H78D; R43E T74E H78D Q80E; R43T T74E H78D Q80E; T63E H78D; T63E H78E; T63E H78E Q80E; T63E T74E H78D; T63E T74E H78E; T74E H78D Q80E; T74E H78E Q80E; Y70-GG-78H.

Figure 6 presents an alignment of native TIMP-2 and native TIMP-3. Identity of the amino acids in a corresponding residue is indicated by an asterisk beneath the sequences.

Summary of the Invention

In one aspect, the present invention refers to an isolated TIMP-3 mutein having a mature region of TIMP-3 set forth in SEQ ID NO:2, having the mutations of K45S and F57N. In another aspect, the present invention refers to an isolated nucleic acid that encodes a TIMP-3 mutein according to the present invention. In another aspect, the present invention refers to an expression vector comprising the isolated nucleic acid of the present invention. In another aspect, the present invention refers to an isolated host cell transformed or transfected with the expression vector of the present invention. In another aspect, the present invention refers to a method of producing a recombinant TIMP-3 mutein comprising culturing the transformed or transfected host cell of the present invention under conditions promoting expression of the TIMP-3 mutein, and recovering the TIMP-3 mutein. In another aspect, the present invention refers to a composition comprising the TIMP-3 mutein of the present invention and a physiologically acceptable diluent, excipient or carrier. In another aspect, the present invention refers to a composition according to the present invention for use as a medicament. In still another aspect, the present invention refers to the use of a composition according to the present invention in the manufacture of a medicament for treating a condition selected from the group consisting of inflammatory conditions, osteoarthritis, myocardial ischemia, reperfusion injury, and progression to congestive heart failure. In one example, described herein is an isolated TIMP-3 mutein having a mature region that is at least 95 % identical in amino acid sequence to the mature region of TIMP-3 set forth in SEQ ID NO:2, having at least one mutation, the mutation being selected from the group consisting of K45E; K45N; K45S; V47T; K49N; K49E; K49S; K50N; L51T; L51N; V52T; K53T; P56N; F57N; G58T; T63E; T63N; K65T; K65N; M67T; K68S; T74E; K75N; P77T; H78D; H78E; H78N; Q80E; Q80T; K94N; E96T; E96N; V97N; N98T; K99T; D110N; K112T; Q126N; K133S; R138T; R138N;

H140T; T158N; K160T; T166N; M168T; G173T; H181N; A183T; R186N; R186Q, R186E, K188T; K188Q, K188E, P201N; K203T; I205F, I205Y, A208G, A208V, and A208Y.

In another example, described herein is an isolated TIMP-3 mutein having a mature region that is at least 95 % identical in amino acid sequence to the mature region of TIMP-3 set forth in SEQ ID NO:2, having, having the mutation F57N and at least one further mutation, wherein the mutation is substitution at one or more of K residues of TIMP-3. In a further example, the further mutation introduces an N-linked glycosylation site into the amino acid sequence.

Also described herein is an isolated TIMP-3 mutein having a mature region that is at least 90 % identical in amino acid sequence to the mature region of TIMP-3 set forth in SEQ ID NO:2, wherein the mutein has at least one mutation that introduces at least one N-linked glycosylation site into the amino acid sequence. In an additional example, the TIMP-3 mutein has two, three, four, five or six N-linked glycosylation sites; in a still further example, the number of N-linked glycosylation sites introduced is seven, eight, nine or ten.

In one example, the N-linked glycosylation site is introduced at a region of the TIMP-3 amino acid sequence selected from the group consisting of: the region comprising amino acids 48 – 54; the region comprising amino acids 93 – 100; the region comprising amino acids 121 – 125; the region comprising amino acids 143 – 152; amino acids 156 – 164; the region comprising amino acids 183 – 191; and combinations thereof. In an additional example, the TIMP-3 mutein has two, three, four, or five N-linked glycosylation sites; in a still further embodiment, the number of N-linked glycosylation sites introduced is six, seven, eight, nine or ten.

