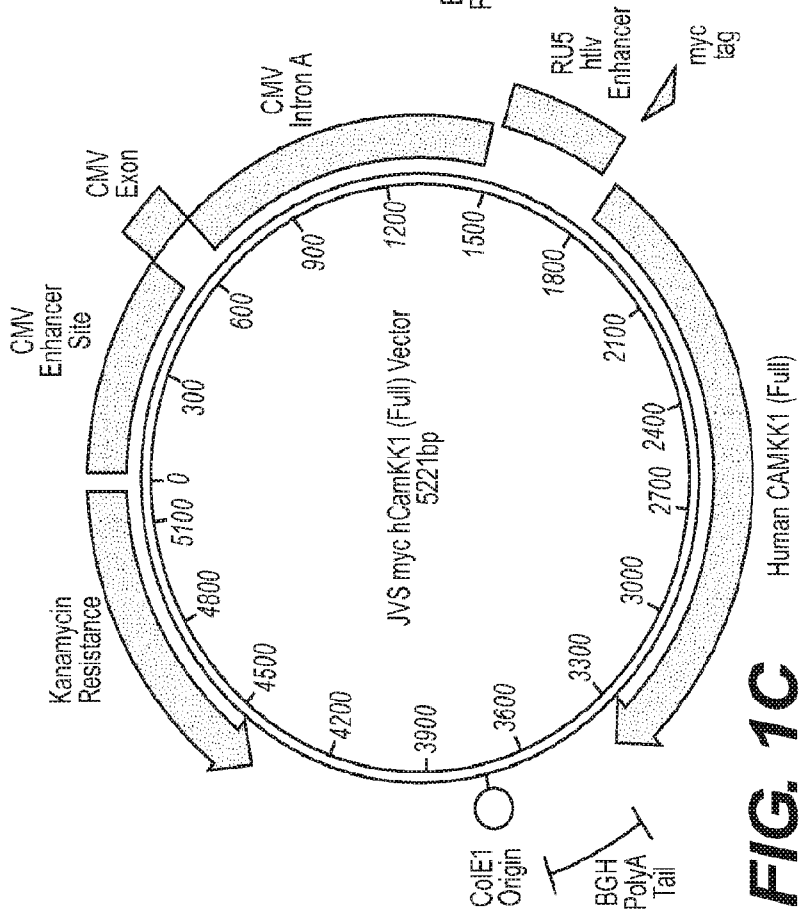

JVS myc hCamKK1 (Full) Vector
5221bp
CMV Enhancer Site
CMV Exon
CMV Intron A
RU5 htlv Enhancer
myc tag
Human CAMKK1 (Full)
Kanamycin Resistance
ColE1 Origin
BGH PolyA Tail

