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DESCRIPTION

[0001] The U.S. Government through the National Institute of Health provided financial assistance for this project under NIH/NHLBI Grant Number R01 HL58027. Therefore, the United States Government may own certain rights to this invention.

Field of the Invention

[0002] The invention is in the fields of cell and molecular biology, polypeptides, drug discovery, and therapeutic methods of use.

Summary of the Invention

[0003] The present invention provides a polypeptide consisting of the amino acid sequence YARAAARQARAKALNRQLAVAA or YARAAARQARAKALNRQLGVA, as well as biomedical devices and compositions comprising the same.

[0004] The present disclosure includes a polypeptide comprising or consisting of a sequence according to general formula I:

Z1-X1-X2-X3-X4 X5-X6-X7-X8-X9-X10-Z2

wherein Z1 and Z2 are independently absent or are transduction domains;

X1 is selected from the group consisting of KA, KKA, and KKKA, or is absent;

X2 is selected from the group consisting of G, L, A, V, I, M, Y, W, and F, or is an aliphatic amino acid;

X3 is selected from the group consisting of V, L, I, A, G, Q, N, S, T, and C, or is an aliphatic amino acid;

X4 is selected from the group consisting of Q, N, H, R and K;

X5 is selected from the group consisting of Q and N;

X6 is selected from the group consisting of C, A, G, L, V, I, M, Y, W, and F or is an aliphatic amino acid;

X7 is selected from the group consisting of S, A, C, T, and G or is an aliphatic amino acid;

X8 is selected from the group consisting of V, L, I, and M;

X9 is absent or is any amino acid; and

X10 is absent or is any amino acid;

wherein at least one of the following is true:

1. (a) X3 is N and X6 is not G;
2. (b) X6 is G and X3 is not N;
3. (c) X2 is not L;
4. (d) X4 is not R;
5. (e) X5 is not Q;
6. (f) X6 is not L;
7. (g) X8 is not V;
8. (h) X10 is absent; or
9. (i) X9 and X10 are absent; and wherein when X1 is absent, then Z1 is a transduction domain ending in KA.

[0005] In various further disclosures, X4 is R; X5 is Q, and/or X8 is V. In various further disclosures, X3 is selected from the group consisting of A, G, Q, and N.

[0006] In one disclosure, at least one of Z1 and Z2 is a transduction domain.

In another aspect, the present invention provides compositions, comprising the polypeptide YARAAARQARAKALNRQLAVAA or YARAAARQARAKALNRQLGVA and a pharmaceutically acceptable carrier.

[0007] The disclosure includes isolated nucleic acid sequences encoding a polypeptide of the present invention. In further disclosures, the present invention discloses recombinant expression vectors comprising the disclosed nucleic acid sequences, and host cells transfected with the recombinant expression vectors.

[0008] In another aspect, the invention provides biomedical devices, wherein the biomedical devices comprise the polypeptide YARAAARQARAKALNRQLAVAA or YARAAARQARAKALNRQLGVA disposed on or in the biomedical device. In various embodiments, such biomedical devices are selected from the group consisting of stents, grafts, shunts, stent grafts, angioplasty devices, balloon catheters, fistulas, wound dressings, and any implantable drug delivery device.

[0009] Also disclosed are methods for one or more of the following therapeutic uses (a) reducing smooth muscle cell proliferation and/or migration; (b) promoting smooth muscle relaxation; (c) increasing the contractile rate in heart muscle; (d) increasing the rate of heart muscle relaxation; (e) promoting wound healing; (f) treating and/or reducing fibrotic disorders and/or keloids.; (g) reducing scar formation; (h) disrupting focal adhesions; (i) regulating actin polymerization; and (j) treating or reducing incidence of one or more of intimal hyperplasia, stenosis, restenosis, atherosclerosis, smooth muscle cell tumors and metastasis, smooth muscle spasm, angina, Prinzmetal's angina (coronary vasospasm), ischemia, stroke, bradycardia, hypertension, cardiac hypertrophy and other end-organ damage associated with hypertension (including but not limited to renal failure and stroke), pulmonary (lung) hypertension, asthma (bronchospasm), toxemia of pregnancy, pre-term labor, pre-eclampsia/eclampsia, Raynaud's disease or phenomenon, hemolytic-uremia, non-occlusive mesenteric ischemia, anal fissure, achalasia, impotence, migraine, ischemic muscle injury associated with smooth muscle spasm, vasculopathy, such as transplant vasculopathy; bradyarrythmia, bradycardia, congestive heart failure, stunned myocardium, pulmonary hypertension, diastolic dysfunction, gliosis; chronic obstructive pulmonary disease, osteopenia, endothelial dysfunction, and inflammation; wherein the method comprises administering to a subject in need thereof an effective amount to carry out the one or more therapeutic uses of one or more inventive or disclosed polypeptides or compositions, or functional equivalents thereof.

DETAILED DESCRIPTION OF THE INVENTION

[0010] Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: Molecular Cloning: A Laboratory Manual (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), Gene Expression Technology (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in Methods in Enzymology (M.P. Deutshcer, ed., (1990) Academic Press, Inc.); PCR Protocols: A Guide to Methods and Applications (Innis, et al. 1990. Academic Press, San Diego, CA), Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed. (R.I. Freshney. 1987. Liss, Inc. New York, NY), and Gene Transfer and Expression Protocols, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.)

[0011] The single letter designation for amino acids is used predominately herein. As is well known by one of skill in the art, such single letter designations are as follows: A is alanine; C is cysteine; D is aspartic acid; E is glutamic acid; F is phenylalanine; G is glycine; H is histidine; I is isoleucine; K is lysine; L is leucine; M is methionine; N is asparagine; P is proline; Q is gluatamine; R is arginine; S is serine; T is threonine; V is valine; W is tryptophan; and Y is tyrosine.

[0012] As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. For example, reference to a "polypeptide" means one or more polypeptides.

[0013] The present invention provides a polypeptide consisting of the amino acid sequence YARAAARQARAKALNRQLAVAA or YARAAARQARAKALNRQLGVA, as well as biomedical devices and compositions comprising the same.

[0014] The present disclosure includes a polypeptide comprising or consisting of a sequence according to general formula I:

Z1-X1-X2-X3-X4 X5-X6-X7-X8-X9-X10-Z2

wherein Z1 and Z2 are independently absent or are transduction domains;

X1 is selected from the group consisting of KA, KKA, and KKKA, or is absent;

X2 is selected from the group consisting of G, L, A, V, I, M, Y, W, and F, or is an aliphatic amino acid;

X3 is selected from the group consisting of V, L, I, A, G, Q, N, S, T, and C, or is an aliphatic amino acid;

X4 is selected from the group consisting of Q, N, H, R and K;

X5 is selected from the group consisting of Q and N;

X6 is selected from the group consisting of C, A, G, L, V, I, M, Y, W, and F or is an aliphatic amino acid;

X7 is selected from the group consisting of S, A, C, T, and G or is an aliphatic amino acid;

X8 is selected from the group consisting of V, L, I, and M;

X9 is absent or is any amino acid; and

X10 is absent or is any amino acid;

wherein at least one of the following is true:

1. (a) X3 is N and X6 is not G;
2. (b) X6 is G and X3 is not N;
3. (c) X2 is not L;
4. (d) X4 is not R;
5. (e) X5 is not Q;
6. (f) X6 is not L;
7. (g) X8 is not V;
8. (h) X10 is absent; or
9. (i) X9 and X10 are absent; and

wherein when X1 is absent, then Z1 is a transduction domain ending in KA..

[0015] In addition to the recited amino acids, X2, X3, X6 and X7 can be any aliphatic amino acid (whether naturally occurring or not), including but not limited to beta-alanine and 2-aminocyclohexane-1-carboxylic acid.

[0016] In various further disclosures, X4 is R; X5 is Q, and/or X8 is V. In various further disclosures, X3 is selected from the group consisting of V, L, I, A, G, Q, and N. In further disclosures, X6 is selected from the group consisting of C, A, G, L, V, I, M, Y, W, and F. In various further disclosures, X7 is selected from the group consisting of S, A, C, T, and G.

[0017] In one disclosure, at least one of Z1 and Z2 are a transduction domain.

