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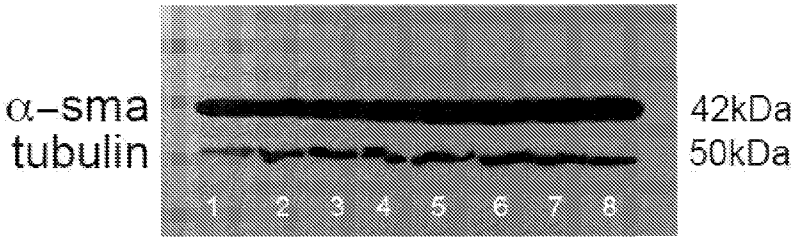

FIG. 1

(57) Abstract: Provided herein are compositions comprising modified caveolin-1 (Cav-1) peptides. Further provided are methods of using the modified Cav-1 peptides for the treatment of lung infections or acute or chronic lung injury, particularly lung fibrosis.

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DESCRIPTION

MODIFIED PEPTIDE FRAGMENTS OF CAV-1 PROTEIN AND THE USE THEREOF IN THE TREATMENT OF FIBROSIS

[0001] This application claims the benefit of United States Provisional Patent Application No. 62/728,997, filed September 10, 2018, the entirety of which is incorporated herein by reference.

[0001] The present invention was made as a result of activities undertaken within the scope of a joint research agreement that was in effect at the time the present invention was made. The parties to said joint research agreement are Board of Regents of the University of Texas System and Lung Therapeutics.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The present invention relates generally to the fields of molecular biology and medicine. More particularly, it concerns compositions and methods for the delivery of therapeutic polypeptide compositions to subjects, such as by delivery to the respiratory system.

2. Description of Related Art

[0003] During lung injury, p53 expression increases, inducing plasminogen activator inhibitor-1 (PAI-1) while inhibiting expression of urokinase-type plasminogen activator (uPA) and its receptor (uPAR), resulting in apoptosis of lung epithelial cells (LECs). The mechanism of injury involves cell surface signaling interactions between uPA, uPAR, caveolin-1 ("Cav-1") and β1-integrin (Shetty *et al.*, 2005). Compositions that modulate these interactions could be used in methods for inhibiting apoptosis of injured or damaged lung epithelial cells and for treating acute lung injury and consequent pulmonary fibrosis. Thus, there is a need for polypeptides that could be used to prevent or treat lung injury and, in particular, formulations and methods for therapeutic delivery of such polypeptides.

SUMMARY OF THE INVENTION

[0004] In accordance with the present disclosure, there is provided a peptide comprising the amino acid sequence of SEQ ID NO: 2, wherein the peptide comprises at least one N- or C-terminal addition. The N- or C- terminal additions may be standard amino acids, non-standard amino acids, or chemical modifications. There are provided peptide multimers of the peptide of the disclosure. Also provided is a pharmaceutical composition of the peptide. Peptides of the present disclosure may be used to treat lung injuries, infections or diseases. In further aspects, peptides of the embodiments can be used to treat fibrotic conditions, *e.g.,* organ fibrosis, or inflammation.

[0005] In some embodiments, the present disclosure provides a peptide comprising the amino acid sequence ASFTTFTVT (SEQ ID NO: 3), wherein the peptide comprises at least one N- or C-terminal addition lacking identity to SEQ ID NO: 1. In some aspects, the peptide comprises at least one amino acid added to the N-terminus. In some aspects, the peptide comprises at least one amino acid added to the C-terminus. In some aspects, the peptide comprises at least one amino acid added to the N-terminus and the C-terminus. In some aspects, the peptide maintains the biological activity of caveolin-1 (Cav-1). In further aspects, a peptide of the embodiments can be comprise one or more deuterated residues.

[0006] In some aspects, the peptide comprises L-amino acids. In some aspects, the peptide comprises D-amino acids. In some aspects, the peptide comprises both L- and D-amino acids.

[0007] In some aspects, the peptide comprises at least one non-standard amino acid. In some aspects, the peptide comprises 2 or more non-standard amino acids. In some aspects, the peptide comprises 4 or more non-standard amino acids. In some aspects, the non-standard amino acid is ornithine. In some aspects, the non-standard amino acid is D-alanine.

[0008] In some aspects, the peptide comprises N- or C-terminal modifications. In some aspects, the peptide comprises a N-terminal modification. In some aspects, the peptide comprises a C-terminal modification. In some aspects, the peptide comprises a N- and C-terminal modification. In some aspects, the N-terminal modification is acylation. In some aspects, the C-terminal modification is amidation.

[0009] In some aspects, the peptide comprises the amino acid sequence KASFTTFTVTKGS (SEQ ID NO: 4). In some aspects, the peptide comprises the amino acid sequence aaEGKASFTTFTVTKGSaa (SEQ ID NO: 6). In other aspects, the peptide comprises the amino acid sequence OASFTTFTVTOS (SEQ ID NO: 9). In other aspects, the peptide comprises the amino acid sequence aaEGKASFTTFTVTKGSaa-NH2 (SEQ ID NO: 7). In still other aspects, the peptide comprises the amino acid sequence Ac-aaEGKASFTTFTVTKGSaa-NH2 (SEQ ID NO: 8). In other aspects, the peptide comprises the amino acid sequence OASFTTFTVTOS-NH2 (SEQ ID NO: 10).

[0010] In some aspects, the peptide further comprises a cell-penetrating peptide (CPP). In some embodiments the CPP comprises an amino acid sequence selected from the group comprising: GRKKRRQRRRPPQ (SEQ ID NO: 21), RQIKIWFQNRRMKWKK (SEQ ID NO:22), and GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO:23).

[0011] In some embodiments, the disclosure provides a peptide multimer comprising at least two peptides as disclosed herein. In some aspects, a first peptide of the at least two peptides is essentially identical to a second peptide of the at least two peptides. In other aspects, a first peptide of the at least two peptides is not identical to a second peptide of the at least two peptides.

[0012] In some embodiments, the disclosure provides a composition comprising peptides disclosed herein. In some aspects, the peptides are substantially pure. In some aspects, the peptides are at least 95% pure, at least 96% pure, at least 97% pure, at least 98% pure, or at least 99% pure.

[0013] In some embodiments, the disclosure provides a pharmaceutical composition comprising the peptide a peptide as disclosed herein and a pharmaceutically acceptable carrier. In some aspects, the pharmaceutical composition is formulated for oral, intravenous, intraarticular, parenteral, enteral, topical, subcutaneous, intramuscular, buccal, sublingual, rectal, intravaginal, intrapenile, intraocular, epidural, intracranial, or inhalational administration. In some aspects, the pharmaceutical composition is formulated for lung instillation. In some aspects, the pharmaceutical composition is formulated as a nebulized solution.

[0014] In some embodiments, the disclosure provides a polynucleotide comprising a nucleic acid sequence encoding the peptide as described herein.

[0015] In certain aspects, a peptide composition of the embodiments can be used in a method of treating or preventing disease in subject. In some aspects the disease is a fibrotic or inflammatory disease. For example, the fibrotic disease can be organ fibrotic disease, can be kidney, liver, lung or heart fibrosis. In some aspects, the inflammatory disease is an inflammatory eye disease. Compositions of the embodiments can be administered systemically or locally (*e.g.*, at the site of diseased tissues).

[0016] In some embodiments, the disclosure provides a method of treating or preventing acute lung injury, lung infection or lung disease in a subject comprising administering to the subject an effective amount of the peptide as described herein. In some aspects, the subject has pulmonary inflammation. In some aspects, the subject is undergoing chemotherapy or radiation therapy. In some aspects, the subject has an acute lung injury or infection. In some aspects, the subject has a chemical-induced lung injury. In some aspects, the subject has plastic bronchitis, chronic obstructive pulmonary disease, bronchitis, bronchiolitis, bronchiolitis obliterans, asthma, acute respiratory distress syndrome (ARDS) or inhalational smoke induced acute lung injury (ISALI). In some aspects, the lung disease is a fibrotic condition of the lungs. In some aspects, the lung disease is interstitial lung disease. In some aspects, the lung disease is Idiopathic Pulmonary Fibrosis (IPF) or lung scarring. In some aspects, the administering comprises nebulizing a solution comprising the peptide. In some aspects, the method further comprises administering at least one additional anti-fibrotic therapeutic. In some aspects, the at least one additional anti-fibrotic is NSAID, steroid, DMARD, immunosuppressive, biologic response modulators, or bronchodilator. In some aspects, the subject is a human.

[0017] It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0019] **FIG. 1: Western blot of SMA and tubulin idiopathic pulmonary fibrosis cells treated with Cav-1 peptides.** IPF cells were treated with: 1: Untreated, 2:10 μM LTI-03, 3: 90 μM LTI-03, 4: 10 μM APi2350, 5: 10 μM APi2354, 6: 10 μM APi2355, 7: 10 μM APi2356, and 8: DMSO, and SMA and tubulin expression was evaluated by western blot.

[0020] **FIG. 2: Treatment with Cav-1 peptides increases SMA relative to tubulin in IPF cells.** Graphical representation of the ratio of SMA to tubulin in cells receiving the indicated treatments.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0021] The present disclosure overcomes challenges associated with current technologies by providing modified caveolin-1 (Cav-1) peptides and the use thereof for disease treatment and prevention, particularly lung fibrosis. In some aspects, pharmaceutical formulations of the modified Cav-1 peptides are provided. For example, in some aspects, the peptide is formulated for delivery to the respiratory system. For instance, peptides can be prepared for administration to a subject's airway by formulation in an aqueous solution and nebulizing the solution using a nebulizer. In other aspects, peptides can be formulated for injection. Also provided herein is a method of treating lung injuries and diseases, by administering to the subject (*e.g.*, *via* the airway) a therapeutically effective amount of a modified Cav-1 peptide.

I. Definitions

[0022] As used herein, "essentially free," in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.01%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical methods.

[0023] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one.

[0024] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

[0025] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0026] The term "peptide" as used herein typically refers to a sequence of amino acids made up of a single chain of amino acids joined by peptide bonds. Generally, peptides contain at least two amino acid residues and are less than about 50 amino acids in length, unless otherwise defined.

[0027] A "biologically active" caveolin-1 (Cav-1) peptide refers to a peptide that increases p53 protein levels, reduces urokinase plasminogen activator (uPA) and uPA receptor (uPAR), and/or increases plasminogen activator inhibitor-1 (PAI-1) expression in cells, such as fibrotic lung fibroblasts. In some aspects, the biologically active peptide has at least 20% of the biological or biochemical activity of native Cav-1 polypeptide of SEQ ID NO: 1 (*e.g.*, as measured by an *in vitro* or an *in vivo* assay). In some aspects, the biological active peptide has an increase biological or biochemical activity as compared to the native Cav-1 polypeptide.

[0028] The term "identity" or "homology" shall be construed to mean the percentage of amino acid residues in the candidate sequence that are identical with the residue of a corresponding sequence to which it is compared, after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent identity for the entire sequence, and not considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity or homology. Methods and computer programs for the alignment are well known in the art. Sequence identity may be measured using sequence analysis software.

[0029] The term "polypeptide" or "protein" is used in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, *e.g.* ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. The term "peptidomimetic" or "peptide mimic" means that a peptide according to the invention is modified in such a way that it includes at least one non-peptidic bond such as, for example, urea bond, carbamate bond, sulfonamide bond, hydrazine bond, or any other covalent bond. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If the peptide chain is long, the peptide is commonly called a polypeptide or a protein.

[0030] The terms "subject" and "individual" and "patient" are used interchangeably herein, and refer to an animal, for example a human or non-human animal (*e.g.*, a mammal) , to whom treatment, including prophylactic treatment, with a pharmaceutical composition as disclosed herein, is provided. The term "subject" as used herein refers to human and non-human animals. The term "non-human animals" includes all vertebrates, *e.g.*, mammals, such as non-human primates, (particularly higher primates), sheep, dogs, rodents (*e.g.* mouse or rat), guinea pigs, goats, pigs, cats, rabbits, cows, and non-mammals such as chickens, amphibians, reptiles etc. In one embodiment, the subject is human. In another embodiment, the subject is an experimental animal or animal substitute as a disease model. Non-human mammals include mammals such as non-human primates, (particularly higher primates), sheep, dogs, rodents (*e.g.* mouse or rat), guinea pigs, goats, pigs, cats, rabbits and cows. In some aspects, the non-human animal is a companion animal such as a dog or a cat.