FIG. 1C

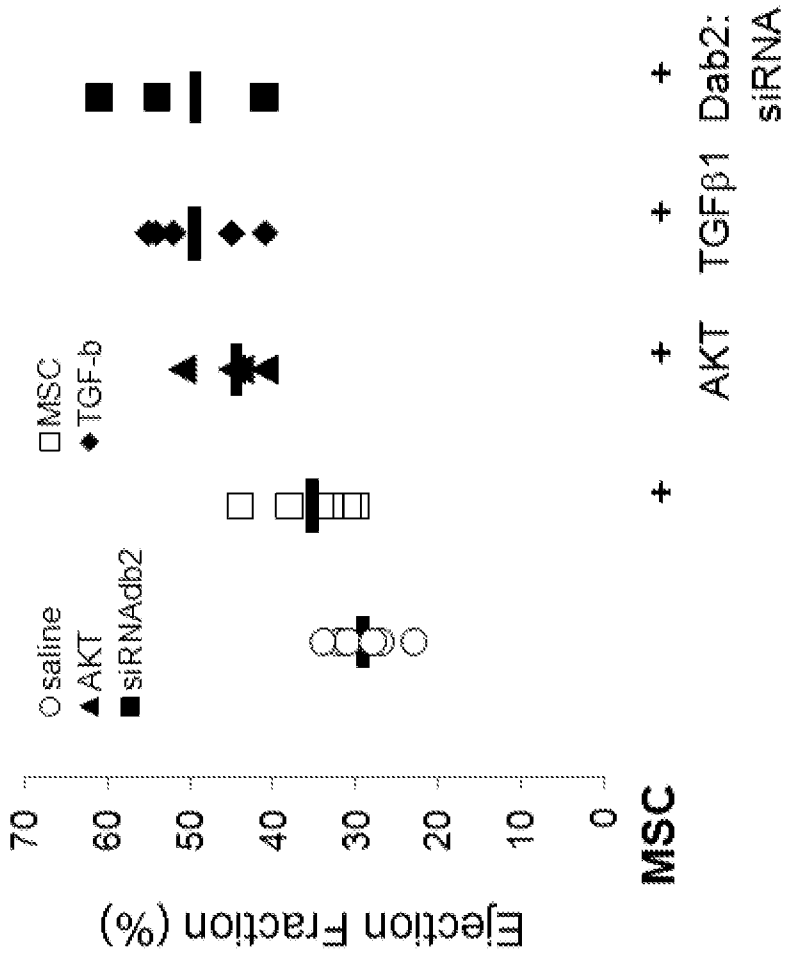

Ejection Fraction (%)
70
60
50
40
30
20
10
0
saline
AKT
siRNAdb2
MSC
TGF-b
MSC
+
+
+
+
AKT
TGFβ1
Dab2: siRNA