[0018] Thus, according to these various disclosures, polypeptides according to general formula I include, but are not limited to the following:

Z1-X1-X2-X3-**K**-X5-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-X3-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-X3-**R-N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-X3-**R-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-X3-R-N-X6-X7-V-X9-X10-Z2

Z1-X1-X2-X3-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-X3-**K-Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-X3-**K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-X3-**K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-X3-**K-N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-X3-**K-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A-R**-X5-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**A-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**A**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-A-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**A**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A-R-Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**A-R**-X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A-R-Q**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A-R-N-**X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**A-R-N-**X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A**-X4-**Q**-X6-X7-**V**-X9-X10-22

Z1-X1-X2-**A-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**A-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**A-K-N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**A-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G-R-**X5-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**G-K-**X5-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**G**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**G**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**G**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G-R-Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**G-R**-X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G-R-Q**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G-R-N**-X6-X7-X8-X9-X10-Z2

Z1-Xl-X2-**G-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2**-G-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G**-X4-**Q**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**G-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**G-K-N-**X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**G-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**Q-R**-X5-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**Q-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**Q**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**Q**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**Q**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**Q-R-Q-**X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**Q-R-**X5-X6-X7-**V**-X9-X0-Z2

Z1-X1-X2-**Q-R-Q**-X6-X7-V-X9-X10-Z2

Z1-X1-X2-**Q-R-N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**Q-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**Q-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**Q**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**Q**-X4-**Q**-X6-X7-**V**-X9-X10-22

Z1-X1-X2-**Q-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**Q-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**Q-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**Q-K-N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**Q-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**N-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**N**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**N-R-N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**N-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**N-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**N**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**N-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**N-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**N-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-X1-X2-**N-K-N**-X6-X7-X8-X9-X10-Z2

Z1-X1-X2-**N-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-X3-**K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-X3-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-X3-**R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-X3-**R-N**-X6-X7-V-X9-X10-Z2

Z1-**KKKA**-X2-X3-**R-N**-X6-X7-V-X9-X10-Z2

Z1-**KKKA**-X2-X3-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-X3-**K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-X3-**K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA-**X2-X3-**K-Q-**X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-X3-**K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-X3-**K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A-R**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**A-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**A**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**A**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**A**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A-R-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**A-R**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A-R-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A-R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**A-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A**-X4-**Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**A-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**A-K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**A-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G-R**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**G-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**G**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**G**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**G**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G-R-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**G-R**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G-R-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G-R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**G-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G**-X4-**Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA-**X2-**G-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**G-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**G-K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**G-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**Q-R**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KKKA-**X2-**Q-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**Q**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**Q**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**Q**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**Q-R-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**Q-R**-X5-X6-X7-V-X9-X10-Z2

Z1-**KKKA**-X2-**Q-R-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**Q-R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA-**X2-**Q-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**Q-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**Q**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**Q**-X4-**Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA-**X2-**Q-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**Q-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**Q-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**Q-K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA-**X2-**Q-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**N-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**N**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**N-R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**N-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**N-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**N**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**N-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**N-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**N-K-Q-**X6-X7-**V**-X9-X10-Z2

Z1-**KKKA**-X2-**N-K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KKKA**-X2-**N-K-N**-X6-X7-V-X9-X10-Z2

Z1-**KA**-X2-X3-**K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-X3-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-X3-**R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA-**X2-X3-**R-N-**X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-X3-**R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-X3-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-X3-**K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-X3-**K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-X3-**K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-X3-**K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-X3-**K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**A-R**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**A-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**A**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**A**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**A**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**A-R-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**A-R**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**A-R-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**A-R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**A-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**A-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**A**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA-**X2-**A**-X4-**Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA-**X2-**A-K-Q-**X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**A-K-**X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**A-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**A-K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**A-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA-**X2-**G-R-**X5-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**G-K-**X5-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**G**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**G**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**G**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G-R-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**G-R**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G-R-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G-R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**G-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G**-X4-**Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**G-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**G-K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**G-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q-R**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**Q-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**Q**-X4-**Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**Q**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**Q**-X4 X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q-R-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**Q-R**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q-R-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q-R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**Q-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q**-X4-**Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**Q-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**Q-K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**Q-K-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**N-K**-X5-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**N**-X4-**N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**N-R-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**N-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**N-R-N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**N**-X4-**N**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**N-K-Q**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**N-K**-X5-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**N-K-Q**-X6-X7-**V**-X9-X10-Z2

Z1-**KA**-X2-**N-K-N**-X6-X7-X8-X9-X10-Z2

Z1-**KA**-X2-**N-K-N**-X6-X7-**V**-X9-X10-Z2

[0019] The polypeptides YARAAARQARAKALNRQLAVAA and YARAAARQARAKALNRQLGVA are useful, for example, as HSP27 kinase inhibitors, which can be used as therapeutic agents for a variety of disorders, as disclosed in more detail-below.

[0020] The term "polypeptide" is used in its broadest sense to refer to a sequence of subunit amino acids, amino acid analogs, or peptidomimetics. The subunits are linked by peptide bonds, except where noted. The polypeptides described herein may be chemically synthesized or recombinantly expressed.

[0021] Preferably, the inventive or disclosed polypeptides are chemically synthesized. Synthetic polypeptides, prepared using the well known techniques of solid phase, liquid phase, or peptide condensation techniques, or any combination thereof, can include natural and unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (Nα-amino protected N-α-t-butyloxycarbonyl) amino acid resin with the standard deprotecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield (1963, J. Am. Chem. Soc. 85:2149-2154), or the base-labile Nα-amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids first described by Carpino and Han (1972, J. Org. Chem. 37:3403-3409). Both Fmoc and Boc Nα-amino protected amino acids can be obtained from Sigma, Cambridge Research Biochemical, or other chemical companies familiar to those skilled in the art. In addition, the polypeptides can be synthesized with other Nα-protecting groups that are familiar to those skilled in this art.

[0022] Solid phase peptide synthesis may be accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, 1984, Solid Phase Synthesis, Second Edition, Pierce Chemical Co., Rockford, Ill.; Fields and Noble, 1990, Int. J. Pept. Protein Re. 35:161-214, or using automated synthesizers. The polypeptides of the invention may comprise D-amino acids (which are resistant to L-amino acid-specific proteases in vivo), a combination of D- and L-amino acids, and various "designer" amino acids (e.g., β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids, etc.) to convey special properties. Synthetic amino acids include ornithine for lysine, and norleucine for leucine or isoleucine.

[0023] In addition, the polypeptides can have peptidomimetic bonds, such as ester bonds, to prepare peptides with novel properties. For example, a peptide may be generated that incorporates a reduced peptide bond, i.e., R_1CH_2-NH-R_2, where R_1 and R_2 are amino acid residues or sequences. A reduced peptide bond may be introduced as a dipeptide subunit. Such a polypeptide would be resistant to protease activity, and would possess an extended half-live in vivo.

[0024] In one disclosure, at least one of Z1 and Z2 is a transduction domain. As used herein, the term "transduction domain" means one or more amino acid sequence or any other molecule that can carry the active domain across cell membranes. These domains can be linked to other polypeptides to direct movement of the linked polypeptide across cell membranes. In some cases the transducing molecules do not need to be covalently linked to the active polypeptide. In a preferred embodiment, the transduction domain is linked to the rest of the polypeptide via peptide bonding. (See, for example, Cell 55:1179-1188,1988; Cell 55: 1189-1193, 1988; Proc Natl Acad Sci USA 91: 664-668, 1994; Science 285: 1569-1572, 1999; J Biol Chem 276: 3254-3261, 2001; and Cancer Res 61: 474-477, 2001) In a further disclosure, both X1 and X3 are transduction domains. In a further disclosure, the transduction domain(s) is/are selected from the group consisting of $(R)_{4-9}$; GRKKRRQRRRPPQ; RQRRKKRG; GRKKRRQR; AYARAAARQARA; DAATATRGRSAASRPTERPRAPARSASRPRRPVE; GWTLNSAGYLLGLINLKALAALAKKIL; PLSSIFSRIGDP; AAVALLPAVLLALLAP; AAVLLPVLLAAP; VTVLALGALAGVGVG; GALFLGWLGAAGSTMGAWSQP; GWTLNSAGYLLGLINLKALAALAKKIL; KLALKLALKALKAALKLA; KETWWETWWTEWSQPKKKRKV; KAFAKLAARLYRKA; KAFAKLAARLYRAA; AAFAKLAARLYRKA;KAFAALAARLYRKA;KAFAKLAARLYRKAGC; KAFAKLAARLYRAAGC; AAFAKLAARLYRKAGC; KAFAALAARLYRKAGC; KAFAKLAAQLYRKAGC, AGGGGYGRKKRRQRRR, and YARAAARQARA, YGRKKRRQRRR, WLRRIKAWLRRIKA; and WLRRIKAWLRRIKAWLRRIKA.