[0031] "Treating" a disease or condition in a subject or "treating" a patient having a disease or condition refers to subjecting the individual to a pharmaceutical treatment, *e.g.*, the administration of a drug, such that at least one symptom of the disease or condition is decreased or stabilized. Typically, when the peptide is administered therapeutically as a treatment, it is administered to a subject who presents with one or more symptoms of lung injury or lung fibrosis.

[0032] By "isolated" it is meant that the polypeptide has been separated from any natural environment, such as a body fluid, *e.g.*, blood, and separated from the components that naturally accompany the peptide.

[0033] By isolated and "substantially pure" is meant a polypeptide that has been separated and purified to at least some degree from the components that naturally accompany it. Typically, a polypeptide is substantially pure when it is at least about 60%, or at least about 70%, at least about 80%, at least about 90%, at least about 95%, or even at least about 99%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. For example, a substantially pure polypeptide may be obtained by extraction from a natural source, by expression of a recombinant nucleic acid in a cell that does not normally express that protein, or by chemical synthesis.

[0034] The term "variant" as used herein refers to a polypeptide or nucleic acid that differs from the polypeptide or nucleic acid by one or more amino acid or nucleic acid

deletions, additions, substitutions or side-chain modifications, yet retains one or more specific functions or biological activities of the naturally occurring molecule. Amino acid substitutions include alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an amino acid residue contained in a polypeptide is replaced with another naturally occurring amino acid of similar character either in relation to polarity, side chain functionality or size. Such conservative substitutions are well known in the art. Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a peptide is substituted with an amino acid having different properties, such as naturally-occurring amino acid from a different group (*e.g.*, substituting a charged or hydrophobic amino; acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non- conventional amino acid. In some embodiments, amino acid substitutions are conservative. Also encompassed within the term variant when used with reference to a polynucleotide or polypeptide, refers to a polynucleotide or polypeptide that can vary in primary, secondary, or tertiary structure, as compared to a reference polynucleotide or polypeptide, respectively (*e.g.*, as compared to a wild- type polynucleotide or polypeptide).

[0035] The term "insertions" or "deletions" are typically in the range of about 1 to 5 amino acids. The variation allowed can be experimentally determined by producing the peptide synthetically while systematically making insertions, deletions, or substitutions of nucleotides in the sequence using recombinant DNA techniques.

[0036] The term "substitution" when referring to a peptide, refers to a change in an amino acid for a different entity, for example another amino acid or amino-acid moiety. Substitutions can be conservative or non-conservative substitutions.

[0037] An "analog" of a molecule such as a peptide refers to a molecule similar in function to either the entire molecule or to a fragment thereof. The term "analog" is also intended to include allelic species and induced variants. Analogs typically differ from naturally occurring peptides at one or a few positions, often by virtue of conservative substitutions. Analogs typically exhibit at least 80 or 90% sequence identity with natural peptides. Some analogs also include unnatural amino acids or modifications of N or C terminal amino acids. Examples of unnatural amino acids are, for example but not limited to; disubstituted amino acids, N-alkyl amino acids, lactic acid, 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-

trimethyllysine, ε-N-acetyllysine, O- phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N- methylarginine. Fragments and analogs can be screened for prophylactic or therapeutic efficacy in transgenic animal models as described below.

[0038] By "covalently bonded" is meant joined either directly or indirectly (*e.g.*, through a linker) by a covalent chemical bond. In some aspects of all the embodiments of the invention, the fusion peptides are covalently bonded.

[0039] The term "fusion protein" as used herein refers to a recombinant protein of two or more proteins. Fusion proteins can be produced, for example, by a nucleic acid sequence encoding one protein is joined to the nucleic acid encoding another protein such that they constitute a single open-reading frame that can be translated in the cells into a single polypeptide harboring all the intended proteins. The order of arrangement of the proteins can vary. Fusion proteins can include an epitope tag or a half-life extender. Epitope tags include biotin, FLAG tag, c-myc, hemaglutinin, His6, digoxigenin, FITC, Cy3, Cy5, green fluorescent protein, V5 epitope tags, GST, β-galactosidase, AU1, AU5, and avidin. Half-life extenders include Fc domain and serum albumin.

[0040] The term "airway" refers herein to any portion of the respiratory tract including the upper respiratory tract, the respiratory airway, and the lungs. The upper respiratory tract includes the nose and nasal passages, mouth, and throat. The respiratory airway includes the larynx, trachea, bronchi and bronchioles. The lungs include the respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli.

[0041] The terms "inhalational smoke induced acute lung injury" and "ISALI" are used interchangeably herein and refer to a form of acute lung injury (ALI) caused by smoke inhalation. ALI is also referred to as "mild Acute Respiratory Distress Syndrome; ARDS." ARDS can be defined by finding one or more of the following conditions in a subject: 1) bilateral pulmonary infiltrates on chest x-ray, 2) when measured by right heart catheterization as clinically indicated, pulmonary capillary wedge pressure < 18 mmHg (2.4 kPa), and 3) PaO_2/FiO_2 <300 mmHg (40 kPa). In some embodiments, treatment of ISALI includes treatment of one or more of the following conditions: reduced oxygenation, airway obstruction (including a severe airway obstruction), fibrinous airway casts or debris, and alveolar fibrin deposition.

[0042] The terms "nebulizing," "nebulized" and other grammatical variations, refer herein to the process of converting a liquid into small aerosol droplets. In some embodiments, the aerosol droplets have a median diameter of approximately 2-10 μm. In some embodiments, the aerosol droplets have a median diameter of approximately 2 - 4 μm.

II. Caveolin-1 peptides

[0043] Embodiments of the present disclosure provide peptide variants of the caveolin-1 (Cav-1) protein. The Caveolin-1 (Cav-1) scaffolding domain or polypeptide interferes with Cav-1 interaction with Src kinases mimics the combined effect of uPA and anti-β1-integrin antibody. Native human Cav-1 has a length of 178 amino acids and a molecular weight of 22 kDa. The amino acid sequence of Cav-1 is shown below (SEQ ID NO:1).

1 MSGGKYVDSE GHLYTVPIRE QGNIYKPNNK AMADELSEKQ VYDAHTKEID LVNRDPKHLN
61 DDVVKIDFED VIAEPEGTHS FDGIWKASFT TFTVTKYWFY RLLSALFGIP MALIWGIYFA
121 ILSFLHIWAV VPCIKSFLIE IQCISRVYSI YVHTVCDPLF EAVGKIFSNV RINLQKEI

[0044] In some aspects, the peptide is a scaffolding domain peptide which comprises an amino acid sequence at least about 40%, 50%, 60%, 70%, 80%, 85%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 2, FTTFTVT. The peptide may comprise 1, 2, 3, 4 or more amino acid substitutions, deletions, or insertions relative to the sequence of SEQ ID NO:1, such as to derive a polypeptide of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 residues. In particular aspects, the peptides are truncations of the native Cav-1 polypeptide, such as the exemplary polypeptides shown in Table 1.

Table 1: Exemplary Cav-1 peptides.

Sequence	**ID**
ASFTTFTVT	SEQ ID NO:3
KASFTTFTVTKGS	SEQ ID NO:4
KASFTTFTVTKGS-NH2	SEQ ID NO: 5
aaEGKASFTTFTVTKGSaa	SEQ ID NO: 6
aaEGKASFTTFTVTKGSaa-NH2	SEQ ID NO: 7
Ac-aaEGKASFTTFTVTKGSaa-NH2	SEQ ID NO: 8
OASFTTFTVTOS	SEQ ID NO: 9
OASFTTFTVTOS-NH2	SEQ ID NO: 10
FTTFTVT-NH2	SEQ ID NO: 11
FTTFTVTK-NH2	SEQ ID NO: 12
KASFTTFTVTK-NH2	SEQ ID NO: 13
Ac-KASFTTFTVTK-NH2	SEQ ID NO: 14
OASFTTFTVTK-NH2	SEQ ID NO: 15
Ac-OASFTTFTVTK-NH2	SEQ ID NO: 16
Ac-KASFTTFTVTKGS-NH2	SEQ ID NO: 17
DSGKASFTTFTVTK-NH2	SEQ ID NO: 18
Ac-DSGKASFTTFTVTK-NH2	SEQ ID NO: 19
Ac-OASFTTFTVTOS-NH2	SEQ ID NO: 20

(a=D-Alanine, O=Ornithine)

[0045] The peptides provided in the present disclosure are biologically active derivatives which have the activity of the native CAV-1 polypeptide in *in vitro* or *in vivo* assays of binding or of biological activity. In particular aspects, the peptide inhibits or prevents apoptosis of LECs induced by BLM *in vitro* or *in vivo* with activity at least about 20% of the activity of the native CAV-1 polypeptide, or at least about 30%, 40%, 50%, 60 %, 65%, 70%, 75%, 80%, 85%, 90%, about 95%, 97%, 99%, and any range derivable therein, such as, for

example, from about 70% to about 80%, and more preferably from about 81% to about 90%; or even more preferably, from about 91% to about 99%. The peptide may have 100% or even greater activity than the native CAV-1 polypeptide. Assays for testing biological activity, *e.g.*, anti-fibrotic activity, the ability to affect expression of uPA, uPAR and PAI-1 mRNAs, or inhibit proliferation of lung fibroblasts, are well-known in the art.

[0046] The peptides of the present disclosure are peptides of the native Cav-1 polypeptide or modified versions thereof. The peptides can be synthetic, recombinant, or chemically modified peptides isolated or generated using methods well known in the art. Modifications can be made to amino acids on the N-terminus, C-terminus, or internally. N-terminal modfications may be, for example but not limited to, acylation, acetylation, or C-terminal amidation. Peptides can include conservative or non-conservative amino acid changes, as described below. Polynucleotide changes can result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence. Peptides can also include insertions, deletions or substitutions of amino acids, including insertions and substitutions of amino acids (and other molecules) that do not normally occur in the peptide sequence that is the basis of the modified variant, for example but not limited to insertion L-amino acids, or non-standard amino acids such as ornithine, which do not normally occur in human proteins. The term conservative substitution, when describing a peptide, refers to a change in the amino acid composition of the peptide that does not substantially alter the peptide's activity. For example, a conservative substitution refers to substituting an amino acid residue for a different amino acid residue that has similar chemical properties. Conservative amino acid substitutions include replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

[0047] Conservative amino acid substitutions result from replacing one amino acid with another having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine. Thus, a conservative substitution of a particular amino acid sequence refers to substitution of those amino acids that are not critical for polypeptide activity or substitution of amino acids with other amino acids having similar properties (*e.g.*, acidic, basic, positively or negatively charged, polar or non-polar, etc.) such that the substitution of even critical amino acids does not reduce the activity of the peptide. Conservative substitution tables providing functionally similar amino acids are well known in the art. For example, the following six groups each

contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Serine (S), Threonine (T); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W). (See also Creighton, Proteins, W. H. Freeman and Company (1984), incorporated by reference in its entirety.) In some embodiments, individual substitutions, deletions or additions that alter, add or delete a single amino acid or a small percentage of amino acids can also be considered conservative substitutions if the change does not reduce the activity of the peptide. Insertions or deletions are typically in the range of about 1 to 5 amino acids. The choice of conservative amino acids may be selected based on the location of the amino acid to be substituted in the peptide, for example if the amino acid is on the exterior of the peptide and expose to solvents, or on the interior and not exposed to solvents.

[0048] In alternative embodiments, one can select the amino acid which will substitute an existing amino acid based on the location of the existing amino acid, *i.e.* its exposure to solvents (*i.e.* if the amino acid is exposed to solvents or is present on the outer surface of the peptide or polypeptide as compared to internally localized amino acids not exposed to solvents). Selection of such conservative amino acid substitutions are well known in the art, for example as disclosed in Dordo *et al, J. Mol Biol*, 1999, 217, 721-739 and Taylor *et al, J. Theor. Biol.* 119(1986); 205-218 and S. French and B. Robson, *J. Mol. Evol.* 19(1983)171. Accordingly, one can select conservative amino acid substitutions suitable for amino acids on the exterior of a protein or peptide (*i.e.* amino acids exposed to a solvent), for example, but not limited to, the following substitutions can be used: substitution of Y with F, T with S or K, P with A, E with D or Q, N with D or G, R with K, G with N or A, T with S or K, D with N or E, I with L or V, F with Y, S with T or A, R with K, G with N or A, K with R, A with S, K or P.