FIG. 2

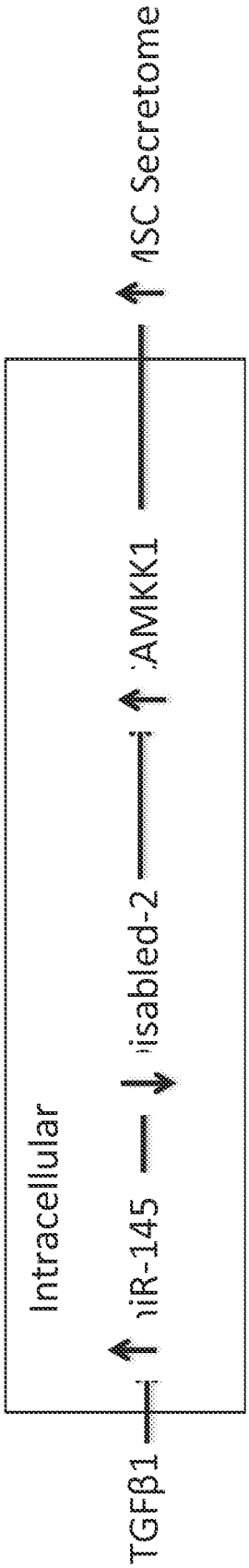

TGFβ1
miR-145
Disabled-2
AMKK1
MSC Secretome
Intracellular

FIG. 3

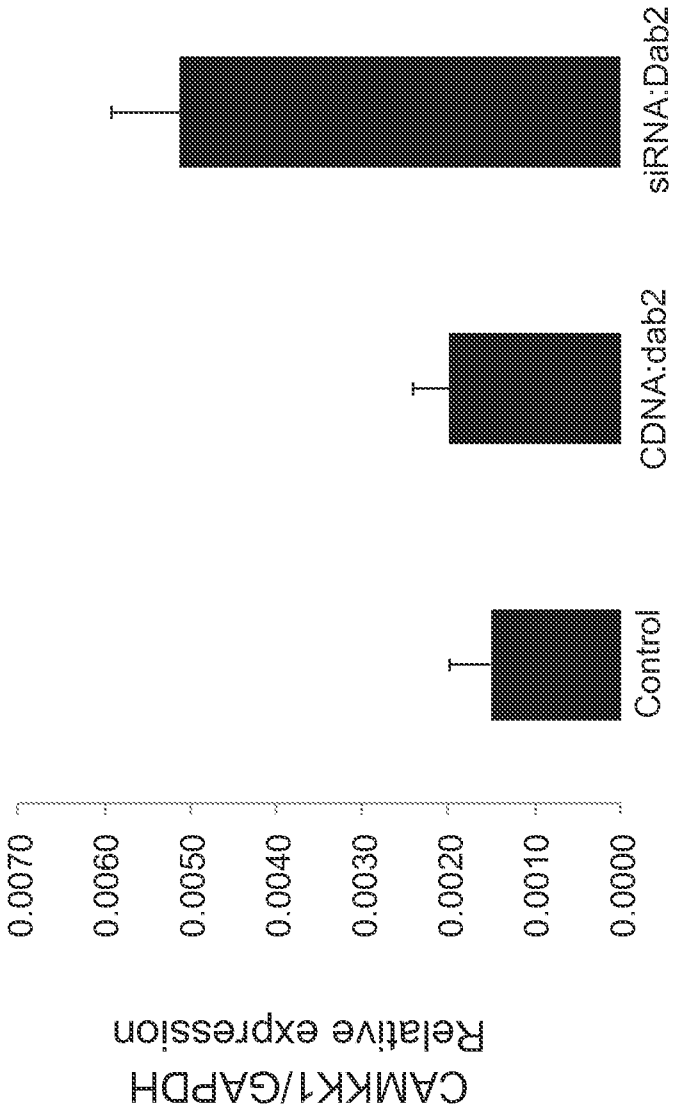

CAMKK1/GAPDH
Relative expression
0.0070
0.0060
0.0050
0.0040
0.0030
0.0020
0.0010
0.0000
Control
CDNA:dab2
siRNA:Dab2

FIG. 4

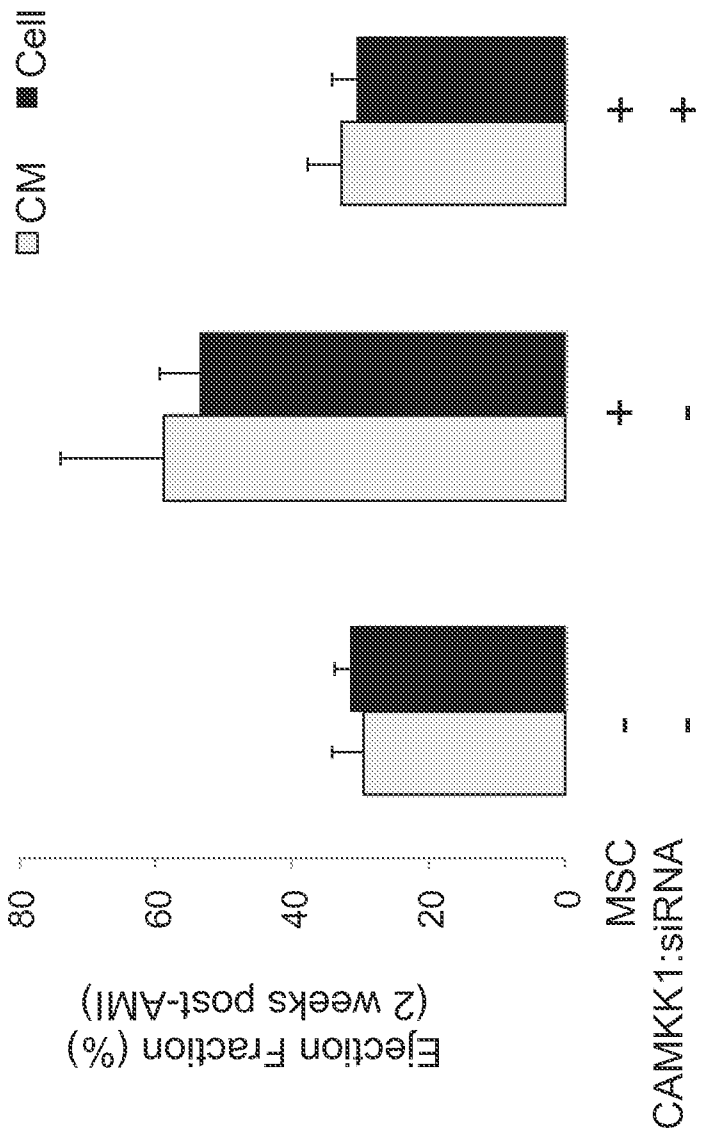

CM
Cell
Ejection Fraction (%)
(2 weeks post-AMI)
80
60
40
20
0
MSC
CAMKK1:siRNA
-
-
+
-
+
+