[0025] Further exemplary polypeptides include, but are not limited to any of those listed above, wherein one or both of Z1 and Z2 are selected from the group consisting of WLRRIKAWLRRIKA; WLRRIKAWLRRIKAWLRRIKA; YGRKKRRQRRR; YARAAARQARA; RQRRKKRG; and GRKKRRQR as well as
;

YGRKKRRQRRRKALNRQLGVA;

GRKKRRQRKALNRQLGVA;

RQRRKKRGKALNRQLGVA;

WLRRIKAWLRRIKAKALNRQLGVA;

WLRRIKAWLRRIKAWLRRIKAKALNRQLGVA.

YARAAARQARAKKKALNRQLGVA;

YGRKKRRQRRRKKKALNRQLGVA;

RQRRKKRGKKKALNRQLGVA;

GRKKRRQRKKKALNRQLGVA;

WLRRIKAWLRRIKAKKKALNRQLGVA;

WLRRIKAWLRRIKAWLRRIKAKKKALNRQLGVA;

YARAAARQARAKKKALNRGLGVA;

YGRKKRRQRRRKKKALNRGLGVA;

RQRRKKRGKKKALNRGLGVA;

GRKKRRQRKKKALNRGLGVA;

WLRRIKAWLRRIKAKKKALNRGLGVA;

WLRRIKAWLRRIKAWLRRIKAKKKALNRGLGVA;

YARAAARQARAKKKALNRQLAVA;

YGRKKRRQRRRKKKALNRQLAVA;

RQRRKKRGKKKALNRQLAVA;

GRKKRRQRKKKALNRQLAVA;

WLRRIKAWLRRIKAKKKALNRQLAVA;

WLRRIKLAWLRRIKAWLRRIKAKKKALNRQLAVA;

YARAAARQARAKKKALARQLGVA;

YGRKKRRQRRRKKKALARQLGVA;

RQRRKKRGKKKALARQLGVA;

GRKKRRQRKKKALARQLGVA;

WLRRIKAWLRRIKAKKKALARQLGVA;

WLRRI KAWLRRI KAWLRRI KAKKKALARQLGVA;

YARAAARQARAKALNRGLGVA;

YGRKKRRQRRRKALNRGLGVA;

RQRRKKRGKALNRGLGVA;

GRKKRRQRKALNRGLGVA;

WLRRIKAWLRRIKAKALNRGLGVA;

WLRRIKAWLRRIKAWLRRIKAKALNRGLGVA;

YARAAARQARAKALNRQLAVA;

YGRKKRRQRRRKALNRQLAVA;

RQRRKKRGKALNRQLAVA;

GRKKRRQRKALNRQLAVA;

WLRRIKAWLRRIKAKALNRQLAVA;

WLRRIKAWLRRIKAWLRRIKAKALNRQLAVA;

YARAAARQARAKALARQLGVA;

YGRKKRRQRRRKALARQLGVA;

RQRRKKRGKALARQLGVA;

GRKKRRQRKALARQLGVA;

WLRRIKAWLRRIKAKALARQLGVA;

WLRRIKAWLRRIKAWLRRIKAKALARQLGVA;

YARAAARQARAKKKALNRGLGVAA;

YGRKKRRQRRRKKKALNRGLGVAA;

RQRRKKRGKKKALNRGLGVAA;

GRKKRRQRKKKALNRGLGVAA;

WLRRIKAWLRRIKAKKKALNRGLGVAA;

WLRRIKAWLRRIKAWLRRIKAKKKALNRGLGVAA;

YARAAARQARAKKKALNRQLAVAA;

YGRKKRRQRRRKKKALNRQLAVAA;

RQRRKKRGKKKALNRQLAVAA;

GRKKRRQRKKKALNRQLAVAA;

WLRRIKAWLRRIKAKKKALNRQLAVAA;

WLRRIKAWLRRIKAWLRRIKAKKKALNRQLAVAA;

YARAAARQARAKKKALARQLGVAA;

YGRKKRRQRRRKKKALARQLGVAA;

RQRRKKRGKKKALARQLGVAA;

GRKKRRQRKKKALARQLGVAA;

WLRRIKAWLRRIKAKKKALARQLGVAA;

WLRRIKAWLRRIKAWLRRIKAKKKALARQLGVAA;

YARAAARQARAKALNRGLGVAA;

YGRKKRRQRRRKALNRGLGVAA;

RQRRKKRGKALNRGLGVAA;

GRKKRRQRKALNRGLGVAA;

WLRRIKAWLRRIKAKALNRGLGVAA;

WLRRIKAWLRRIKAWLRRIKAKALNRGLGVAA;
;

YGRKKRRQRRRKALNRQLAVAA;

RQRRKKRGKALNRQLAVAA;

GRKKRRQRKALNRQLAVAA;

WLRRIKAWLRRIKAKALNRQLAVAA;

WLRRIKAWLRRIKAWLRRIKAKALNRQLAVAA;

YARAAARQARAKALARQLGVAA;

YGRKKRRQRRRKALARQLGVAA;

RQRRKKRGKALARQLGVAA;

GRKKRRQRKALARQLGVAA;

WLRRIKAWLRRIKAKALARQLGVAA; and

WLRRI KAWLRRI KAWLRRI KAKALARQLGVAA.

[0026] In another aspect, the present invention provides compositions, comprising the polypeptide YARAAARQARAKALNRQLAVAA or YARAAARQARAKALNRQLGVA, and a pharmaceutically acceptable carrier. Such pharmaceutical compositions are especially useful for carrying out the disclosed methods below. For administration, the polypeptides are ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidine, dextran sulfate, heparin-containing gel or non-gel compositions or coatings, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds of this invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art. The polypeptides may be linked to other compounds to promote an increased half-life in vivo, such as polyethylene glycol. Such linkage can be covalent or non-covalent as is understood by those of skill in the art.

[0027] The polypeptides may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (e.g., solutions, suspensions, or emulsions). The polypeptides of the invention may be applied in a variety of solutions. Suitable solutions for use in accordance with the invention are sterile, dissolve sufficient amounts of the polypeptides, and are not harmful for the proposed application.

[0028] The present disclosure includes an isolated nucleic acid encoding a polypeptide of the present invention. Appropriate nucleic acids according to this disclosure will be apparent to one of skill in the art based on the disclosure provided herein and the general level of skill in the art.

[0029] The present disclosure includes an expression vector comprising DNA control sequences operably linked to the disclosed isolated nucleic acids, as disclosed above. "Control sequences" operably linked to the nucleic acids are nucleic acid sequences capable of effecting the expression of the disclosed nucleic acids. The control sequences need not be contiguous with the nucleic acids, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed

sequences can be present between a promoter sequence and the nucleic acid and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors can be of any type known in the art, including but not limited to plasmid and viral-based expression vectors.
The present disclosure includes genetically engineered host cells comprising the disclosed expression vectors. Such host cells can be prokaryotic cells or eukaryotic cells, and can be either transiently or stably transfected, or can be transduced with viral vectors.