[0049] In alternative embodiments, one can also select conservative amino acid substitutions encompassed suitable for amino acids on the interior of a protein or peptide, for example one can use suitable conservative substitutions for amino acids is on the interior of a protein or peptide (*i.e.* the amino acids are not exposed to a solvent), for example but not limited to, one can use the following conservative substitutions: where Y is substituted with F, T with A or S, I with L or V, W with Y, M with L, N with D, G with A, T with A or S, D with N, I

with L or V, F with Y or L, S with A or T and A with S, G, T or V. In some embodiments, non-conservative amino acid substitutions are also encompassed within the term of variants.

[0050] In some aspects, the polypeptides are derivatives of the native Cav-1 polypeptide. The term "derivative" as used herein refers to peptides which have been chemically modified, for example but not limited to by techniques such as acetylation, ubiquitination, labeling, pegylation (derivatization with polyethylene glycol), lipidation, glycosylation, amidation, or addition of other molecules. A molecule is also a "derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties can alter the pH or improve the molecule's solubility, absorption, biological half-life, *etc.* The moieties can alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed in Remington's Pharmaceutical Sciences, 18th edition, A. R. Gennaro, Ed., MackPubl., Easton, PA (1990), incorporated herein, by reference, in its entirety.

[0051] The term "functional" when used in conjunction with "derivative" or "variant" refers to a polypeptide of the invention which possesses a biological activity (either functional or structural) that is substantially similar to a biological activity of the entity or molecule it is a functional derivative or functional variant thereof. The term functional derivative is intended to include the fragments, analogues or chemical derivatives of a molecule.

[0052] In some aspects, amino acid substitutions can be made in a polypeptide at one or more positions wherein the substitution is for an amino acid having a similar hydrophilicity. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Thus such conservative substitution can be made in a polypeptide and will likely only have minor effects on their activity. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (0.5); histidine -0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

These values can be used as a guide and thus substitution of amino acids whose hydrophilicity values are within ±2 are preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. Thus, any of the polypeptides described herein may be modified by the substitution of an amino acid, for different, but homologous amino acid with a similar hydrophilicity value. Amino acids with hydrophilicities within +/- 1.0, or +/- 0.5 points are considered homologous.

[0053] The modified Cav-1 peptides may comprise co-translational and post-translational (C-terminal peptide cleavage) modifications, such as, for example, disulfide-bond formation, glycosylation, acetylation, phosphorylation, proteolytic cleavage (*e.g.*, cleavage by furins or metalloproteases), and the like to the extent that such modifications do not affect the anti-inflammatory properties of the isolated peptides or their capacity to improve glycemic control.

[0054] In some aspects, the modified Cav-1 peptide comprises non-naturally occurring amino acids. The polypeptides can comprise a combination of naturally occurring and non-naturally occurring amino acids, or may comprise only non-naturally occurring amino acids. The non-naturally occurring amino acids can include synthetic non-native amino acids, substituted amino acids, or one or more D-amino acids into the peptides (or other components of the composition, with exception for protease recognition sequences) is desirable in certain situations. D-amino acid- containing peptides exhibit increased stability in vitro or in vivo compared to L-amino acid- containing forms. Thus, the construction of peptides incorporating D-amino acids can be particularly useful when greater in vivo or intracellular stability is desired or required. More specifically, D- peptides are resistant to endogenous peptidases and proteases, thereby providing better oral trans-epithelial and transdermal delivery of linked drugs and conjugates, improved bioavailability of membrane -permanent complexes (see below for further discussion), and prolonged intravascular and interstitial lifetimes when such properties are desirable. The use of D- isomer peptides can also enhance transdermal and oral trans-epithelial delivery of linked drugs and other cargo molecules. Additionally, D-peptides cannot be processed efficiently for major histocompatibility complex class Il-restricted presentation to T helper cells, and are therefore less likely to induce humoral immune responses in the whole organism. Peptide conjugates can therefore be constructed using, for example, D-isomer forms of cell penetrating peptide sequences, L-isomer forms of cleavage sites, and D-isomer forms of therapeutic peptides.

[0055] In addition to the 20 "standard" L-amino acids, D-amino acids or non-standard, modified or unusual amino acids which are well-defined in the art are also contemplated for use in the present disclosure. Phosphorylated amino acids (Ser, Thr, Tyr), glycosylated amino acids (Ser, Thr, Asn), β-amino acids, GABA, ω- amino acids are further contemplated for use in the present disclosure. These include, for example, include β-alanine (β-Ala) and other ω-amino acids such as 3-aminopropionic acid, 2,3-diaminopropionic acid (Dpr), 4-aminobutyric acid and so forth; α-aminoisobutyric acid (Aib); ε-aminohexanoic acid (Aha); δ-aminovaleric acid (Ava); N-methylglycine or sarcosine (MeGly); ornithine (Orn); citrulline (Cit); t-butylalanine (t-BuA); t-butylglycine (t-BuG); N-methylisoleucine (MeIle); phenylglycine (Phg); norleucine (Nle); 4-chlorophenylalanine (Phe(4-Cl)); 2-fluorophenylalanine (Phe(2-F)); 3-fluorophenylalanine (Phe(3-F)); 4-fluorophenylalanine (Phe(4-F)); penicillamine (Pen); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic); homoarginine (hArg); N-acetyl lysine (AcLys); 2,4-diaminobutyric acid (Dbu); 2,4-diaminobutyric acid (Dab); p-aminophenylalanine (Phe(pNH_2)); N-methyl valine (MeVal); homocysteine (hCys), homophenylalanine (hPhe) and homoserine (hSer); hydroxyproline (Hyp), homoproline (hPro), N-methylated amino acids and peptoids (N-substituted glycines).

[0056] Carboxy terminal modifications include acylation with carboxylic acids: formic, acetic, propionic, fatty acids (myristic, palmitic, stearic), succinic, benzoic, carbobenzoxy (Cbz); acetylation and biotinylation. Amino terminal modifications include: (i) acylation with carboxylic acids: formic, acetic, propionic, fatty acids (myristic, palmitic, stearic, etc) succinic, benzoic, carbobenzoxy (Cbz); (ii) biotinylation; (iii) amidation; (iv) attachment of dyes such as fluorescein (FITC, FAM, etc.), 7-hydroxy-4- methylcoumarin-3 -acetic acid, 7-hydroxycoumarin-3 -acetic acid, 7-metoxycoumarin-3 -acetic acid and other coumarins; rhodamines (5-carboxyrhodamine 110 or 6G, 5(6)-TAMRA, ROX); N-[4-(4-dimethylamino)phenylazo]bezoic acid (Dabcyl), 2,4-dinitrobenzene (Dnp), 5 - dimethylaminonaphthalene - 1 - sulfonic acid (Dansyl) and other dyes; and (v) polyethyleneglycol.

[0057] The polypeptide may be capped at its N and C termini with an acyl (abbreviated "Ac") -and an amido (abbreviated "Am") group, respectively, for example acetyl (CH_3CO-) at the N terminus and amido (-NH_2) at the C terminus. A broad range of N-terminal capping functions, preferably in a linkage to the terminal amino group, is contemplated, for example: formyl;

alkanoyl, having from 1 to 10 carbon atoms, such as acetyl, propionyl, butyryl;

alkenoyl, having from 1 to 10 carbon atoms, such as hex-3-enoyl;

alkynoyl, having from 1 to 10 carbon atoms, such as hex-5-ynoyl;

aroyl, such as benzoyl or 1-naphthoyl;

heteroaroyl, such as 3-pyrroyl or 4-quinoloyl;

alkylsulfonyl, such as methanesulfonyl;

arylsulfonyl, such as benzenesulfonyl or sulfanilyl;

heteroarylsulfonyl, such as pyridine-4-sulfonyl;

substituted alkanoyl, having from 1 to 10 carbon atoms, such as 4-aminobutyryl;

substituted alkenoyl, having from 1 to 10 carbon atoms, such as 6-hydroxy-hex-3-enoyl;

substituted alkynoyl, having from 1 to 10 carbon atoms, such as 3-hydroxy-hex-5-ynoyl;

substituted aroyl, such as 4-chlorobenzoyl or 8-hydroxy-naphth-2-oyl;

substituted heteroaroyl, such as 2,4-dioxo-1,2,3,4-tetrahydro-3-methyl-quinazolin-6-oyl;

substituted alkylsulfonyl, such as 2-aminoethanesulfonyl;

substituted arylsulfonyl, such as 5-dimethylamino-1-naphthalenesulfonyl;

substituted heteroarylsulfonyl, such as 1-methoxy-6-isoquinolinesulfonyl;

carbamoyl or thiocarbamoyl;

substituted carbamoyl (R'-NH-CO) or substituted thiocarbamoyl (R'-NH-CS) wherein R' is alkyl, alkenyl, alkynyl, aryl, heteroaryl, substituted alkyl, substituted alkenyl, substituted alkynyl, substituted aryl, or substituted heteroaryl;

substituted carbamoyl (R'-NH-CO) and substituted thiocarbamoyl (R'-NH-CS) wherein R' is alkanoyl, alkenoyl, alkynoyl, aroyl, heteroaroyl, substituted alkanoyl, substituted alkenoyl, substituted alkynoyl, substituted aroyl, or substituted heteroaroyl, all as above defined.

The C-terminal capping function can either be in an amide or ester bond with the terminal carboxyl. Capping functions that provide for an amide bond are designated as NR^1R^2 wherein R^1 and R^2 may be independently drawn from the following group: hydrogen;

alkyl, preferably having from 1 to 10 carbon atoms, such as methyl, ethyl, isopropyl;

alkenyl, preferably having from 1 to 10 carbon atoms, such as prop-2-enyl;

alkynyl, preferably having from 1 to 10 carbon atoms, such as prop-2-ynyl;

substituted alkyl having from 1 to 10 carbon atoms, such as hydroxyalkyl, alkoxyalkyl, mercaptoalkyl, alkylthioalkyl, halogenoalkyl, cyanoalkyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, alkanoylalkyl, carboxyalkyl, carbamoylalkyl;

substituted alkenyl having from 1 to 10 carbon atoms, such as hydroxyalkenyl, alkoxyalkenyl, mercaptoalkenyl, alkylthioalkenyl, halogenoalkenyl, cyanoalkenyl, aminoalkenyl, alkylaminoalkenyl, dialkylaminoalkenyl, alkanoylalkenyl, carboxyalkenyl, carbamoylalkenyl;

substituted alkynyl having from 1 to 10 carbon atoms, such as hydroxyalkynyl, alkoxyalkynyl, mercaptoalkynyl, alkylthioalkynyl, halogenoalkynyl, cyanoalkynyl, aminoalkynyl, alkylaminoalkynyl, dialkylaminoalkynyl, alkanoylalkynyl, carboxyalkynyl, carbamoylalkynyl;

aroylalkyl having up to 10 carbon atoms, such as phenacyl or 2-benzoylethyl;

aryl, such as phenyl or 1-naphthyl;

heteroaryl, such as 4-quinolyl;

alkanoyl having from 1 to 10 carbon atoms, such as acetyl or butyryl;

aroyl, such as benzoyl;

heteroaroyl, such as 3-quinoloyl;

OR' or NR'R'' where R' and R'' are independently hydrogen, alkyl, aryl, heteroaryl, acyl, aroyl, sulfonyl, sulfinyl, or SO_2-R''' or SO-R''' where R''' is substituted or unsubstituted alkyl, aryl, heteroaryl, alkenyl, or alkynyl.

[0058] Capping functions that provide for an ester bond are designated as OR, wherein R may be: alkoxy; aryloxy; heteroaryloxy; aralkyloxy; heteroaralkyloxy; substituted alkoxy; substituted aryloxy; substituted heteroaryloxy; substituted aralkyloxy; or substituted heteroaralkyloxy.

[0059] Either the N-terminal or the C-terminal capping function, or both, may be of such structure that the capped molecule functions as a prodrug (a pharmacologically inactive derivative of the parent drug molecule) that undergoes spontaneous or enzymatic transformation within the body in order to release the active drug and that has improved delivery properties over the parent drug molecule (Bundgaard H, Ed: *Design of Prodrugs*, Elsevier, Amsterdam, 1985).