FIG. 5A

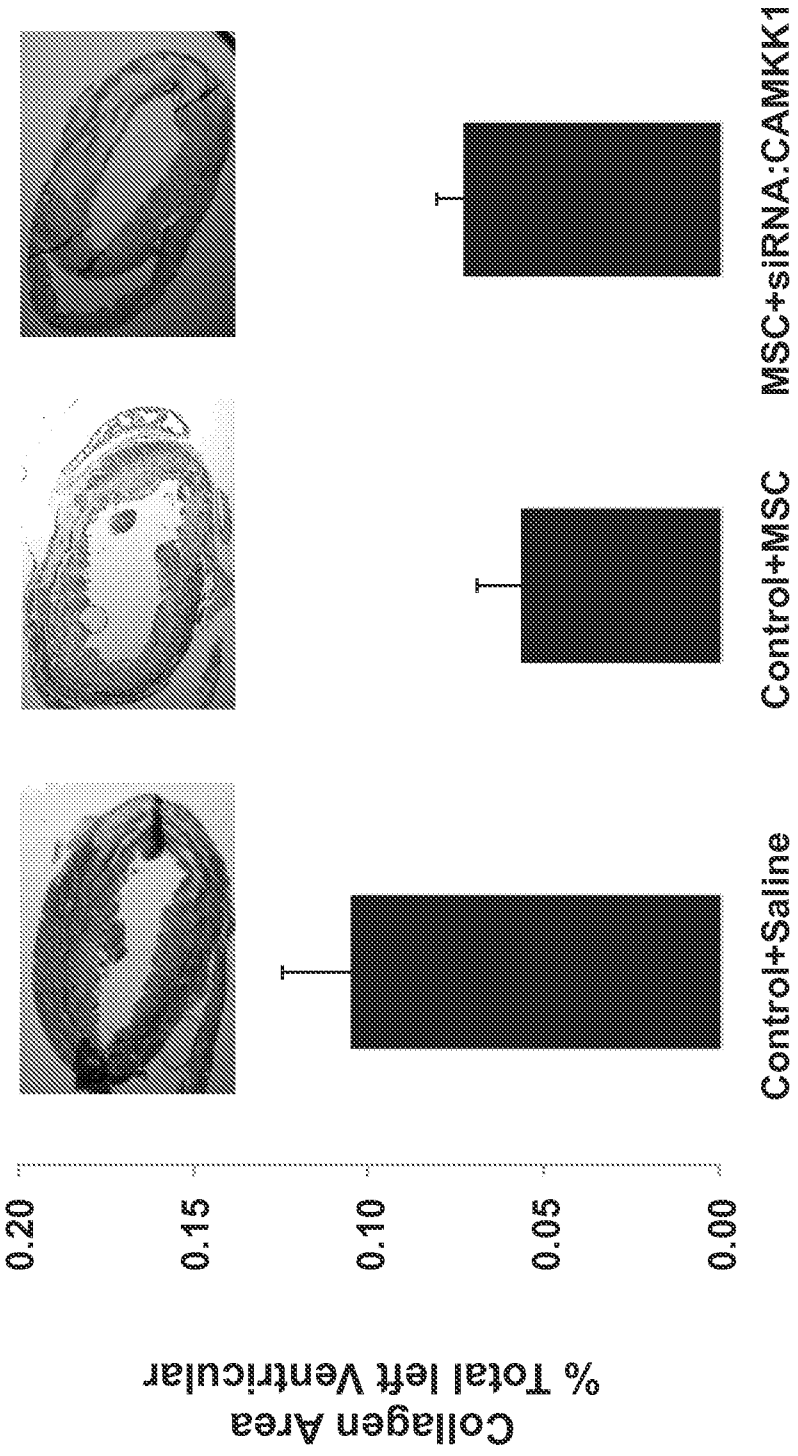

Collagen Area
% Total left Ventricular
0.20
0.15
0.10
0.05
0.00
Control+Saline
Control+MSC
MSC+siRNA:CAMKK1