[0030] In another aspect, the invention provides biomedical devices comprising the polypeptides YARAAARQARAKALNRQLAVAA or YARAAARQARAKALNRQLGVA disposed on or in the biomedical device. As used herein, a "biomedical device" refers to a device to be implanted into a subject, for example, a human being, in order to bring about a desired result. Particularly preferred biomedical devices according to this aspect of the invention include, but are not limited to, stents (including but not limited to coronary stents), grafts (including but not limited to vascular grafts), shunts, stent grafts, fistulas, angioplasty devices, balloon catheters, venous catheters, implantable drug delivery devices, adhesion barriers (including but not limited to carboxymethylcellulose, hyaluronic acid, and PTFE sheets) to separate tissue, wound dressings such as films (e.g., polyurethane films), hydrocolloids (hydrophilic colloidal particles bound to polyurethane foam), hydrogels (cross-linked polymers containing about at least 60% water), other viscous liquids and hydrogel-like species (including but not limited to, those disclosed in US 20030190364), foams (hydrophilic or hydrophobic), calcium alginates (nonwoven composites of fibers from calcium alginate), cellophane, pluronics (ie: poly(ethylene glycol)-block-poly(propylene glycol), and biological polymers.

[0031] As used herein, the term "grafts" refers to both natural and prosthetic grafts and implants. In a preferred embodiment, the graft is a vascular graft.

[0032] As used herein, the term "stent" includes the stent itself, as well as any sleeve or other component that may be used to facilitate stent placement.

[0033] As used herein, "disposed on or in" means that the one or more polypeptides can be either directly or indirectly in contact with an outer surface, an inner surface, or embedded within the biomedical device. "Direct" contact refers to disposition of the polypeptides directly on or in the device, including but not limited to soaking a biomedical device in a solution containing the one or more polypeptides, spin coating or spraying a solution containing the one or more polypeptides onto the device, implanting any device that would deliver the polypeptide, and administering the polypeptide through a catheter directly on to the surface or into any organ.

[0034] "Indirect" contact means that the one or more polypeptides do not directly contact the biomedical device. For example, the one or more polypeptides may be disposed in a matrix, such as a gel matrix (such as a heparin coating) or a viscous fluid, which is disposed on the biomedical device. Such matrices can be prepared to, for example, modify the binding and release properties of the one or more polypeptides as required. In one non-limiting example, a heparin coating is disposed on the biomedical device (such as a poly(tetrafluoroethylene) (PTFE) vascular device or sheet) and the one or more polypeptides are disposed on or in a heparin coating; in this example, the one or more polypeptides can be delivered to a subject in need thereof in a controlled manner. In one non-limiting example, the release of the one or more polypeptides from interstitial surfaces of poly(tetrafluoroethylene) (PTFE) vascular devices or sheets can be controlled by first adsorbing or bonding heparin to the surface and/or interstices of the PTFE device followed by adsorption of polypeptide. Alternating layers of heparin and the polypeptide can also be used to increase the polypeptide dose and/or time of release. Under physiological conditions within the body, the kinetics of the association and dissociation of polypeptides disclosed herein to and from heparin will lead to a delayed release profile as compared to release of the polypeptide from a bare PTFE device. In addition, the release profile can be further altered through changes in local temperature, pH or ionic strength. Such controlled release is of great value for use in the various therapeutic treatments for which the biomedical devices can be used, as discussed below.

[0035] Heparin coatings on various medical devices are known in the art. Applications in humans include central venous catheters, coronary stents, ventricular assist devices, extracorporeal blood circuits, blood sampling devices, and vascular grafts. Such coatings can be in a gel or non-gel form. As used herein "heparin coating" includes heparin adsorbed to the surface, heparin bonded to the surface, and heparin imbedded in the PTFE polymer surface. An example of a method for bonding the heparin would be to use ammonia plasma to treat, for example, a PTFE surface and reacting the resultant amines with oxidized heparin. Layer-by-layer buildup of the heparin and one or more polypeptides could then be used to increase polypeptide on the surface and expand the delivery time. Gel forms of the heparin coating can include, but are not limited to, any hydrogel containing heparin either covalently or physically bound to the gel. The heparin coating is disposed on the biomedical device, which includes direct contact with an outer surface or an inner surface of the biomedical device, or embedded within the biomedical device. "Direct" contact refers to disposition directly on or in the device, including but not limited to soaking a biomedical device in a

heparin coating solution (wherein the polypeptides may be added as part of the heparin coating solution, or may be subsequently disposed on or in the heparin coating after it is contacted with the device), spin coating or spraying a heparin coating solution onto the device (wherein the polypeptides may be added as part of the heparin coating solution, or may be subsequently disposed on or in the heparin coating after it is contacted with the device), and administering the heparin coating solution containing the polypeptides through a catheter directly on to the surface or into any organ. The physical characteristics and specific composition of the heparin layer can be any that provides the desired release profile of the one or more polypeptides. See, for example, Seal and Panitch, Biomacromolecules 2003(4): 1572-1582 (2003); US20030190364; and Carmeda BioActive Surface (CBAS™) the product of Carmeda AB in Stockholm, Sweden. "Indirect" contact means that the heparin coating is not directly in contact with the device such as, for example, when an intervening coating is placed between the device surface and the heparin coating. In one non-limiting example, the one or more polypeptides could be initially adsorbed (directly or indirectly), and then adsorbing a heparin coating; this can optionally be followed by subsequent polypeptide layers, heparin layers, or combinations thereof, as desired. As will be understood by those of skill in the art, any sulfated polysaccharide or negatively charged polymer can be used in like manner to heparin as described above, to provide desired release characteristics.

[0036] The present disclosure includes methods for one or more of the following therapeutic uses

1. (a) reducing smooth muscle cell proliferation and/or migration; (b) promoting smooth muscle relaxation; (c) increasing the contractile rate in heart muscle; (d) increasing the rate of heart muscle relaxation; (e) promoting wound healing; (f) treating and/or reducing fibrotic disorders and/or keloids.; (g) reducing scar formation; (h) disrupting focal adhesions; (i) regulating actin polymerization; and (j) treating or reducing incidence of one or more of intimal hyperplasia, stenosis, restenosis, atherosclerosis, smooth muscle cell tumors and metastasis, smooth muscle spasm, angina, Prinzmetal's angina (coronary vasospasm), ischemia, stroke, bradycardia, hypertension, cardiac hypertrophy and other end-organ damage associated with hypertension (including but not limited to renal failure and stroke), pulmonary (lung) hypertension, asthma (bronchospasm), toxemia of pregnancy, pre-term labor, pre-eclampsia/eclampsia, Raynaud's disease or phenomenon, hemolytic-uremia, non-occlusive mesenteric ischemia, anal fissure, achalasia, impotence, migraine, ischemic muscle injury associated with smooth muscle spasm, vasculopathy, such as transplant vasculopathy; bradyarrythmia, bradycardia, congestive heart failure, stunned myocardium, pulmonary hypertension, diastolic dysfunction, gliosis (proliferation of astrocytes, and may include deposition of extracellular matrix, including but not limited to such proliferation and ECM deposition in damaged areas of the central nervous system; chronic obstructive pulmonary disease (COPD) (ie, respiratory tract diseases characterized by airflow obstruction or limitation; includes but is not limited to chronic bronchitis and emphysema), bone resorption (osteopenia) associated with aging or immobilization (which leads to bone fractures); limiting endothelial dysfunction, and inflammation;wherein the method comprises administering to a subject in need thereof an effective amount to carry out the one or more therapeutic uses of one or more inventive or disclosed polypeptides or compositions or functional equivalents thereof.

[0037] While not being bound by any specific mechanism, the inventors believe that the polypeptides YARAAARQARAKALNRQLAVAA and YARAAARQARAKALNRQLGVA provide their therapeutic effect as a result of inhibiting HSP27 phosphorylation by HSP27 kinase (MAPKAP2), although alternative mechanisms, including but not limited to inhibition of HSP27 phosphorylation by MAPKAP3, and MAPKAP5 are also encompassed by the present invention.

[0038] Since MAPKAP2 is downstream of p38 MAP kinase, any therapeutic uses for which p38 MAPK inhibitors are useful are within the scope of the present disclosure as well.

[0039] In one disclosure, the individual is a mammal; in another disclosure, the individual is a human.

[0040] As used herein, "treat" or "treating" means accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting development of symptoms characteristic of the disorder(s) being treated; (c) limiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting recurrence of symptoms in patients that were previously symptomatic for the disorder(s).