[0060] Judicious choice of capping groups allows the addition of other activities on the peptide. For example, the presence of a sulfhydryl group linked to the N- or C-terminal cap will permit conjugation of the derivatized peptide to other molecules.

[0061] In yet a further aspect, the peptides or fragments or derivatives thereof can be "retro-inverso peptides." A "retro-inverso peptide" refers to a peptide with a reversal of the direction of the peptide bond on at least one position, *i.e.*, a reversal of the amino- and carboxy-termini with respect to the side chain of the amino acid. Thus, a retro-inverso analogue has reversed termini and reversed direction of peptide bonds while approximately maintaining the topology of the side chains as in the native peptide sequence. The retro-inverso peptide can contain L-amino acids or D-amino acids, or a mixture of L-amino acids and D-amino acids, up to all of the amino acids being the D- isomer. Partial retro-inverso peptide analogues are polypeptides in which only part of the sequence is reversed and replaced with enantiomeric amino acid residues. Since the retro- inverted portion of such an analogue has reversed amino and carboxyl termini, the amino acid residues flanking the retro-inverted portion are replaced by side -chain-analogous a-substituted geminal-diaminomethanes and malonates, respectively. Retro-inverso forms of cell penetrating peptides have been found to work as efficiently in translocating across a membrane as the natural forms. Synthesis of retro-inverso peptide analogues are described in Bonelli, F. *et al., Int J Pept Protein Res.* 24(6):553-6 (1984); Verdini, A and Viscomi, G. C, J. *Chem. Soc. Perkin Trans.* 1 :697-701 (1985); and U.S. Patent No. 6,261,569, which are incorporated herein in their entirety by reference. Processes for the solid-phase synthesis of partial retro-inverso peptide analogues have been described (EP 97994-B) which is also incorporated herein in its entirety by reference.

[0062] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 80%, 85%, 90%, or 95%) of "sequence identity" or “homology” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Current Protocols In Molecular Biology (F. M. Ausubel *et al.*, eds., 1987) Supplement 30, section 7.7.18, Table 7.7.1. Preferably, default parameters are used for alignment. A preferred alignment program is BLAST, using default parameters. In particular, preferred programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10;

Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR.

A. Multimeric Polypeptides

[0063] Embodiments of the present disclosure also include longer polypeptides built from repeating units of a modified Cav-1 variant polypeptide. A polypeptide multimer may comprise different combinations of polypeptide. Such multimeric polypeptides can be made by chemical synthesis or by recombinant DNA techniques as discussed herein. When produced by chemical synthesis, the oligomers preferably have from 2-5 repeats of a core polypeptide sequence, and the total number of amino acids in the multimer should not exceed about 160 residues, preferably not more than 100 residues (or their equivalents, when including linkers or spacers).

B. Peptidomimetics

[0064] The modified Cav-1 peptide may be a peptidomimetic compound which mimics the biological effects of the native Cav-1 polypeptide. A peptidomimetic agent may be an unnatural peptide or a non-peptide agent that recreates the stereospatial properties of the binding elements of the native Cav-1 polypeptide such that it has the binding activity and biological activity of the native Cav-1 polypeptide. Similar to a native Cav-1 polypeptide or polypeptide multimer, a peptidomimetic will have a binding face (which interacts with any ligand to which native Cav-1 binds) and a non-binding face.

[0065] In some aspects, the present disclosure also includes compounds that retain partial peptide characteristics. For example, any proteolytically unstable bond within a peptide of the invention could be selectively replaced by a non-peptidic element such as an isostere (N-methylation; D-amino acid) or a reduced peptide bond while the rest of the molecule retains its peptidic nature.

[0066] Peptidomimetic compounds, either agonists, substrates or inhibitors, have been described for a number of bioactive peptides/polypeptides such as opioid peptides, VIP, thrombin, HIV protease, *etc.* Methods for designing and preparing peptidomimetic compounds are known in the art (Hruby, VJ, *Biopolymers 33*:1073-1082 (1993); Wiley, RA *et al.*, *Med. Res. Rev. 13*:327-384 (1993); Moore *et al.*, *Adv. in Pharmacol 33*:91-141 (1995); Giannis *et*

al., Adv. in Drug Res. 29:1-78 (1997). Certain mimetics that mimic secondary structure are described in Johnson *et al.*, In: *Biotechnology and Pharmacy*, Pezzuto *et al.*, Chapman and Hall (Eds.), NY, 1993. These methods are used to make peptidomimetics that possess at least the binding capacity and specificity of the native Cav-1 polypeptide and preferably also possess the biological activity. Knowledge of peptide chemistry and general organic chemistry available to those skilled in the art are sufficient, in view of the present disclosure, for designing and synthesizing such compounds.

[0067] For example, such peptidomimetics may be identified by inspection of the three-dimensional structure of a polypeptide of the invention either free or bound in complex with a ligand (*e.g.*, soluble uPAR or a fragment thereof). Alternatively, the structure of a polypeptide of the invention bound to its ligand can be gained by the techniques of nuclear magnetic resonance spectroscopy. Greater knowledge of the stereochemistry of the interaction of the peptide with its ligand or receptor will permit the rational design of such peptidomimetic agents. The structure of a peptide or polypeptide of the invention in the absence of ligand could also provide a scaffold for the design of mimetic molecules.

C. PEGylation

[0068] The modified Cav-1 peptides may be conjugated with heterologous polypeptide segments or polymers, such as polyethylene glycol. The polypeptides may be linked to PEG to increase the hydrodynamic radius of the enzyme and hence increase the serum persistence. The polypeptides may be conjugated to any targeting agent, such as a ligand having the ability to specifically and stably bind to an external receptor (U.S. Patent Publ. 2009/0304666).

[0069] In certain aspects, methods and compositions of the embodiments related to PEGylation of disclosed polypeptides. PEGylation is the process of covalent attachment of poly(ethylene glycol) polymer chains to another molecule, normally a drug or therapeutic protein. PEGylation is routinely achieved by incubation of a reactive derivative of PEG with the target macromolecule. The covalent attachment of PEG to a drug or therapeutic protein can "mask" the agent from the host's immune system (reduced immunogenicity and antigenicity) or increase the hydrodynamic size (size in solution) of the agent, which prolongs its circulatory time by reducing renal clearance. PEGylation can also provide water solubility to hydrophobic drugs and proteins.

[0070] The first step of the PEGylation is the suitable functionalization of the PEG polymer at one or both terminals. PEGs that are activated at each terminus with the same reactive moiety are known as "homobifunctional," whereas if the functional groups present are different, then the PEG derivative is referred as "heterobifunctional" or "heterofunctional." The chemically active or activated derivatives of the PEG polymer are prepared to attach the PEG to the desired molecule.

[0071] The choice of the suitable functional group for the PEG derivative is based on the type of available reactive group on the molecule that will be coupled to the PEG. For proteins, typical reactive amino acids include lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, and tyrosine. The N-terminal amino group and the C-terminal carboxylic acid can also be used.

[0072] The techniques used to form first generation PEG derivatives are generally reacting the PEG polymer with a group that is reactive with hydroxyl groups, typically anhydrides, acid chlorides, chloroformates, and carbonates. In the second generation PEGylation chemistry more efficient functional groups, such as aldehyde, esters, amides, *etc.*, are made available for conjugation.

[0073] As applications of PEGylation have become more and more advanced and sophisticated, there has been an increase in need for heterobifunctional PEGs for conjugation. These heterobifunctional PEGs are very useful in linking two entities, where a hydrophilic, flexible, and biocompatible spacer is needed. Preferred end groups for heterobifunctional PEGs are maleimide, vinyl sulfones, pyridyl disulfide, amine, carboxylic acids, and NHS esters.

[0074] The most common modification agents, or linkers, are based on methoxy PEG (mPEG) molecules. Their activity depends on adding a protein-modifying group to the alcohol end. In some instances polyethylene glycol (PEG diol) is used as the precursor molecule. The diol is subsequently modified at both ends in order to make a hetero- or homo-dimeric PEG-linked molecule.

[0075] Proteins are generally PEGylated at nucleophilic sites, such as unprotonated thiols (cysteinyl residues) or amino groups. Examples of cysteinyl-specific modification reagents include PEG maleimide, PEG iodoacetate, PEG thiols, and PEG vinylsulfone. All four are strongly cysteinyl-specific under mild conditions and neutral to slightly alkaline pH

but each has some drawbacks. The thioether formed with the maleimides can be somewhat unstable under alkaline conditions so there may be some limitation to formulation options with this linker. The carbamothioate linkage formed with iodo PEGs is more stable, but free iodine can modify tyrosine residues under some conditions. PEG thiols form disulfide bonds with protein thiols, but this linkage can also be unstable under alkaline conditions. PEG-vinylsulfone reactivity is relatively slow compared to maleimide and iodo PEG; however, the thioether linkage formed is quite stable. Its slower reaction rate also can make the PEG-vinylsulfone reaction easier to control.

[0076] Site-specific PEGylation at native cysteinyl residues is seldom carried out, since these residues are usually in the form of disulfide bonds or are required for biological activity. On the other hand, site-directed mutagenesis can be used to incorporate cysteinyl PEGylation sites for thiol-specific linkers. The cysteine mutation must be designed such that it is accessible to the PEGylation reagent and is still biologically active after PEGylation.

[0077] Amine-specific modification agents include PEG NHS ester, PEG tresylate, PEG aldehyde, PEG isothiocyanate, and several others. All react under mild conditions and are very specific for amino groups. The PEG NHS ester is probably one of the more reactive agents; however, its high reactivity can make the PEGylation reaction difficult to control on a large scale. PEG aldehyde forms an imine with the amino group, which is then reduced to a secondary amine with sodium cyanoborohydride. Unlike sodium borohydride, sodium cyanoborohydride will not reduce disulfide bonds. However, this chemical is highly toxic and must be handled cautiously, particularly at lower pH where it becomes volatile.

[0078] Due to the multiple lysine residues on most proteins, site-specific PEGylation can be a challenge. Fortunately, because these reagents react with unprotonated amino groups, it is possible to direct the PEGylation to lower-pK amino groups by performing the reaction at a lower pH. Generally the pK of the alpha-amino group is 1-2 pH units lower than the epsilon-amino group of lysine residues. By PEGylating the molecule at pH 7 or below, high selectivity for the N-terminus frequently can be attained. However, this is only feasible if the N-terminal portion of the protein is not required for biological activity. Still, the pharmacokinetic benefits from PEGylation frequently outweigh a significant loss of *in vitro* bioactivity, resulting in a product with much greater *in vivo* bioactivity regardless of PEGylation chemistry.

[0079] There are several parameters to consider when developing a PEGylation procedure. Fortunately, there are usually no more than four or five key parameters. The "design of experiments" approach to optimization of PEGylation conditions can be very useful. For thiol-specific PEGylation reactions, parameters to consider include: protein concentration, PEG-to-protein ratio (on a molar basis), temperature, pH, reaction time, and in some instances, the exclusion of oxygen. (Oxygen can contribute to intermolecular disulfide formation by the protein, which will reduce the yield of the PEGylated product.) The same factors should be considered (with the exception of oxygen) for amine-specific modification except that pH may be even more critical, particularly when targeting the N-terminal amino group.

[0080] For both amine- and thiol-specific modifications, the reaction conditions may affect the stability of the protein. This may limit the temperature, protein concentration, and pH. In addition, the reactivity of the PEG linker should be known before starting the PEGylation reaction. For example, if the PEGylation agent is only 70 percent active, the amount of PEG used should ensure that only active PEG molecules are counted in the protein-to-PEG reaction stoichiometry.

D. Fusion Proteins

[0081] Certain embodiments of the present invention concern fusion proteins of the modified Cav-1 peptides. These molecules may have the polypeptides of the embodiments linked at the N- or C-terminus to a heterologous domain. For example, fusions may also employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Fusion proteins can comprise a half-life extender. Another useful fusion includes the addition of a protein affinity tag, such as a serum albumin affinity tag or six histidine residues, or an immunologically active domain, such as an antibody epitope, preferably cleavable, to facilitate purification of the fusion protein. Non-limiting affinity tags include polyhistidine, chitin binding protein (CBP), maltose binding protein (MBP), and glutathione-S-transferase (GST).