FIG. 5B

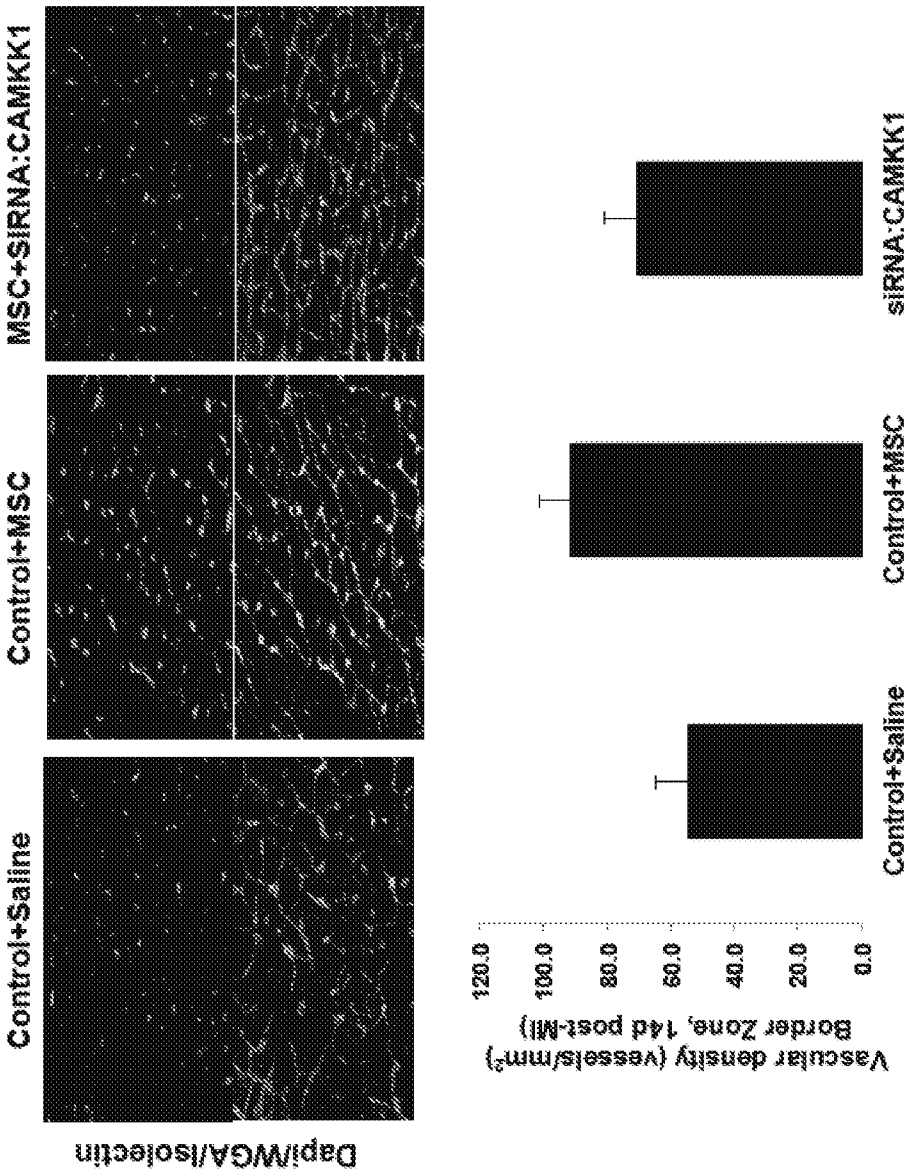

Control+Saline
Control+MSC
MSC+SIRNA:CAMKK1
Dapi/WGA/Isolectin
Vascular density (vessels/mm²)
Border Zone, 14d post-MI)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Control+Saline
Control+MSC
siRNA:CAMKK1