[0041] As used herein, the term "reduce" or "reducing" means to limit occurrence of the disorder in individuals at risk of developing the disorder.

[0042] As used herein, "administering" includes in vivo administration, as well as administration directly to tissue ex vivo, such as vein grafts.

[0043] Intimal hyperplasia is a complex process that leads to graft failure, and is the most common cause of failure of arterial bypass grafts. While incompletely understood, intimal hyperplasia is mediated by a sequence of events that include endothelial cell injury and subsequent vascular smooth muscle proliferation and migration from the media to the intima. This process is associated with a phenotypic modulation of the smooth muscle cells from a contractile to a synthetic phenotype. The "synthetic" smooth muscle cells secrete extracellular matrix proteins, which leads to pathologic narrowing of the vessel lumen leading to graft stenoses and ultimately graft failure. Such endothelial cell injury and subsequent smooth muscle cell proliferation and migration into the intima also characterize restenosis, most commonly after angioplasty to clear an obstructed blood vessel.

[0044] In some disclosures of the methods of the invention, such as those relating to reducing occurrence of smooth muscle cell proliferation and/or migration, or promoting smooth muscle relaxation, the administering may be direct, by contacting a blood vessel in a subject being treated with one or more polypeptides of the invention. For example, a liquid preparation of one or more polypeptides according to the invention can be forced through a porous catheter, or otherwise injected through a catheter to the injured site, or a gel or viscous liquid containing the one or more polypeptides according to the invention can be spread on the injured site. In these disclosures of direct delivery, it is most preferred that the one or more inventive or disclosed polypeptides be delivered into smooth muscle cells at the site of injury or intervention. This can be accomplished, for example, by delivering the recombinant expression vectors (most preferably a viral vector, such as an adenoviral vector) of the invention to the site. More preferably, delivery into smooth muscle cells is accomplished by using the one or more inventive or disclosed polypeptides that include at least one transduction domain to facilitate entry into the smooth muscle cells.

[0045] In various other disclosures of the methods, particularly those that involve reducing occurrence of smooth muscle cell proliferation and/or migration, the method is performed on a subject who has undergone, is undergoing, or will undergo a procedure selected from the group consisting of angioplasty, vascular stent placement, endarterectomy, atherectomy, bypass surgery (such as coronary artery bypass surgery; peripheral vascular bypass surgeries), vascular grafting, organ transplant, prosthetic device implanting, microvascular reconstructions, plastic surgical flap construction, and catheter emplacement.

[0046] In another disclosure, the methods comprise treating or reducing occurrence of one or more disorder selected from the group consisting of intimal or neointimal hyperplasia, stenosis, restenosis, and atherosclerosis, comprising contacting a subject in need thereof with an amount effective to treat or reduce intimal or neointimal hyperplasia, stenosis, restenosis, and/or atherosclerosis of one or more polypeptides according to the invention.

[0047] In a further disclosure, the method is used to treat tumors and/or metastasis, including but not limited to smooth muscle tumors. In one disclosure, the tumor is a leiomyosarcoma, which is defined as a malignant neoplasm that arises from muscle. Since leiomyosarcomas can arise from the walls of both small and large blood vessels, they can occur anywhere in the body, but peritoneal, uterine, and gastro-intestinal (particularly esophageal) leiomyosarcomas are more common. Alternatively, the smooth muscle tumor can be a leiomyoma, a non-malignant smooth muscle neoplasm. In a further disclosure, the method can be combined with other treatments for smooth muscle cell tumors and/or metastasis, such as chemotherapy, radiation therapy, and surgery to remove the tumor. While not being limited by any specific mechanism, the inventors believe that administration of the inventive or disclosed polypeptides can be used to treat tumors and/or metastasis by any or all of the following mechanisms: preventing drug resistance to anticancer drugs or promoting susceptibility to anticancer drugs, promoting apoptosis of cancer cells, decreasing cell invasion through decreased matrix metalloproteinase expression and decreased migration of cancer cells, and through suppressing viral oncogenesis.

[0048] In a further disclosure, the methods are used for treating or reducing occurrence of smooth muscle spasm, comprising contacting a subject or graft in need thereof with an amount effective to reduce smooth muscle spasm of one or more inventive or disclosed polypeptides.

[0049] Smooth muscles are found in the walls of blood vessels, airways, the gastrointestinal tract, and the genitourinary tract. Pathologic tonic contraction of smooth muscle constitutes spasm. Many pathological conditions are associated with spasm of vascular smooth muscle ("vasospasm"), the smooth muscle that lines blood vessels. This can cause symptoms such as angina and ischemia (if a heart artery is involved), or stroke as in the case of subarachnoid hemorrhage induced vasospasm if a brain vessel is involved. Hypertension (high blood pressure) is caused by excessive vasoconstriction, as well as thickening, of the vessel wall, particularly in the smaller vessels of the circulation.

[0050] Thus, in a further disclosure, the muscle cell spasm comprises a vasospasm, and the methods of the invention are used to treat or reduce occurrence of vasospasm. Disclosures of the method include, but are not limited to, methods to treat or inhibit angina, coronary vasospasm, Prinzmetal's angina (episodic focal spasm of an epicardial coronary artery), ischemia, stroke, bradycardia, and hypertension.

[0051] In another disclosureof the methods, occurrence of smooth muscle spasm is reduced by treatment of a graft, such as a vein or arterial graft, with the one or more inventive or disclosed polypeptides. One of the ideal conduits for peripheral vascular and coronary reconstruction is the greater saphenous vein. However, the surgical manipulation during harvest of the conduit often leads to vasospasm. The exact etiology of vasospasm is complex and most likely multifactorial. Most investigations have suggested that vasospasm is either due to enhanced constriction or impaired relaxation of the vascular smooth muscle in the media of the vein. Numerous vasoconstricting agents such as endothelin-1 and thromboxane are increased during surgery and result in vascular smooth muscle contraction. Other vasoconstrictors such as norepinephrine, 5-hydroxytryptamine, acetylcholine, histamine, angiotensin II, and phenylephrine have been implicated in vein graft spasm. Papaverine is a smooth muscle vasodilator that has been used. In circumstances where spasm occurs even in the presence of papaverine, surgeons use intraluminal mechanical distension to break the spasm. This leads to injury to the vein graft wall and subsequent intimal hyperplasia. Intimal hyperplasia is the leading cause of graft failure.

[0052] Thus, in this disclosure, the graft can be contacted with the one or more polypeptides according to the invention, during harvest from the graft donor, subsequent to harvest (before implantation), and/or during implantation into the graft recipient (ie: ex vitro or in vivo). This can be accomplished, for example, by delivering the recombinant expression vectors (most preferably a viral vector, such as an adenoviral vector) of the invention to the site, and transfecting the smooth muscle cells. More preferably, delivery into smooth muscle is accomplished by using the one or more polypeptides according to the invention that include at least one transduction domain to facilitate entry into the smooth muscle cells. During graft implantation, it is preferred that the subject receiving the graft be treated systemically with heparin, as heparin has been shown to bind to protein transduction domains and prevent them from transducing into cells. This approach will lead to localize protein transduction of the graft alone, and not into peripheral tissues. The methods according to this disclosure reduce occurrence of vein graft spasm during harvest and/or implantation of the graft, and thus improve both short and long term graft success.

[0053] In various other disclosures of the methods, the muscle cell spasm is associated with a disorder including, but not limited to pulmonary (lung) hypertension, asthma (bronchospasm), toxemia of pregnancy, pre-term labor, pre-eclampsia/eclampsia, Raynaud's disease or phenomenon, hemolytic-uremia, non-occlusive mesenteric ischemia (ischemia of the intestines that is caused by inadequate blood flow to the intestines), anal fissure (which is caused by persistent spasm of the internal anal sphincter), achalasia (which is caused by persistent spasm of the lower esophageal sphincter), impotence (which is caused by a lack of relaxation of the vessels in the penis, erection requires vasodilation of the corpra cavemosal (penile) blood vessels), migraine (which is caused by spasm of the intracranial blood vessels), ischemic muscle injury associated with smooth muscle spasm, and vasculopathy, such as transplant vasculopathy (a reaction in the transplanted vessels which is similar to atherosclerosis, it involves constrictive remodeling and ultimately obliteration of the transplanted blood vessels, this is the leading cause of heart transplant failure).