Further described herein is an isolated TIMP-3 mutein having a mature region that is at least 95 % identical in amino acid sequence to the mature region of TIMP-3 set forth in SEQ ID NO:2, wherein the mutein has at least one mutation selected from the group consisting of: (a) one or more mutations in a TIMP-3 surface exposed positively charged patch that results in changes in the characteristics of the TIMP-3 charged patch that mimic a TIMP-2 charged surface; (b) one or more mutations that reduce susceptibility to proteolytic cleavage; (c) one or more mutations that result in decreased interaction of the TIMP-3 mutein with the scavenger receptor LRP-1; (d) one or more mutations that result in decreased interaction of the TIMP-3 mutein heparin or extracellular matrix components; (e) addition of one or more cysteinyl residues to the native TIMP-3 sequence; (f) improved pharmacokinetic and/o pharmacodynamic properties; and (g) combinations of the mutations set forth in (a) – (f). In one example the mutation or mutations is or are introduced at a region of the TIMP-3 amino acid sequence selected from the group consisting of: the region comprising amino acids 48 – 54; the region comprising amino acids 93 – 100; the region comprising amino acids 121 – 125; the region comprising amino acids 143 – 152; amino acids 156 – 164; the region comprising amino acids 183 – 191; and combinations thereof.

One example described herein is an isolated nucleic acid that encodes a TIMP-3 mutein according to any one of the aforementioned TIMP-3 muteins. Other examples described herein refer to an expression vector comprising such isolated nucleic acid; an isolated host cell transformed or transfected with the expression vector; and a method of producing a recombinant TIMP-3 mutein comprising culturing the transformed or transfected host cell of under conditions promoting expression of the TIMP-3 mutein, and recovering the TIMP-3 mutein.

Further described herein is a composition comprising the TIMP-3 mutein described herein, as well as a method of treating a condition in which matrix metalloproteases (MMPs) and/or other proteinases that are inhibited or inhibitable by TIMP-3 play a causative or exacerbating role, comprising administering to an individual afflicted with such a condition, an amount of such composition sufficient to treat the condition.

In one example, the condition is selected from the group consisting of inflammatory conditions, osteoarthritis, myocardial ischemia, reperfusion injury, and progression to congestive heart failure. In another embodiment, the condition is selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF), inflammatory bowel disease (for example, ulcerative colitis, Crohn's disease, and celiac disease), psoriases, myocarditis including viral myocarditis, inflammation related to atherosclerosis, and arthritic conditions including rheumatoid arthritis and psoriatic arthritis.

In a further example, the condition is selected from the group consisting of dystrophic epidermolysis bullosa, osteoarthritis, Reiter's syndrome, pseudogout, rheumatoid arthritis including juvenile rheumatoid arthritis, ankylosing spondylitis, scleroderma, periodontal disease, ulceration including corneal, epidermal, or gastric ulceration, wound healing after surgery, restenosis, emphysema, Paget's disease of bone, osteoporosis, scleroderma, pressure atrophy of bone or tissues as in bedsores, cholesteatoma, abnormal wound healing, rheumatoid arthritis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), dermatomyositis, psoriatic arthritis, scleroderma, systemic lupus erythematosus, vasculitis, myolitis, polymyolitis, dermatomyolitis, osteoarthritis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, polymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjogren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus (SLE), myasthenia gravis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, Celiac disease (nontropical Sprue), enteropathy associated with seronegative arthropathies, microscopic or collagenous colitis, eosinophilic gastroenteritis, or pouchitis resulting after proctocolectomy and ileoanal anastomosis, pancreatitis, insulin-

What is claimed is:

1. An isolated TIMP-3 mutein having a mature region of TIMP-3 set forth in SEQ ID NO:2, having the mutations of K45S and F57N.

2. An isolated nucleic acid that encodes a TIMP-3 mutein according to claim 1.

3. An expression vector comprising the isolated nucleic acid of claim 2.

4. An isolated host cell transformed or transfected with the expression vector of claim 3.

5. A method of producing a recombinant TIMP-3 mutein comprising culturing the transformed or transfected host cell of claim 4 under conditions promoting expression of the TIMP-3 mutein, and recovering the TIMP-3 mutein.

6. A composition comprising the TIMP-3 mutein of claim 1 and a physiologically acceptable diluent, excipient or carrier.

7. A composition according to claim 6 for use as a medicament.

8. The composition according to claim 7, wherein the medicament is for treating a condition in which matrix metalloproteases (MMPs) and/or other proteinases that are inhibited or inhibitable by TIMP-3 play a causative or exacerbating role.

9. Use of a composition according to claim 6 in the manufacture of a medicament for treating a condition selected from the group consisting of inflammatory conditions, osteoarthritis, myocardial ischemia, reperfusion injury, and progression to congestive heart failure.