FIG. 5C

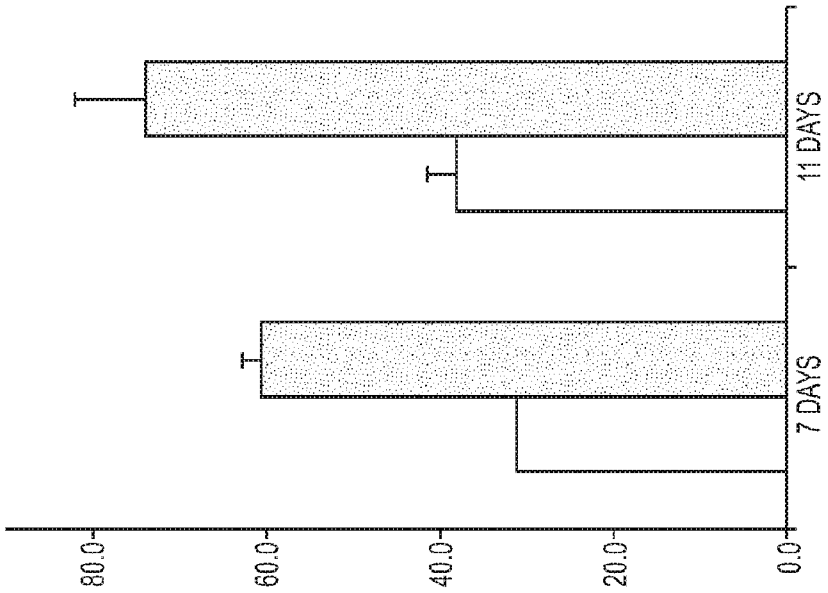


FIG. 6

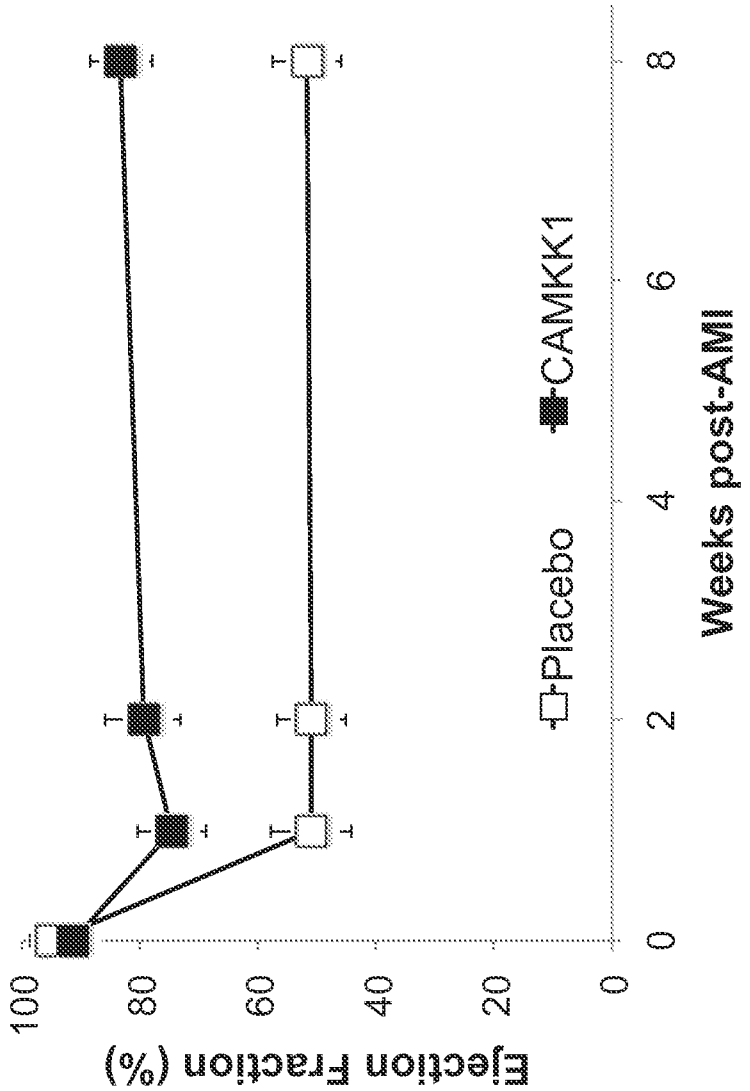

Ejection Fraction (%)
100
80
60
40
20
0
0
2
4
6
8
Weeks post-AMI
Placebo
CAMKK1

FIG. 7