[0054] In other disclosures, the methods of the invention are used for one or more of promoting wound healing, reducing scar formation, treating and/or reducing fibrotic disorders and treating and/or reducing keloids. In these disclosures, an "individual in need thereof is an individual that has suffered or will suffer (for example, via a surgical procedure) a wound that may result in scar formation, or has resulted in scar formation. As used herein, the term "wound" refers broadly to injuries to the skin and subcutaneous tissue. Such wounds include, but are not limited to lacerations; burns; punctures; pressure sores; bed sores; canker sores; trauma, bites; fistulas; ulcers; lesions caused by infections; periodontal wounds; endodontic wounds; burning mouth syndrome; laparotomy wounds; surgical wounds; incisional wounds; contractures after burns; tissue fibrosis, including but not limited to idiopathic pulmonary fibrosis, hepatic fibrosis, renal fibrosis, retroperitoneal fibrosis, and cystic fibrosis, but excluding blood vessel fibrosis or heart tissue fibrosis; and wounds resulting from cosmetic surgical procedures. In these disclosures, it is preferred that the one or more polypeptides or compositions are disposed on or in a wound dressing or other topical administration. Such wound dressings can be any used in the art, including but not limited to films (e.g., polyurethane films), hydrocolloids (hydrophilic colloidal particles bound to polyurethane foam), hydrogels (cross-linked polymers containing about at least 60% water), foams (hydrophilic or hydrophobic), calcium alginates (nonwoven composites of fibers from calcium alginate), cellophane, and biological polymers such as those described in US patent application publication number 20030190364, published October 9, 2003.

[0055] As used herein, the phrase "reducing scar formation" means any decrease in scar formation that provides a therapeutic or cosmetic benefit to the patient. Such a therapeutic or cosmetic benefit can be achieved, for example, by decreasing the size and/or depth of a scar relative to scar formation in the absence of treatment with the methods of the invention, or by reducing the size of an existing scar. As used herein, such scars include scars of all types, including but not limited to keloids; hypertrophic scars; and adhesion formation between organ surfaces, including but not limited to those occurring as a result of surgery.

[0056] The methods according to these disclosures are clinically useful for treating all types of wounds to reduce scar formation,

both for reducing initial scar formation, and for therapeutic treatment of existing scars (i.e.: cutting out the scar after its formation, treating it with the compounds of the invention, and letting the scar heal more slowly). In one disclosure, individuals in need of treatment or limiting of scarring (such as keloids or hypertrophic scarring) are highly pigmented individuals, including but not limited to individuals of Asian or African descent, that are susceptible to keloids, and thus can benefit from the methods of the invention for prophylactic therapy to limit development of keloids, as well as for treating keloids. In various other disclosures, individuals in need of therapy for treating or limiting fibrotic disorders are those suffering from or at risk of one or more fibrotic disorders associated with TGFβ-induced CTGF expression, including but not limited to tissue fibrosis (including but not limited to idiopathic pulmonary fibrosis, hepatic fibrosis, renal fibrosis, retroperitoneal fibrosis, cystic fibrosis, blood vessel fibrosis, CNS fibrosis, and heart tissue fibrosis); diabetic nephropathy, glomerulosclerosis, and IgA nephropathy (causes of kidney failure and the need for dialysis and retransplant); diabetic retinopathy and macular degeneration (fibrotic diseases of the eye and leading causes of blindness); cirrhosis and biliary atresia (leading causes of liver fibrosis and failure); congestive heart failure; lung fibrosis; scleroderma; abdominal adhesions; and interstitial fibrosis.

[0057] In various other disclosures, individuals in need of therapy for treating and/or limiting fibrotic disorders and/or keloids are those with elevated levels of one or more of the following biomarkers:

TGFβ1 expression;

Collagen I;

CTGF expression; and

alpha smooth muscle actin.

[0058] Elevated levels of such biomarkers can be detected using standard techniques, including but not limited to immunological techniques (ELISA, immunocytochemistry, etc.) using commercially available antibodies against the one or more biomarkers.

[0059] As disclosed below, the polypeptides of the invention inhibit TGFβ1-induced CTGF and collagen expression in human keloid fibroblasts, which are elevated in fibrotic conditions, indicating that individuals with elevated levels of one or more of these biomarkers can especially benefit from the methods of the present invention. As used herein, an "elevated" level of the one or more biomarkers means any increase above normal for that individual or similarly situated individuals in a relevant target tissue. Such target tissues are those affected by fibrotic conditions, including but not limited to blood, wound exudate, and biopsies taken from tissues affected by fibrosis including but not limited to those disclosed above (skin, kidney, lung, liver, peritoneum, blood vessel, heart, retina, etc.) In various further embodiments, an individual in need thereof is one that has a level of one or more of the recited biomarkers 5%, 10%, 15%, 20%, 25%, 50%, 75%, 100%, or more above normal levels. Determining the level of the one or more biomarkers can be done using standard techniques in the art for measuring protein and/or gene expression, including but not limited to those disclosed below.

[0060] A "normal" level of these one or more biomarkers may be established by any suitable means, including but not limited to determining a normal level in that individual or similarly situated individuals in the absence of fibrotic conditions and/or keloids, or any other suitable means to establish a standard for reference.

[0061] Preferred routes of delivery for these various indications of the different disclosuresof the methods of the invention vary. Topical administration is preferred for methods involving treatment or reducing the incidence of vein graft spasm, intimal hyperplasia, restenosis, prosthetic graft failure due to intimal hyperplasia, stent, stent graft failure due to intimal hyperplasia/constrictive remodeling, microvascular graft failure due to vasospasm, transplant vasculopathy, scarring, fibrosis, keloid formation, male and female sexual dysfunction, and for promoting wound healing. As used herein, "topical administration" refers to delivering the polypeptide onto the surface of the organ.

[0062] Intrathecal administration, defined as delivering the polypeptide into the cerebrospinal fluid is the preferred route of delivery for treating or reducing incidence of stroke and subarachnoid hemorrhage induced vasospasm. Intraperitoneal administration, defined as delivering the polypeptide into the peritoneal cavity, is the preferred route of delivery for treating or reducing incidence of non-occlusive mesenteric ischemia. Oral administration is the preferred route of delivery for treating or reducing incidence of achalasia. Intravenous administration is the preferred route of delivery for treating or reducing incidence of hypertension and bradycardia. Administration via suppository is preferred for treating or reducing incidence of anal fissure. Aerosol delivery is preferred for treating or reducing incidence of asthma (ie: bronchospasm). Intrauterine administration is preferred for treating or reducing incidence of pre-term labor and pre-eclampsia/eclampsia.

[0063] In another disclosureof the methods described herein, the methods are used to increase the contractile rate in heart muscle. Individuals that can benefit from such treatment include those who exhibit a reduced heart rate relative to either a normal heart rate for the individual, or relative to a "normal" heart rate for a similarly situated individual. As used herein, the phrase "increasing the contractile rate in heart muscle" means any increase in contractile rate that provides a therapeutic benefit to the patient. Such a therapeutic benefit can be achieved, for example, by increasing the contractile rate to make it closer to a normal contractile rate for the individual, a normal contractile rate for a similarly situated individual, or some other desired target contractile rate. In one disclosure, the methods result in an increase of at least 5% in the contractile rate of the patient in need of such treatment. In further disclosures, the methods result in an increase of at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, and/or 50% in the contractile rate of the patient in need of such treatment. In another disclosure, increasing the contractile rate in heart muscle is accomplished by increasing the heart muscle relaxation rate (ie: if the muscles relax faster, they beat faster). In another disclosure, the methods result in an increase of at least 5% in the heart muscle relaxation rate of the patient in need of such treatment. In further disclosures, the methods result in an increase of at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, and/or 50% in the heart muscle relaxation rate of the patient in need of such treatment.