[0082] In a particular embodiment, the peptide of the embodiments may be linked to a peptide that increases the *in vivo* half-life, such as an XTEN® polypeptide (Schellenberger *et al.*, 2009), IgG Fc domain, albumin, or albumin binding peptide.

[0083] Methods of generating fusion proteins are well known to those of skill in the art. Such proteins can be produced, for example, by *de novo* synthesis of the complete fusion

protein, or by attachment of the DNA sequence encoding the heterologous domain, followed by expression of the intact fusion protein.

[0084] Production of fusion proteins that recover the functional activities of the parent proteins may be facilitated by connecting genes with a bridging DNA segment encoding a peptide linker that is spliced between the polypeptides connected in tandem. The linker would be of sufficient length to allow proper folding of the resulting fusion protein.

1. Linkers

[0085] In certain embodiments, the polypeptide of the embodiments may be chemically conjugated using bifunctional cross-linking reagents or fused at the protein level with peptide linkers.

[0086] Bifunctional cross-linking reagents have been extensively used for a variety of purposes, including preparation of affinity matrices, modification and stabilization of diverse structures, identification of ligand and receptor binding sites, and structural studies. Suitable peptide linkers may also be used to link the polypeptide of the embodiments, such as Gly-Ser linkers.

[0087] Homobifunctional reagents that carry two identical functional groups proved to be highly efficient in inducing cross-linking between identical and different macromolecules or subunits of a macromolecule, and linking of polypeptide ligands to their specific binding sites. Heterobifunctional reagents contain two different functional groups. By taking advantage of the differential reactivities of the two different functional groups, cross-linking can be controlled both selectively and sequentially. The bifunctional cross-linking reagents can be divided according to the specificity of their functional groups, *e.g.*, amino-, sulfhydryl-, guanidine-, indole-, carboxyl-specific groups. Of these, reagents directed to free amino groups have become especially popular because of their commercial availability, ease of synthesis, and the mild reaction conditions under which they can be applied.

[0088] A majority of heterobifunctional cross-linking reagents contain a primary amine-reactive group and a thiol-reactive group. In another example, heterobifunctional cross-linking reagents and methods of using the cross-linking reagents are described (U.S. Pat. No. 5,889,155, specifically incorporated herein by reference in its entirety). The cross-linking reagents combine a nucleophilic hydrazide residue with an electrophilic maleimide residue,

allowing coupling, in one example, of aldehydes to free thiols. The cross-linking reagent can be modified to cross-link various functional groups.

[0089] Additionally, any other linking/coupling agents and/or mechanisms known to those of skill in the art may be used to combine polypeptides of the embodiments, such as, for example, antibody-antigen interaction, avidin biotin linkages, amide linkages, ester linkages, thioester linkages, ether linkages, thioether linkages, phosphoester linkages, phosphoramide linkages, anhydride linkages, disulfide linkages, ionic and hydrophobic interactions, bispecific antibodies and antibody fragments, or combinations thereof.

[0090] It is preferred that a cross-linker having reasonable stability in blood will be employed. Numerous types of disulfide-bond containing linkers are known that can be successfully employed to conjugate targeting and therapeutic/preventative agents. Linkers that contain a disulfide bond that is sterically hindered may prove to give greater stability *in vivo*. These linkers are thus one group of linking agents.

[0091] In addition to hindered cross-linkers, non-hindered linkers also can be employed in accordance herewith. Other useful cross-linkers, not considered to contain or generate a protected disulfide, include SATA, SPDP, and 2-iminothiolane (Wawrzynczak and Thorpe, 1987). The use of such cross-linkers is well understood in the art. Another embodiment involves the use of flexible linkers.

[0092] Once chemically conjugated, the peptide generally will be purified to separate the conjugate from unconjugated agents and from other contaminants. A large number of purification techniques are available for use in providing conjugates of a sufficient degree of purity to render them clinically useful.

[0093] Purification methods based upon size separation, such as gel filtration, gel permeation, or high performance liquid chromatography, will generally be of most use. Other chromatographic techniques, such as Blue-Sepharose separation, may also be used. Conventional methods to purify the fusion proteins from inclusion bodies may be useful, such as using weak detergents, such as sodium N-lauroyl-sarcosine (SLS).

2. Cell Penetrating and Membrane Translocation Peptides

[0094] Furthermore, in certain aspects, the modified Cav-1 peptides may further comprise a cell-binding domain or cell penetrating peptide (CPP). As used herein the terms

"cell penetrating peptide" and "membrane translocation domain" are used interchangeably and refer to segments of polypeptide sequence that allow a polypeptide to cross the cell membrane (*e.g.*, the plasma membrane in the case a eukaryotic cell). Examples of CPP segments include, but are not limited to, segments derived from HIV Tat (*e.g.*, GRKKRRQRRRPPQ (SEQ ID NO: 21)), herpes virus VP22, the *Drosophila* Antennapedia homeobox gene product, protegrin I, Penetratin (RQIKIWFQNRRMKWKK (SEQ ID NO: 22)) or melittin (GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO: 23)). In certain aspects the CPP comprises the T1 (TKIESLKEHG (SEQ ID NO: 24)), T2 (TQIENLKEKG (SEQ ID NO: 25)), 26 (AALEALAEALEALAEALEALAEAAAA (SEQ ID NO: 26)) or INF7 (GLFEAIEGFIENGWEGMIEGWYGCG (SEQ ID NO: 27)) CPP sequence.

III. Methods of Use

[0095] One aspect of the present invention relates to the use of polypeptides described herein and mutants, variants, analogs or derivatives thereof. Specifically, these methods relate to administering any one of the polypeptides as described herein or their pharmaceutically acceptable modifications in a pharmaceutically acceptable carrier to a subject, a composition for use in the treatment of treating or preventing a disease, injury or infection of the lungs (*e.g.*, a fibrotic condition of the lungs), said composition comprising a polypeptide of the embodiments in pharmaceutically acceptable carrier.

A. Pharmaceutical Compositions

[0096] It is contemplated that the modified Cav-1 peptides can be administered systemically or locally to inhibit cell apoptosis and for the treatment and prevention damage to lung tissues. They can be administered intravenously, intrathecally, and/or intraperitoneally. In particular aspects, the polypeptides are delivered locally to the airway, such as administration of a nebulized formulation or a dry powder formulation for inhalation. They can be administered alone or in combination with anti-fibrotic compounds.

[0097] The modified Cav-1 peptide may be administered in combination, simultaneously or sequentially with at least one additional therapeutic for lung fibrosis. The additional therapeutic may be an NSAID, steroid, DMARD, immunosuppressive, biologic response modulators, bronchodilator or antifibrotic agent such as pirfenedone, an agent whose antifibrotic mechanism of action is not fully understood but may involve blockade of TGF-beta, nintedanib, a broad tyrosine kinase blocker or any other antifibrotic agent. Suitable

NSAIDS are selected from the non-selective COX-inhibitors acetylsalicyclic acid, mesalazin, ibuprofen, naproxen, flurbiprofen, fenoprofen, fenbufen, ketoprofen, indoprofen, pirprofen, carprofen, oxaprozin, pranoprofen, miroprofen, tioxaprofen, suprofen, alminoprofen, tiaprofenic acid, fluprofen, indomethacin, sulindac, tolmetin, zomepirac, nabumetone, diclofenac, fenclofenac, alclofenac, bromfenac, ibufenac, aceclofenac, acemetacin, fentiazac, clidanac, etodolac, oxpinac, mefenamic acid, meclofenamic acid, flufenamic acid, nifluminic acid, tolfenamic acid, diflunisal, flufenisal, piroxicam, tenoxicam, lornoxicam and nimesulide and the pharmaceutically acceptable salts thereof, the selective COX 2-inhibitors meloxicam, celecoxib and rofecoxib and the pharmaceutically acceptable salts thereof. Suitable steroids are prednisone, prednisolone, methylprednisolone, dexamethasone, budenoside, fluocortolone and triamcinolone. Suitable DMARDs are sulfasalazine, olsalazine, chloroquin, gold derivatives (Auranofin), D-penicillamine and cytostatics such as methotrexate and cyclophosphamide. Suitable immunsuppressives are cyclosporine A and derivatives thereof, mycophenolatemofetil, FK 506, OKT-3, ATG, 15-desoxyspergualin, mizoribine, misoprostol, rapamycin, reflunomide and azathioprine. Suitable biologic response modifiers are interferon β, anti-TNF-α (Etanercept), IL-10, anti-CD3 or anti-CD25. Suitable bronchodilators are ipratropiumbromide, oxytropiumbromide, tiotropiumbromide, epinephrinehydrochloride, salbutamole, terbutalinsulfate, fenoterolhydrobromide, salmeterole and formoterole. In such combinations each active ingredient can be administered either in accordance with its usual dosage range or a dose below its usual dosage range. The dosage for the combined NSAIDs, steroids, DMARDs, immunosuppressives and biologic response modifiers is appropriately 1/50 of the lowest dose normally recommended up to 1/1 of the normally recommended dosage, preferably 1/20 to 1/2 and more preferably 1/10 to 1/5. The normally recommended dose for the combined drug should be understood to be the dose disclosed for example in Rote Liste® 2002, Editio Cantor Verlag Aulendorf, Germany, or in Physician's Desk Reference.

[0098] Where clinical applications are contemplated, it may be necessary to prepare pharmaceutical compositions comprising proteins, antibodies, and drugs in a form appropriate for the intended application. Generally, pharmaceutical compositions may comprise an effective amount of one or more of the polypeptides of the embodiments or additional agents dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, such

as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one polypeptide of the embodiments isolated by the method disclosed herein, or additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed., 1990, incorporated herein by reference. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety, and purity standards as required by the FDA Office of Biological Standards.

[0099] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed., 1990, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the pharmaceutical compositions is contemplated.

[00100] Certain embodiments of the present invention may comprise different types of carriers depending on whether it is to be administered in solid, liquid, or aerosol form, and whether it needs to be sterile for the route of administration, such as injection. The compositions can be administered intravenously, intrathecally, intradermally, transdermally, intrathecally, intraarterially, intraperitoneally, intranasally, intravaginally, intrarectally, intramuscularly, subcutaneously, mucosally, orally, topically, locally, by inhalation (*e.g.*, inhalation of a nebulized or dry powder formulation), by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, *via* a catheter, *via* a lavage, in lipid compositions (*e.g.*, liposomes), or by other methods or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed., 1990, incorporated herein by reference).

[00101] The modified polypeptides may be formulated into a composition in a free base, neutral, or salt form. Pharmaceutically acceptable salts include the acid addition salts, *e.g.*, those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids, such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, or mandelic acid. Salts formed with the free carboxyl groups

can also be derived from inorganic bases, such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine, or procaine. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as formulated for parenteral administrations, such as injectable solutions, or aerosols for delivery to the lungs, or formulated for alimentary administrations, such as drug release capsules and the like.

[00102] Further in accordance with certain aspects of the present invention, the composition suitable for administration may be provided in a pharmaceutically acceptable carrier with or without an inert diluent. The carrier should be assimilable and includes liquid, semi-solid, *i.e.*, pastes, or solid carriers. Except insofar as any conventional media, agent, diluent, or carrier is detrimental to the recipient or to the therapeutic effectiveness of a composition contained therein, its use in administrable composition for use in practicing the methods is appropriate. Examples of carriers or diluents include fats, oils, water, saline solutions, lipids, liposomes, resins, binders, fillers, and the like, or combinations thereof. The composition may also comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives, such as various antibacterial and antifungal agents, including but not limited to parabens (*e.g.*, methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

[00103] In accordance with certain aspects of the present invention, the composition is combined with the carrier in any convenient and practical manner, *i.e.*, by solution, suspension, emulsification, admixture, encapsulation, absorption, and the like. Such procedures are routine for those skilled in the art.