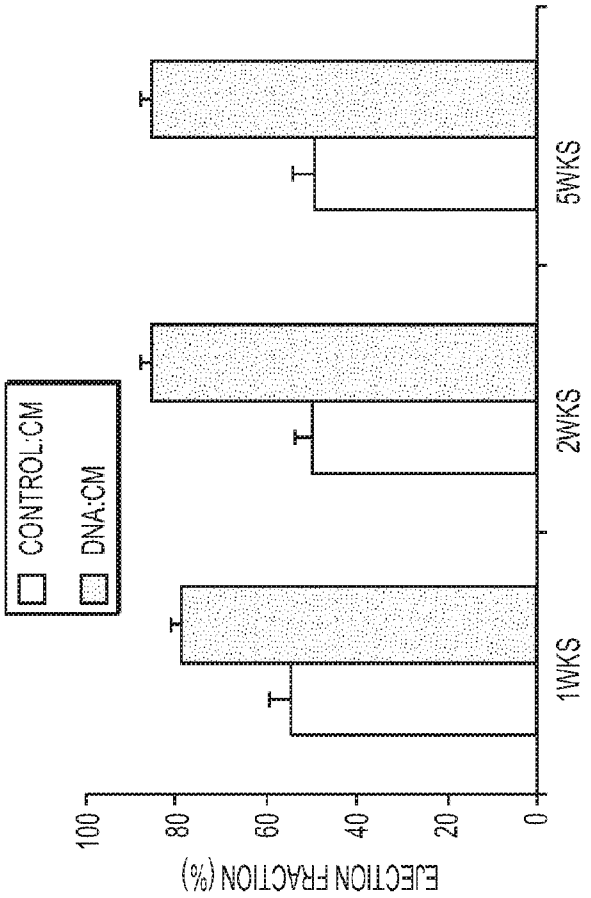

CONTROL:CM
DNA:CM
EJECTION FRACTION (%)
100
80
60
40
20
0
1WKS
2WKS
5WKS
WEEKS AFTER AMI

FIG. 8B

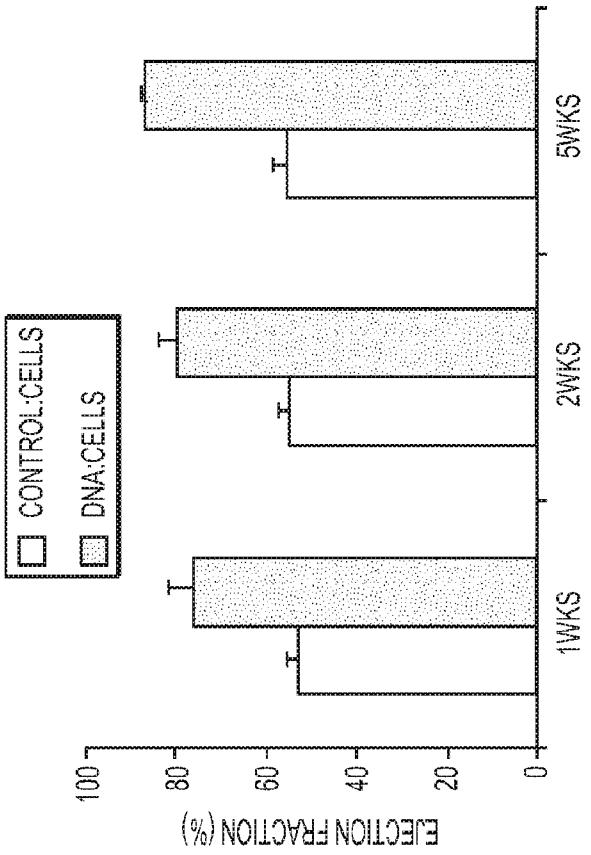
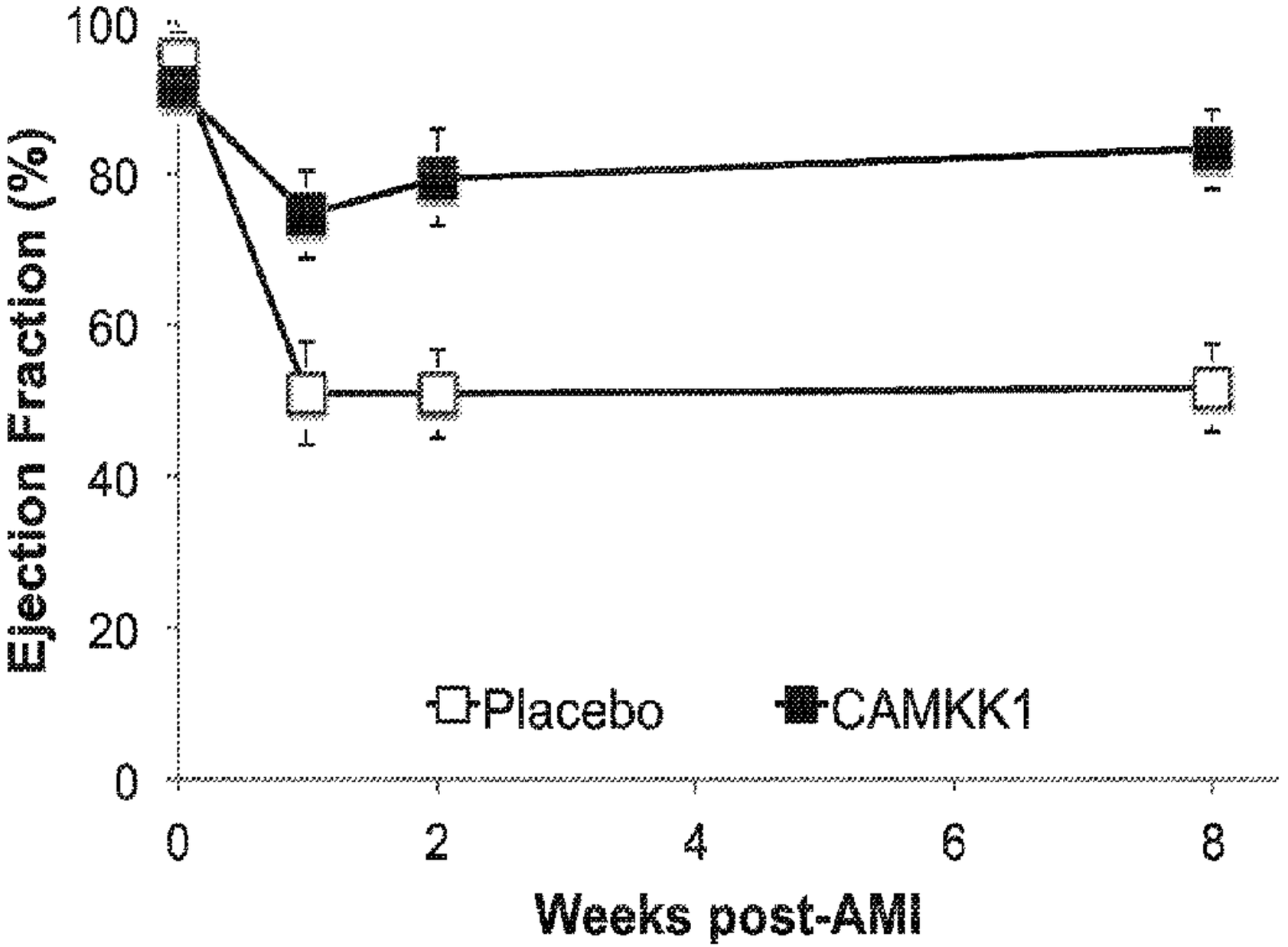

CONTROL:CELLS
DNA:CELLS
EJECTION FRACTION (%)
100
80
60
40
20
0
1WKS
2WKS
5WKS
WEEKS AFTER AMI

FIG. 8A


100
80
60
40
20
0
Ejection Fraction (%)
Placebo
CAMKK1
0
2
4
6
8
Weeks post-AMI