[0064] In a further disclosureof the methods, the methods are performed to treat one or more cardiac disorders that can benefit from increasing the contractile rate in heart muscle. Such cardiac disorders include bradyarrythmias, bradycardias congestive heart failure, pulmonary hypertension, stunned myocardium, and diastolic dysfunction. As used herein, "bradyarrythmia" means an abnormal decrease of the rate of the heartbeat to less than 60 beats per minute, generally caused by a disturbance in the electrical impulses to the heart. A common cause of bradyarrythmias is coronary heart disease, which leads to the formation of atheromas that limit the flow of blood to the cardiac tissue, and thus the cardiac tissue becomes damaged. Bradyarrythmias due to coronary artery disease occur more frequently after myocardial infarction. Symptoms include, but are not limited to, loss of energy, weakness, syncope, and hypotension. As used herein, "Congestive heart failure" means an inability of the heart to pump adequate supplies of blood throughout the body. Such heart failure can be due to a variety of conditions or disorders, including but not limited to hypertension, anemia, hyperthyroidism, heart valve defects including but not limited to aortic stenosis, aortic insufficiency, and tricuspid insufficiency; congenital heart defects including but not limited to coarctation of the aorta, septal defects, pulmonary stenosis, and tetralogy of Fallot; arrythmias, myocardial infarction, cardiomyopathy, pulmonary hypertension, and lung disease including but not limited to chronic bronchitis and emphysema. Symptoms of congestive heart failure include, but are not limited to, fatigue, breathing difficulty, pulmonary edema, and swelling of the ankles and legs.

[0065] As used herein, "Stunned myocardium" means heart muscle that is not functioning (pumping/beating) due to cardiac ischemia (lack of blood flow/oxygen to the vessels supplying the heart muscle).

[0066] As used herein, "Diastolic dysfunction" means an inability of the heart to fill with blood during diastole (the resting phase of heart contraction). This condition usually occurs in the setting of left ventricular hypertrophy. The heart muscle becomes enlarged and stiff such that it cannot fill adequately. Diastolic dysfunction can result in heart failure and inadequate heart function.

[0067] As used herein, "Pulmonary hypertension" means a disorder in which the blood pressure in the arteries supplying the lungs is abnormally high. Causes include, but are not limited to, inadequate supply of oxygen to the lungs, such as in chronic bronchitis and emphysema; pulmonary embolism, and intestinal pulmonary fibrosis. Symptoms and signs of pulmonary hypertension are often subtle and nonspecific. In the later stages, pulmonary hypertension leads to right heart failure that is associated with liver enlargement, enlargement of veins in the neck and generalized edema.

[0068] In a further disclosureof the methods, the methods are used for treating a heart muscle disorder comprising administering to an individual suffering from one or more of bradyarrythmia, bradycardia, congestive heart failure, stunned myocardium, pulmonary hypertension, and diastolic dysfunction, an amount effective to increase heart muscle contractile rate of one or more inventive or disclosed polypeptides.

[0069] Treating bradyarrythmia includes one or more of the following (a) improving the rate of the heartbeat to closer to normal levels for the individual, closer to a desired rate, or increasing to at least above 60 beats per minute; (b) reducing the occurrence of one or more of loss of energy, weakness, syncope, and hypotension in patients suffering from bradyarrythmia; (c) reducing worsening of one or more of loss of energy, weakness, syncope, and hypotension in patients suffering from bradyarrythmia and its symptoms; (d) reducing recurrence of bradyarrythmia in patients that previously suffered from bradyarrythmia; and (e) reducing recurrence of one or more of loss of energy, weakness, syncope, and hypotension in patients that previously suffered from bradyarrythmia.

[0070] Similarly, treating congestive heart failure includes one or more of the following (a) improving the heart's ability to pump

adequate supplies of blood throughout the body to closer to normal levels for the individual, or closer to a desired pumping capacity; (b) reducing development of one or more of fatigue, breathing difficulty, pulmonary edema, and swelling of the ankles and legs in patients suffering from congestive heart failure; (c) reducing worsening of one or more of fatigue, breathing difficulty, pulmonary edema, and swelling of the ankles and legs in patients suffering from congestive heart failure and its symptoms; (d) reducing recurrence of congestive heart failure in patients that previously suffered from congestive heart failure; and (e) reducing recurrence of one or more of fatigue, breathing difficulty, pulmonary edema, and swelling of the ankles and legs in patients that previously suffered from congestive heart failure.

[0071] Treating stunned myocardium means one or more of (a) improving the ability of the heart muscle to pump by improving the oxygenation of the ischemic muscle, or by decreasing the need of the myocardial cells for oxygen and (b) reducing recurrence of stunned myocardium in patients that previously suffered from stunned myocardium.

[0072] Similarly, treating diastolic dysfunction includes one or more of (a) reducing occurrence of heart failure and/or inadequate heart function by allowing the heart to relax and fill more completely; (b) reducing recurrence of diastolic dysfunction in patients that previously suffered from diastolic dysfunction; and (c) reducing recurrence of heart failure and/or inadequate heart function in patients that previously suffered from diastolic dysfunction.

[0073] Treating pulmonary hypertension includes one or more of the following (a) decreasing blood pressure in the arteries supplying the lungs to closer to normal levels for the individual, or closer to a desired pressure; (b) reducing the occurrence of one or more of enlargement of veins in the neck, enlargement of the liver, and generalized edema in patients suffering from pulmonary hypertension; (c) reducing worsening of one or more of enlargement of veins in the neck, enlargement of the liver, and generalized edema in patients suffering from pulmonary hypertension and its symptoms; (d)reducing recurrence of pulmonary hypertension in patients that previously suffered from pulmonary hypertension; and (e) reducing recurrence of one or more of enlargement of veins in the neck, enlargement of the liver, and generalized edema in patients that previously suffered from pulmonary hypertension.

[0074] The present disclosure also includes methods for reducing occurrence of a heart muscle disorder comprising administering to an individual at risk of developing bradyarrythmia, bradycardia, congestive heart failure, stunned myocardium, pulmonary hypertension, and diastolic dysfunction an amount effective to increase heart muscle contractile rate of one or more inventive or disclosed polypeptides or compositions.

[0075] For example, methods to reduce occurrence of congestive heart failure involve administration of one or more polypeptides or compositions according to the present invention to a subject that suffers from one or more of hypertension, anemia, hyperthyroidism, heart valve defects including but not limited to aortic stenosis, aortic insufficiency, and tricuspid insufficiency; congenital heart defects including but not limited to coarctation of the aorta, septal defects, pulmonary stenosis, and tetralogy of Fallot; arrythmias, myocardial infarction, cardiomyopathy, pulmonary hypertension, and lung disease including but not limited to chronic bronchitis and emphysema.

[0076] Similarly, methods to reduce occurrence of bradyarrythmia involve administration of the one or more inventive or disclosed polypeptides or compositions to a subject that suffer from one or more of coronary heart disease and atheroma formation, or that previously had a myocardial infarction or conduction disorder.

[0077] Similarly, methods to reduce occurrence of pulmonary hypertension involve administration of the one or more inventive or disclosed polypeptides or compositions to a subject that suffers from one or more of chronic bronchitis, emphysema, pulmonary embolism, and intestinal pulmonary fibrosis.

[0078] Reducing occurrence of stunned myocardium involves administration of the one or more inventive or disclosed polypeptides or compositions to a subject that suffers from cardiac ischemia.

[0079] Reducing occurrence of or treating diastolic dysfunction involves administration of the one or more inventive or disclosed polypeptides or compositions to a subject that suffers from left ventricular hypertrophy

[0080] In other disclosures, the methods are used to treat or limit the incidence of inducing neural regeneration for central nervous system injuries. As used herein, "neural regeneration" includes both regenerating a damaged neural connection, as well as promoting an increase in neural function (including but not limited to treatment of Alzheimer's and peripheral neuropathy); such neural regeneration can be in peripheral nervous system or the central nervous system. While not being limited by any specific mechanism of action, the inventors believe that administration of the peptides to a patient in need thereof prevents or limits

activity of the protein rho, which is known to cause growth cone collapse; thus, minimizing rho activity enhances neurite outgrowth

[0081] In other disclosures, the methods are used to treat or limit the incidence of gliosis (proliferation of astrocytes in damaged areas of the central nervous system). Astrocytes are the connective tissue cells of the CNS, and have functions including accumulating in areas with damaged neurons neurons. Gliosis occurs during any traumatic brain injury, insertion of neural electrodes and during spinal cord injury, as well as in various neurodegenerative disorders including but not limited to Korsakoffs syndrome and AIDS dementia complex. While not being limited by any specific mechanism of action, the inventors believe that administration of the peptides to a patient in need thereof prevents or limits the fibrotic response of astrocytes and possibly microglia to inhibit fibrosis.