[00104] In a specific embodiment of the present invention, the composition is combined or mixed thoroughly with a semi-solid or solid carrier. The mixing can be carried out in any convenient manner, such as grinding. Stabilizing agents can be also added in the mixing process in order to protect the composition from loss of therapeutic activity, *i.e.*, denaturation in the stomach. Examples of stabilizers for use in a composition include buffers, amino acids, such as glycine and lysine, carbohydrates or lyoprotectants, such as dextrose, mannose, galactose, fructose, lactose, sucrose, maltose, sorbitol, mannitol, *etc.*

[00105] In some aspects, a pharmaceutical formulation comprises one or more surfactant. Surfactants used in accordance with the disclosed methods include ionic and non-ionic surfactants. Representative non-ionic surfactants include polysorbates such as TWEEN®-20 and TWEEN-80® surfactants (ICI Americas Inc. of Bridgewater, N.J.); poloxamers (*e.g.*, poloxamer 188); TRITON® surfactants (Sigma of St. Louis, Mo.); sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palnidopropyl-, or (*e.g.*, lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; MONAQUAT™ surfactants (Mona Industries Inc. of Paterson, N.J.); polyethyl glycol; polypropyl glycol; block copolymers of ethylene and propylene glycol such as PLURONIC® surfactants (BASF of Mt. Olive, N.J.); oligo (ethylene oxide) alkyl ethers; alkyl (thio) glucosides, alkyl maltosides; and phospholipids. For example, the surfactant can be present in a formulation in an amount from about 0.01% to about 0.5% (weight of surfactant relative to total weight of other solid components of the formulation; "w/w"), from about 0.03% to about 0.5% (w/w), from about 0.05% to about 0.5% (w/w), or from about 0.1% to about 0.5% (w/w). However, in further aspects, a pharmaceutical formulation of the embodiments is essentially free of non-ionic surfactants or essentially free of all surfactants.

[00106] With respect to the therapeutic methods of the invention, it is not intended that the administration of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof and be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention contemplates all modes of administration, including intramuscular, intravenous, intraperitoneal, intravesicular, intraarticular, intralesional, subcutaneous, or any other route sufficient to provide a dose adequate to treat the inflammation-related disorder. The therapeutic may be administered to the patient in a single dose or in multiple doses. When multiple doses are administered, the doses may be separated from one another by, for example, one hour, three hours, six hours, eight hours, one day, two days, one week, two weeks, or one month. For example, the therapeutic may be administered for, *e.g.*, 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or more weeks. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the

compositions. For example, the dosage of the therapeutic can be increased if the lower dose does not provide sufficient therapeutic activity.

[00107] While the attending physician ultimately will decide the appropriate amount and dosage regimen, therapeutically effective amounts of the one or more polypeptides as disclosed herein or a mutant, variant, analog or derivative thereof may be provided at a dose of 0.0001, 0.01, 0.01 0.1, 1, 5, 10, 25, 50, 100, 500, or 1,000 mg/kg or g/kg. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test bioassays or systems.

[00108] Dosages for a particular patient or subject can be determined by one of ordinary skill in the art using conventional considerations, (*e.g.*, by means of an appropriate, conventional pharmacological protocol). A physician may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. The dose administered to a patient is sufficient to effect a beneficial therapeutic response in the patient over time, or, *e.g.*, to reduce symptoms, or other appropriate activity, depending on the application. The dose is determined by the efficacy of the particular formulation, and the activity, stability or serum half- life of the one or more polypeptides as disclosed herein or a mutant, variant, analog or derivative thereof and the condition of the patient, as well as the body weight or surface area of the patient to be treated.

[00109] In some aspects, a subject is given a single dose, given once daily for treating a subject, preferably a mammal, more preferably human who his suffering from or susceptible to pulmonary fibrosis resulting therefrom is between about 0.2 mg/kg and about 250 mg/kg, such as between about 10 mg/kg and about 50 mg/kg, for example, via instillation (by inhalation). Such a dose can be administered daily for anywhere from about 3 days to one or more weeks. Chronic administration is also possible, though the dose may need to be adjusted downward as is well-understood in the art. The foregoing ranges are, however, suggestive, as the number of variables in an individual treatment regime is large, and considerable excursions from these preferred values are expected.

[00110] For continuous administration, *e.g.*, by a pump system such as an osmotic pump that was used in some of the experiments described below, a total dosage for a time course of about 1-2 weeks is preferably in the range of 1 mg/kg to 1 g/kg, preferably 20-300 mg/kg, more preferably 50-200 mg/kg. After such a continuous dosing regimen, the total concentration of

the active compound is preferably in the range of about 0.5 to about 50 μM, preferably about 1 to about 10 μM.

[00111] An effective concentration of the active compound for inhibiting or preventing inhibiting apoptosis *in vitro* is in the range of about 0.5 nM to about 100 nM, more preferably from about 2 nM to about 20 nM. Effective doses and optimal dose ranges may be determined *in vitro* using the methods described herein.

B. Aerosol Dispersion and Nebulizing Devices

[00112] The formulations can be aerosolized using any suitable device, including but not limited to a jet nebulizer, an ultrasonic nebulizer, a metered dose inhaler (MDI), and a device for aerosolization of liquids by forced passage through a jet or nozzle (*e.g.,* AERX® drug delivery devices by Aradigm of Hayward, Calif.). Furthermore, the compounds can be formeulated as dry powders for delivery using a dry powder inhaler device. For delivery of a formulation to a subject, as described further herein below, an pulmonary delivery device can also include a ventilator, optionally in combination with a mask, mouthpiece, mist inhalation apparatus, and/or a platform that guides users to inhale correctly and automatically deliver the drug at the right time in the breath. Representative aerosolization devices that can be used in accordance with the methods of the present invention include but are not limited to those described in U.S. Pat. Nos. 6,357,671; 6,354,516; 6,241,159; 6,044,841; 6,041,776; 6,016,974; 5,823,179; 5,797,389; 5,660,166; 5,355,872; 5,284,133; and 5,277,175 and U.S. Published Patent Application Nos. 20020020412 and 20020020409.

[00113] Using a jet nebulizer, compressed gas from a compressor or hospital air line is passed through a narrow constriction known as a jet. This creates an area of low pressure, and liquid medication from a reservoir is drawn up through a feed tube and fragmented into droplets by the air stream. Only the smallest drops leave the nebulizer directly, while the majority impact on baffles and walls and are returned to the reservoir. Consequently, the time required to perform jet nebulization varies according to the volume of the composition to be nebulized, among other factors, and such time can readily be adjusted by one of skill in the art.

[00114] A metered dose inhalator (MDI) can be used to deliver a composition of the invention in a more concentrated form than typically delivered using a nebulizer. For optimal effect, MDI delivery systems require proper administration technique, which includes coordinated actuation of aerosol delivery with inhalation, a slow inhalation of about 0.5-0.75

liters per second, a deep breath approaching inspiratory capacity inhalation, and at least 4 seconds of breath holding. Pulmonary delivery using a MDI is convenient and suitable when the treatment benefits from a relatively short treatment time and low cost. Optionally, a formulation can be heated to about 25° C. to about 90° C. during nebulization to promote effective droplet formation and subsequent delivery. See e.g., U.S. Pat. No. 5,299,566.

[00115] Aerosol compositions of the embodiments comprise droplets of the composition that are a suitable size for efficient delivery within the lung. In some cases, a surfactant formulation is delivered to lung bronchi, more preferably to bronchioles, still more preferably to alveolar ducts, and still more preferably to alveoli. Aerosol droplets are typically less than about 15 μm in diameter, less than about 10 μm in diameter, less than about 5 μm in diameter, or less than about 2 μm in diameter. For efficient delivery to alveolar bronchi of a human subject, an aerosol composition may preferably comprise droplets having a diameter of about 1 μm to about 5 μm.

[00116] Droplet size can be assessed using techniques known in the art, for example cascade, impaction, laser diffraction, and optical patternation. See McLean *et al.* (2000) *Anal Chem* 72:4796-804, Fults *et al.* (1991) *J Pharm Pharmacol* 43:726-8, and Vecellio None *et al.* (2001) *J Aerosol Med* 14:107-14.

[00117] Protein stability following aerosolization can be assessed using known techniques in the art, including size exclusion chromatography; electrophoretic techniques; spectroscopic techniques such as UV spectroscopy and circular dichroism spectroscopy, and protein activity (measured in vitro or in vivo). To perform in vitro assays of protein stability, an aerosol composition can be collected and then distilled or absorbed onto a filter. To perform in vivo assays, or for pulmonary administration of a composition to a subject, a device for aerosolization is adapted for inhalation by the subject. For example, protein stability can be assessed by determining the level of protein aggregation. Preferably, an aerosol composition of the invention is substantially free of protein aggregates. The presence of soluble aggregates can be determined qualitatively using DLS (DynaPro-801TC, ProteinSolutions Inc. of Charlottesville, Va.) and/or by UV spectrophotometry.

[00118] The term "vibrating mesh nebulizer" refers herein to any nebulizer that operates on the general principle of using a vibrating mesh or plate with multiple aperatures (an aperture plate) to generate a fine-particle, low-velocity aerosol. Some nebulizers may contain a

mesh/membrane with between 1000 and 7000 holes, which mesh/membrane vibrates at the top of a liquid reservoir (see, *e.g.*, U.S. Patent Publn. 20090134235 and Waldrep and Dhand 2008, each incorporated herein by reference). In some embodiments, the vibrating mesh nebulizer is an AERONEB® Professional Nebulizer, Omron MICROAIR®, Pari EFLOW® or an EZ Breathe Atomizer. In some aspects, a vibrating mesh nebulizer has a vibrating frequency of between about 50-250 kHz, 75-200 kHz 100-150 kHz or about 120 kHz. These devices have a high efficiency of delivering aerosol to the lung and the volume of liquid remaining in these devices is minimal, which is an advantage for expensive and potent compounds like plasminogen activators.

[00119] In certain aspects, a nebulized composition of the embodiments is produced using a vibrating mesh nebulizer. For example, the composition can be produced with an active vibrating mesh nebulizer (*e.g.*, an Aeroneb® Professional Nebulizer System). Descriptions of such system and there operation can be found, for instance, in U.S. Patents Nos. 6,921,020; 6,926,208; 6,968,840; 6,978,941; 7,040,549; 7,083,112; 7,104,463; and 7,360,536, each of which is incorporated herein by reference in its entirety. In yet further aspects, a composition of the embodiments can be produced with a passive vibrating mesh nebulizer, such as the Omron MicroAir® or the EZ Breathe Atomizer.

IV. Pulmonary conditions for treatment

[00120] Modified peptides of the present invention can be used to treat a variety of pulmonary conditions. Pulmonary conditions for treatment may be acute or chronic. Acute pulmonary conditions may be acute lung injury, infection or chemical-induced. Chronic pulmonary conditions maybe the result of injury, infection or disease.

A. Lung injuries

[00121] In some aspects, the subject has an acute lung injury (ALI) or infection or a chemical-induced lung injury. In specific aspects, the subject has acute respiratory distress syndrome (ARDS), inhalational smoke induced acute lung injury (ISALI), bronchiectasis, inhalational toxin-induced airway disease (e.g., chlorine or other induced airways disease), exposure to mustard gas, exposure to particulate matter (e.g., silica dust), bronchiolitis obliterans, bronchiolitis obliterans organizing pneumonia, drug induced lung disease and accelerated pulmonary fibrosis (e.g., that occurs after acute lung injury including ARDS).

Acute lung injury (ALI) is a serious medical problem amongst American military personnel. ALI during combat can result from very broad etiologies.

[00122] ALI from inhalational injury has been treated with inhaled anticoagulants, steroids, beta-agonists, high frequency ventilation, and extra-corporeal membrane oxygenation, with variable and, in general, suboptimal results. No effective preventive measures are available other than barriers with respiratory masks. The management of ARDS has progressed significantly but remains largely supportive with watchful waiting for endogenous healing mechanisms to take effect; and in-hospital mortality remains above 40% (Matthay et al., 2012). Survivors of ALI often suffer chronic respiratory disability with reduced quality of life. Any modalities that can accelerate recovery and/or prevent later complications such as chronic respiratory insufficiency and pulmonary fibrosis will be highly desirable. There is a dire need to improve the early diagnosis and much more importantly, prevention and therapy of ALI. The pathophysiology of ALI from direct inhalational lung injury or ARDS consequent to systemic illness is extremely complex and heterogeneous, encompassing systemic as well as local cardiopulmonary factors such as increased membrane permeability, influx of inflammatory cytokines, oxidative cellular damage, compartmental fluid shifts, deranged ion channels, and many others (Matthay et al., 2012). Clearly, novel treatments are needed for treating and preventing lung disorders such as ALI.

[00123] In some embodiments, there is provided a method of treating or preventing acute lung injury, lung infection or lung disease in a subject comprising administering to the subject an effective amount of a variant polypeptide comprising at least one amino acid substitution, deletion of insertion relative to the amino acid sequence of FTTFTVT (SEQ ID NO:2), wherein the variant polypeptide maintains the biological activity of caveolin-1 (Cav-1). In some aspects, a method of administering a pharmaceutical formulation of the embodiments comprises nebulizing a solution comprising a variant polypeptide. In particular aspects, the subject is a human.

B. Lung diseases

[00124] Lung diseases include cystic fibrosis, chronic obstructive pulmonary disease (COPD), asthma, bronchiolitis obliterans, plastic bronchitis, and pulmonary infections, collagen vascular lung disease (e.g., from lupus, scleroderma or mixed connective tissue disease), interstitial lung disease (e.g., idiopathic pulmonary fibrosis or sarcoidosis), as well as

acute and chronic lung injury leading to fibrosis (Murray *et al.*, 1997; Rabe *et al.*, 2007; Tsushima *et al.*, 2009). These diseases constitute the third leading cause of death world-wide.

[00125] Cystic fibrosis is an inherited disease of the exocrine glands and exocrine sweat glands which primarily affects the digestive and respiratory systems. This disease usually characterized by chronic respiratory infections, pancreatic insufficiency, abnormally viscid mucuous secretions and premature death. Cystic fibrosis (CF) is characterized by progressive airflow obstruction. Subsets of individuals with CF also develop airway hyper-responsiveness to inhaled cholinergic agonists (Weinberger, 2002 and Mitchell *et al.*, 1978) and reversibility of airflow limitation in response to bronchodilators (van Haren *et al.*, 1991 and van Haren *et al.*, 1992). The presence of bronchial hyper-responsiveness and airway obstruction suggest a possible shared etiology of disease between CF and other diseases of airway narrowing such as asthma or chronic obstructive pulmonary disease (COPD) where airway smooth muscle dysfunction is thought to contribute to the disease processes.

[00126] A pulmonary infection may be a bacterial infection. The infectious bacteria may be *Pseudomonas aeruginosa, Bacillus anthracis, Listeria monocytogenes, Staphylococcus aureus, Salmenellosis, Yersina pestis, Mycobacterium leprae, M. africanum, M. asiaticum, M. aviuin-intracellulaire, M. chelonei abscessus, M. fallax, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. shimoidei, M. simiae, M. szulgai, M. xenopi, M. tuberculosis, Brucella melitensis, Brucella suis, Brucella abortus, Brucella canis, Legionella pneumonophilia, Francisella tularensis, Pneurnocystis carinii, mycoplasma, or Burkholderia cepacia.* The bacterial infection may result in pneumonia.

[00127] Chronic obstructive pulmonary disease (COPD) is a term used to classify two major airflow obstruction disorders: chronic bronchitis and emphysema. Approximately 16 million Americans have COPD, 80-90% of them were smokers throughout much of their lives. COPD is a leading cause of death in the U.S., accounting for 122,283 deaths in 2003. The cost to the USA for COPD was approximately $20.9 billion in direct health care expenditures in 2003. Chronic bronchitis is inflammation of the bronchial airways. The bronchial airways connect the trachea with the lungs. When inflamed, the bronchial tubes secrete mucus, causing a chronic cough.

[00128] In emphysema, the alveolar sacs are overinflated as a result of damage to the elastin skeleton of the lung. Inflammatory cells in emphysematous lung release elastase

enzymes, which degrade or damage elastin fibers within the lung matrix. Emphysema has a number of causes, including smoking, exposure to environmental pollutants, alpha-one antitrypsin deficiency, and aging.

[00129] Bronchiolitis is most commonly caused by viral lower respiratory tract infections, and primarily characterized by acute inflammation, edema, necrosis of epithelial cells lining small airways, and increased mucus production (Ralston *et al.*, 2014). Signs and symptoms typically begin with rhinitis and cough, which may progress to tachypnea, wheezing, rales, use of accessory muscles, and/or nasal flaring.

[00130] Bronchiolitis obliterans is a progressive airflow reduction as a result of abnormal remodeling of the small airways in the lungs (Meyer *et al.*, 2014). Bronchiolitis obliterans syndrome is a major complication of lung transplantations, and is often used to describe a delayed allograft dysfunction that results in persistent decline in forced expiratory volume and force that is not caused by other known causes (Meyer *et al.*, 2014).

[00131] The term "asthma" may refer to acute asthma, chronic asthma, intermittent asthma, mild persistent asthma, moderate persistent asthma, severe persistent asthma, chronic persistent asthma, mild to moderate asthma, mild to moderate persistent asthma, mild to moderate chronic persistent asthma, allergic (extrinsic) asthma, non-allergic (intrinsic) asthma, nocturnal asthma, bronchial asthma, exercise induced asthma, occupational asthma, seasonal asthma, silent asthma, gastroesophageal asthma, idiopathic asthma and cough variant asthma. During asthma, the airways are persistently inflamed and may occasionally spasm.

V. Examples

[00132] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Cav-1 peptide solubility

[00133] In order to determine which peptides may be most soluble in a liquid formulation, 50 mg of each Cav-1 peptide was dissolved in 5 mL of Tris Buffered Saline, pH 7.51. Each sample was vortexed to help the sample dissolve completely. The absorbance at 600 nm was measured immediately after dissolution of the peptides for insoluble peptides, or after 10 minutes for soluble peptides. Absorbance was measured again after 10 minutes for insoluble peptides except for samples APi2348, APi2352, APi2353 which were measured a second time at 15 minutes, 5 minutes, or 15 minutes after dissolution, respectively. Sample APi2345 was measured only after 20 minutes following dissolution, as the dissolution was incomplete (Table 2). pH was also tested after 24 hours.

[00134] Samples APi2350, APi2354, APi2355, and APi2356 had increased solubility at pH 7.51 compared to other tested peptides (Table 2). pH remained stable at roughly pH 7.5 for all samples after 24 hours.

Table 2. Absorbance of peptides dissolved in TBS, pH 7.51.

Sample name	Peptide sequence	Dissolve (Y/N)	UV (Abs) at dissolution	UV (Abs) after rest
APi2344	FTTFTVT-NH2 (SEQ ID NO: 11)	N	2.409	1.437
APi2345	FTTFTVTK-NH2 (SEQ ID NO: 12)	N		1.058
APi2346	KASFTTFTVTK-NH2 (SEQ ID NO: 13)	N	1.648	1.622
APi2347	Ac-KASFTTFTVTK-NH2 (SEQ ID NO: 14)	N	2.347	2.284
APi2348	OASFTTFTVTK-NH2 (SEQ ID NO: 15)	N	0.846	0.530
APi2349	Ac-OASFTTFTVTK-NH2 (SEQ ID NO: 20)	N	1.870	1.827
APi2350	KASFTTFTVTKGS-NH2 (SEQ ID NO: 4)	Y		0.004
APi2351	Ac-KASFTTFTVTKGS-NH2 (SEQ ID NO: 17)	N	2.523	2.377
APi2352	DSGKASFTTFTVTK-NH2 (SEQ ID NO: 18)	N	2.468	2.398
APi2353	Ac-DSGKASFTTFTVTK-NH2 (SEQ ID NO: 19)	N	3.000	3.000
APi2354	aaEGKASFTTFTVTKGSaa-NH2 (SEQ ID NO: 7)	Y	0.008	0.007
APi2355	Ac-aaEGKASFTTFTVTKGSaa-NH2 (SEQ ID NO: 8)	Y	0.000	0.000
APi2356	OASFTTFTVTOS-NH2 (SEQ ID NO: 9)	Y	0.001	-0.002
APi2357	Ac-OASFTTFTVTOS-NH2 (SEQ ID NO: 10)	N	2.293	2.149
*a= D-Alanine; O= Ornithine				

Example 2 – Cav-1 peptides increase smooth muscle actin production

[00135] Cav-1 peptides were dissolved in DMSO to make 10 mM stock solutions. 10 mM stock solutions of each peptide were then diluted in HBSS to make 900 μM working stock solutions. The DMSO resuspended polypeptides as well as the working stocks were stored at -20degC. For culture media, working stocks were added to DMEM culture media to a final concentration of 10 μM of the Cav-1 peptide.

[00136] Idiopathic Pulmonary Fibrosis (IPF) cell line 2051 was purchased and IPF cells from the fourth passage were seeded in 100 mm plates containing DMEM, 10% FBS, and 1% P/S. IPF cells were washed with 4 mL DMEM + 1%P/S and serum starved overnight. Cells were then treated for 2 days with either 44 uL HBSS (negative control), 10 uM LTI-03 (SEQ ID NO: 2), 90 uM LTI-03 (positive control), 10 uM APi2350, 10 uM APi2354, 10 uM APi2355, 10 uM APi2356 or with 20 uL of DMSO (negative control).

[00137] After 2 days of treatment, cells were washed once in cold, sterile HBSS. HBSS was removed, and 150 uL of lysis buffer with protease inhibitor cocktail was added to the cells. Cells were incubated with lysis buffer for 10 minutes. Cell lysates were scraped from the plates and collected. Cell lysates were then sonicated twice. Following sonication, the lysates were centrifuged at 13,000 RPM for 20 minutes. Lysates were then flash frozen in liquid nitrogen, thawed, vortexed, and centrifuged again at 13,000 RPM for 30 minutes. The supernatant was then collected and the pellet was discarded. The concentration of cell lysates was then determined by BCA assay.

[00138] Western blots were performed to evaluate the presence the effects of treatments. Briefly, 12 ug of each lysate was run on a 10% polyacrylamide gel. The gel was then transferred to a membrane and washed. Primary antibodies against smooth muscle actin (SMA) and Tubulin Results of the western blot can be seen in FIG. 1. The treatment for each of the lysates in the pictured lanes are: 1: Untreated, 2:10 μM LTI-03, 3: 90 μM LTI-03, 4: 10 μM APi2350, 5: 10 μM APi2354, 6: 10 μM APi 2355, 7: 10 μM APi2356, and 8: DMSO.

[00139] Western blots were photographed and analyzed with ImageJ to determine a ratio of smooth muscle actin to tubulin (FIG. 2). As expected, LTI-03 elicited an increase in SMA production relative to tubulin. Treatment with Cav-1 peptides APi2350, APi2354, APi2355, and APi2356 all increased the expression of SMA relative to tubulin as well (FIG. 2).

Example 3 – Cav-1 peptides preserve AEC2 cells of fibrotic lung biopsies

[0002] To assess the effect of Cav-1 peptide APi2355 (SEQ ID NO: 8) on AEC2 cell viability, surgical biopsies were obtained for the preparation of non-specific interstitial pneumonia precision cut lung slices (PCLS). One individual with non-specific interstitial pneumonia (NSIP) and another with end-stage IPF were processed. Lysotracker staining, which stains acidic compartments in live cells and selectively accumulates in the lamellar bodies of lung AEC2 cells (Van der Velden et al., 2013), was performed. Cav-1 peptide was suspended in DMEM/5%FBS, and PCLS slices (n=5 replicates/treatment group) were treated with 10, 100, or 500 μM LTI-03 or APi2355 (Var 55). Lysotracker staining (Green DND-26, Promega) was performed on NSIP PCLS 48 h after a single treatment. A strong dose-dependent increase in AEC2 cell viability was observed. In addition, lysotracker staining (Red DND-99, Promega) was performed on end-stage IPF on days 1, 2, 3, 5, and 7 following daily treatment with LTI-03 or APi2355. A dose-dependent increase in AEC2 cell viability was observed in an end-stage IPF biopsy treated for 7 consecutive days with LTI-03. The treatment effect for APi2355 (Var 55) was observed out to day 3.

* * *

[00140] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

V. References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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WHAT IS CLAIMED IS:

1. A peptide comprising the amino acid sequence ASFTTFTVT (SEQ ID NO: 3), wherein the peptide comprises at least one N- or C-terminal addition lacking identity to SEQ ID NO: 1.

2. The peptide of claim 1, wherein the peptide comprises at least one amino acid added to the N-terminus.

3. The peptide of claim 1, wherein the peptide comprises at least one amino acid added to the C-terminus.

4. The peptide of claim 1, wherein the peptide comprises at least one amino acid added to the N-terminus and the C-terminus.