[0082] In other disclosures, the methods are used to treat or limit the incidence of chronic obstructive pulmonary disease (COPD), which is a group of respiratory tract diseases characterized by airflow obstruction or limitation. COPD can be caused by a variety of factors, including but not limited to tobacco smoking (chronic smokers at risk), exposure to coal dust (coal minining industry workers particularly at risk), congenital defects (including but not limited to alpha 1-antitrypsin deficiency), or it may be idiopathic (no known cause). COPD includes, but is not limited to chronic bronchitis and emphysema. Symptoms characteristic of COPD (for which the methods of the invention can be used to treat or reduce incidence of) include, but are not limited to recurrent respiratory infections, severe cough, constant wheezing, shortness of breath with minimal exertion or rest, hypoxia, and excessive sputum production.

[0083] The inventive or disclosed polypeptides can be used alone or together with other treatments for COPD, including, bronchodilators, antibiotics, and oral or intravenous steroids.

[0084] In other disclosures, the methods are used to treat or limit the incidence of inflammation. As used herein, inflammation means the response of the immune system to infection, irritation, or associated with foreign bodies (introduction of biomaterials) in the body.

[0085] Symptoms characteristic of inflammation (for which the methods of the invention can be used to treat or reduce incidence of) include, but are not limited to redness, heat, swelling, pain, and dysfunction of the organs involved. Specific inflammatory disorders that can be treated, or whose incidence can be reduced, by the methods of the invention include, but are not limited to, asthma, arthritis (rheumatoid or degenerative), sepsis, endotoxemic shock, psoriasis, radiation enteritis, scleroderma, cirrhosis, interstitial fibrosis, Chrohn's disease, appendicitis, gastritis, laryngitis, meningitis, pancreatitis, and otitsis.

[0086] While not being bound by any specific mechanism of action, the inventors believe that administration of the inventive or disclosed polypeptides to a patient in need of antiinflammatory treatment suppresses the response to inflammatory cytokines including but not limited to TGF ß1.

[0087] In all of the above disclosures of the therapeutic methods, the polypeptides of the invention can be used as the sole active agent, or can be combined with one or more other treatments for the indication, as determined by an attending physician.

[0088] As used herein for all of the disclosed methods, an "amount effective" of the one or more polypeptides is an amount that is sufficient to provide the intended benefit of treatment. An effective amount of the polypeptides that can be employed ranges generally between about 0.01 µg/kg body weight and about 10 mg/kg body weight, preferably ranging between about 0.05 µg/kg and about 5 mg/kg body weight. However dosage levels are based on a variety of factors, including the type of injury, the age, weight, sex, medical condition of the individual, the severity of the condition, the route of administration, and the particular compound employed. Thus, the dosage regimen may vary widely, but can be determined routinely by a physician using standard methods.

[0089] The present invention may be better understood with reference to the accompanying examples that are intended for purposes of illustration only.

Example

Example 1 Alanine and d-amino acid scanning mutagenesis

[0090] The peptide transduction and therapeutic domains were optimized to maximize MK2 specificity and inhibition. First, to

further understand the importance of each amino acid in MK2 inhibition, alanine and d-amino acid scans of the therapeutic sequence of the MK2 inhibitor were performed. Using the OmniaTM Lysate Assay for MAPKAP-K2 kit from Invitrogen, the reaction velocity for MK2 was determined in the presence and absence of each of the peptides listed in Table 1. Briefly, inhibitor peptide concentrations at 12.5, 25, 50 and 100 µmol were evaluated. The kit contains a proprietary reaction buffer to which the following are added (final concentrations are given): 1 mM ATP, 0.2 mM DTT, 10 µM MAPKAP-K2 Sox-modified peptide substrate, 5 ng MK2, and the peptide inhibitor of interest (final volume of 50 µL). The reactions were performed in the wells of a low-protein-binding 96-well plate provided with the kit, and fluorescence readings were taken every 30 seconds for 20 minutes in a Molecular Devices M5 Spectrophotometer. The reaction velocities for a MK2 inhibitor concentration of 100 µM are shown in Table 2 and are representative of trends at different concentrations.

[0091] The results of this study show several possible optimizations and elucidate the amino acids that are most crucial for function of this peptide. First of all, the C-terminal alanine does not enhance MK2 inhibition. Removing an alanine will result in a small cost optimization. Furthermore, both the d-amino acid and alanine scans show that the asparagine is not crucial for MK2 inhibition.

Table 1: MK2 Inhibitor Sequence Evaluation - The Importance of Each Amino Acid

Controls	**Alanine Scan**	**d-Amino Acid Scan**
KALNRQLGVA*	KALNRQLGVAA	K**dA**LNRQLGVAA
KKKALNRQLGVAA#	KAANRQLGVAA	KA**dL**NRQLGVAA
$(WLRRIKA)_2$LNRQLGVAA	KAL**A**RQLGVAA	KAL**dN**RQLGVAA
	KALN**A**QLGVAA	KALN**dR**QLGVAA
	KALNR**A**LGVAA	KALNR**dQ**LGVAA
	KALNRQ**A**GVAA	KALNRQ**dL**GVAA
	KALNRQL**A**VAA	
	KALNRQLG**A**AA	KALNRQLG**dV**AA
*= Control to determine the requirement of the final A; # =Control to determine the importance of initial Ks		

Table 2:

Reaction Velocities for MK2 Inhibitor Variants (n=3)	
Peptide Sequence	**% of KALNRQLGVAA Reaction Velocity at an Inhibitor Concentration of 100µM (+/- SEM*)**
KALNRQLGVAA	100% (+/- 3%)
KALNRQLGVA	100% (+/- 3%)
KAANRQLGVAA	152% (+/- 3%)
KALARQLGVAA	39% (+/- 1%)
KALNAQLGVAA	358% (+/- 8%)
KALNRALGVAA	358% (+/- 15%)
KALNRQAGVAA	118% (+/- 4%)
KALNRQLAVAA	72% (+/- 3%)
KALNRQLGAAA	373% (+/- 13%)
KAdLNRQLGVAA	146% (+/- 4%)
KALdNRQLGVAA	95% (+/- 6%)
KALNdRQLGVAA	306% (+/- 4%)
KALNRdQLGVAA	276% (+/- 3%)
KALNRQdLGVAA	357% (+/- 10%)
KALNRQLGdVAA	260% (+/- 14%)
KKKALNRQLGVAA	91% (+/- 4%)
*SEM = Standard Error of the Mean for three values	

[0092] In fact, replacing the asparagine with an alanine may enhance MK2 inhibition and will result in a small cost savings. Table 2 indicates that substitution of alanine for the glycine increases inhibition slightly. Additionally, these results show that stereochemistry is important for inhibitor peptide function. No d-amino acid substitution significantly enhanced MK2 inhibition, and most substitutions greatly decreased the efficacy of the MK2 inhibitor peptide. The alanine scan also shows the importance of several amino acids. According to this alanine scan, arginine, glutamine, and valine are absolutely necessary for MK2 inhibition. While the two leucines are less important amino acids, their removal still diminishes the efficacy of the inhibitor peptide.

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Polypeptid bestående af aminosyresekvensen YARAAARQARAKALNRQLAVAA eller YARAAARQARAKALNRQLGVA.

2. Biomedicinsk indretning, der omfatter polypeptidet ifølge krav 1 placeret på eller i den biomedicinske indretning.

3. Biomedicinsk indretning ifølge krav 2, hvor den biomedicinske indretning er valgt fra gruppen bestående af: en stent, et transplantat, en shunt, et stenttransplantat, en angioplastiindretning, et ballonkateter, en fistel, en sårbandage og en implanterbar indretning til afgivelse af lægemiddel.

4. Sammensætning, der omfatter polypeptidet ifølge krav 1 og en farmaceutisk acceptabel bærer.