5. The peptide of any of claims 1-4, wherein the peptide comprises L-amino acids.

6. The peptide of any of claims 1-4, wherein the peptide comprises D-amino acids.

7. The peptide of any of claims 1-4, wherein the peptide comprises both L- and D-amino acids.

8. The peptide of any of claims 1-4, wherein the peptide comprises deuterated residues.

9. The peptide of any of claims 1-7 wherein the peptide comprises at least one non-standard amino acid.

10. The peptide of claim 9, wherein the peptide comprises 2 non-standard amino acids.

11. The peptide of claim 9, wherein the non-standard amino acid is ornithine.

12. The peptide of any of claims 1-11, wherein the peptide comprises a N-terminal modification.

13. The peptide of any of claims 1-11, wherein the peptide comprises a C-terminal modification.

14. The peptide of any of claims 1-11, wherein the peptide comprises a N- and C-terminal modification.

15. The peptide of claim 12, wherein the N-terminal modification is acylation.

16. The peptide of claim 13, wherein the C-terminal modification is amidation.

17. The peptide of claim 4, wherein the peptide comprises the amino acid sequence KASFTTFTVTKGS (SEQ ID NO: 4).

18. The peptide of claim 7, wherein the peptide comprises the amino acid sequence aaEGKASFTTFTVTKGSaa (SEQ ID NO: 6).

19. The peptide of claim 11, wherein the peptide comprises the amino acid sequence OASFTTFTVTOS (SEQ ID NO: 9).

20. The peptide of claim 16, wherein the peptide comprises the amino acid sequence KASFTTFTVTKGS-NH2 (SEQ ID NO: 5).

21. The peptide of claim 16, wherein the peptide comprises the amino acid sequence aaEGKASFTTFTVTKGSaa-NH2 (SEQ ID NO: 7).

22. The peptide of claim 14, wherein the peptide comprises the amino acid sequence Ac-aaEGKASFTTFTVTKGSaa-NH2 (SEQ ID NO: 8).

23. The peptide of claim 16, wherein the peptide comprises the amino acid sequence OASFTTFTVTOS-NH2 (SEQ ID NO: 10).

24. The peptide of any one of claim 1-23, further comprising a cell-penetrating peptide (CPP).

25. The peptide of claim 25, wherein the CPP comprises an amino acid sequence selected from the group comprising: GRKKRRQRRRPPQ (SEQ ID NO: 21), RQIKIWFQNRRMKWKK (SEQ ID NO:22), and GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO:23).

26. The peptide of any one of claims 1-25, wherein the peptide maintains the biological activity of caveolin-1 (Cav-1).

27. A peptide multimer comprising at least two peptides according to any one of claims 1-26.

28. The peptide multimer of claim 27, wherein a first peptide of the at least two peptides is essentially identical to a second peptide of the at least two peptides.

29. The peptide multimer of claim 25, wherein a first peptide of the at least two peptides is not identical to a second peptide of the at least two peptides.

30. A composition comprising a peptide of any one of claims 1-29.

31. The composition of claim 30, wherein the peptide is substantially pure.

32. The composition of claim 30 or 31, wherein the peptide is at least 95% pure.

33. The composition of any one of claims 30-32, wherein the peptide is at least 98% pure.

34. A pharmaceutical composition comprising a peptide of any one of claims 1-29 and a pharmaceutically acceptable carrier.

35. The pharmaceutical composition of claim 34, wherein the pharmaceutical composition is formulated for oral, intravenous, intraarticular, parenteral, enteral, topical, subcutaneous, intramuscular, buccal, sublingual, rectal, intravaginal, intrapenile, intraocular, epidural, intracranial, or inhalational administration.

36. The pharmaceutical composition of claim 34, wherein the pharmaceutical composition is formulated for lung instillation.

37. The pharmaceutical composition of claim 34, wherein the pharmaceutical composition is formulated as a nebulized solution.

38. A polynucleotide comprising a nucleic acid sequence encoding the peptide of any one of claims 1-29.

39. A method of treating or preventing disease in subject comprising administering to the subject an effective amount of a peptide of any of claims 1-29.

40. The method of claim 39, wherein the subject has a fibrotic or inflammatory disease.

41. The method of claim 40, wherein the subject has organ fibrosis.

42. The method of claim 41, wherein the subject has kidney, liver, lung or heart fibrosis.

43. The method of claim 39, wherein the inflammatory disease is an inflammatory eye disease.

44. The method of claim 39, further defined as a method of treating or preventing pulmonary inflammation, acute lung injury, lung infection or lung disease in a subject.

45. The method of claim 44, wherein the subject has pulmonary inflammation.

46. The method of claim 44, wherein the subject has chronic obstructive pulmonary disorder (COPD).

47. The method of claim 39, wherein the subject is undergoing chemotherapy or radiation therapy.

48. The method of claim 44, wherein the subject has an acute lung injury.

49. The method of claim 44, wherein the subject has a lung infection.

50. The method of claim 44, wherein the subject has a chemical-induced lung injury.

51. The method of claim 44, wherein the subject has plastic bronchitis.

52. The method of claim 44, wherein the subject has asthma.

53. The method of claim 44, wherein the subject has acute respiratory distress syndrome (ARDS).

54. The method of claim 44, wherein the subject has inhalational smoke induced acute lung injury (ISALI).

55. The method of claim 44, wherein the subject has bronchiolitis.

56. The method of claim 44, wherein the subject has bronchiolitis obliterans.

57. The method of claim 44, wherein the lung disease is a fibrotic condition of the lungs.

58. The method of claim 44, wherein the lung disease is interstitial lung disease.

59. The method of claim 44, wherein the lung disease is Idiopathic Pulmonary Fibrosis (IPF) or lung scarring.

60. The method of claim 44, wherein the administering comprises nebulizing a solution comprising the variant polypeptide.

61. The method of claim 39, wherein the peptide is administered systemically.

62. The method of claim 39, wherein the peptide is administered locally to diseased tissue.

63. The method of claim 39, further comprising administering at least one additional anti-fibrotic therapeutic.

64. The method of claim 63, wherein the at least one additional anti-fibrotic is NSAID, steroid, DMARD, immunosuppressive, biologic response modulators, or bronchodilator.

65. The method of claim 39, wherein the subject is a human.

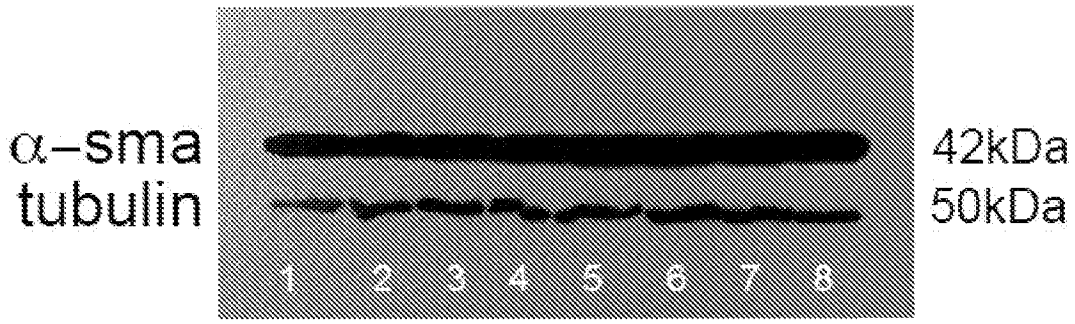
α–sma
tubulin
42kDa
50kDa
1 2 3 4 5 6 7 8

FIG. 1

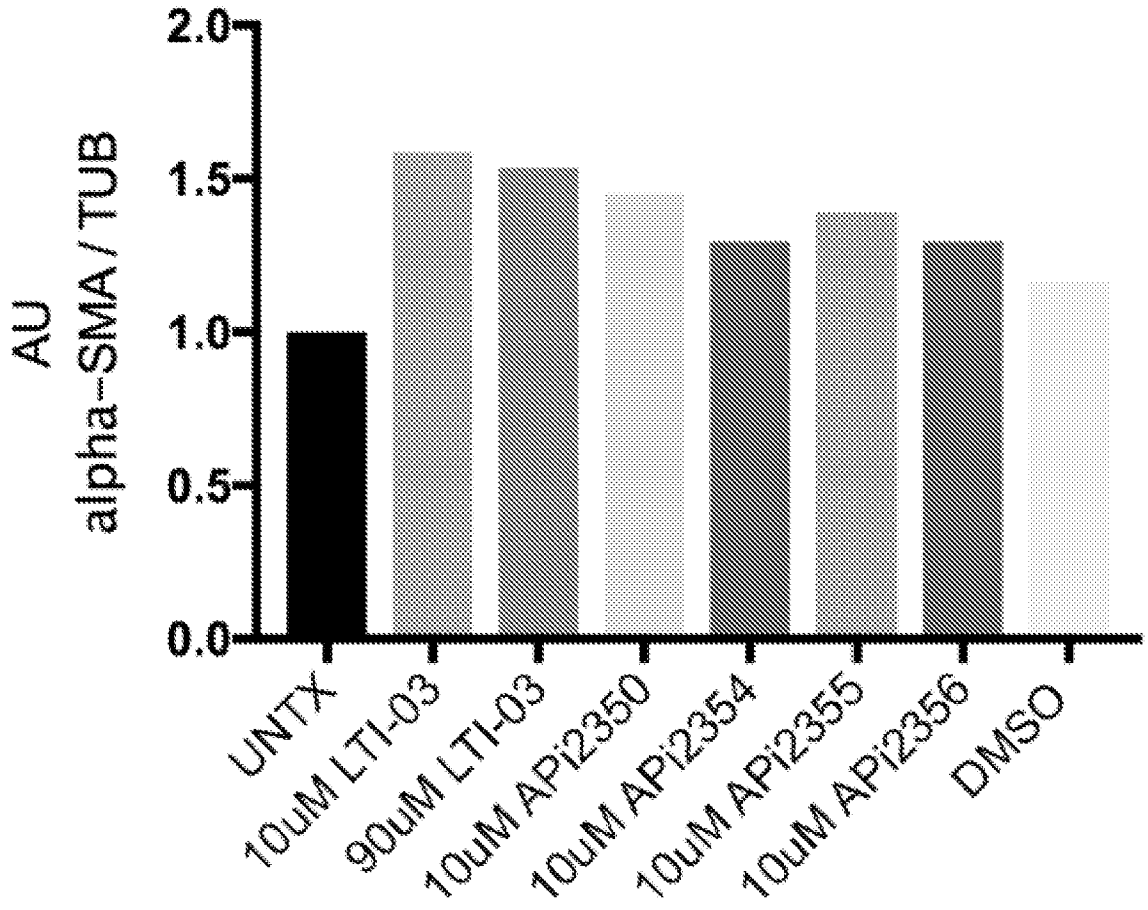
AU
alpha-SMA / TUB
2.0
1.5
1.0
0.5
0.0
UNTX
10uM LTI-03
90uM LTI-03
10uM APi2350
10uM APi2354
10uM APi2355
10uM APi2356
DMSO

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/050332

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/08; A61K 38/10; A61K 38/16; A61K 38/17; A61P 11/00; C07K 7/06 (2019.01)

CPC - A61K 38/08; A61K 38/10; A61K 38/16; A61K 38/1709; A61P 11/00; C07K 7/06; C07K 7/08; C07K 14/00; C07K 14/35; C07K 14/47 (2019.08)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 514/1.1; 530/324; 530/325; 530/326; 530/327; 530/328 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2013/0224163 A1 (HEAD et al) 29 August 2013 (29.08.2013) entire document	1-5, 8 -------- 6, 7
Y	US 2011/0218152 A1 (BELIVEAU et al) 08 September 2011 (08.09.2011) entire document	6, 7
A	WO 2008/046228 A1 (ANGIOCHEM, INC. et al) 24 April 2008 (24.04.2008) entire document	1-8, 17, 18
A	WO 2015/080980 A2 (E&B TECHNOLOGIES LLC) 04 June 2015 (04.06.2015) entire document	1-8, 17, 18
A	WO 2002/020768 A2 (YALE UNIVERSITY et al) 14 March 2002 (14.03.2002) entire document	1-8, 17, 18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 2019	02DEC 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/050332

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1, 3-10, and 21-23 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/050332

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 9-16, 19-65
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.