MCPIP as wound therapy

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Recommended Citation

Disclosed herein are methods and compositions of treating a subject suffering from a wound. In exemplary examples, the method involves elevating MCPIP levels in a subject in need. Elevating MCPIP levels may involve direct administration (e.g., delivery of protein) or indirect administration (e.g., delivery vehicle capable of increasing expression of MCPIP).

7 Claims, 4 Drawing Sheets
Enhanced expression of MCPIP promoted HaCaT cell migration

Figure 1
Knockdown of MCPIP inhibited migration of HaCaT cells

Figure 2
Knockout of MCPIP impaired wound healing in mice

Figure 3
Enhanced expression of MCPIP promoted wound healing in diabetic mice

Figure 4

- A: Western blot analysis showing expression of MCPIP and β-actin in wound healing processes.
- B: Representative images of wound healing at Day 0, Day 3, Day 7, and Day 12 for GFP and MCPIP groups.
- C: Graph indicating percentage of original wound area over time for GFP and MCPIP groups.
- D: mRNA levels (fold change) for MCP-1, TGF-β, IL-6, and INOS.
- E: Gel electrophoresis showing expression of GFP, MCPIP, miRNA, PECAM, VEGF, and β-actin.
MCPIP AS WOUND THERAPY

BACKGROUND

Generally, when the skin of an individual is torn, cut or punctured (wounded), the body naturally reacts to regenerate dermal and epidermal tissue to close the wound. The wound regeneration process typically includes a set of complex biochemical events that take place in a closely orchestrated cascade to repair the damage. These events overlap in time, but may be categorized into different phases, namely the inflammatory, proliferative, and remodeling phases.

In the inflammatory phase, bacteria and debris are phagocytized and removed, and factors are released that cause the migration and division of cells involved in the proliferative phase. In the proliferative phase, the principal steps include angiogenesis, fibroplasia, granulation tissue formation, epithelialization, and wound contraction. Angiogenesis involves the development of new capillary blood vessels for the wound area to provide oxygen and nutrients to the healing tissue. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix (ECM) by excreting collagen and fibronectin. In epithelialization, epithelial cells crawl across the wound bed to cover the bed. In contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. When the cells’ roles are close to complete, unneeded cells undergo apoptosis.

It is known that a number of disease states hinder the normal wound healing process. For example, individuals with diabetes often experience problems with what are termed “diabetic foot ulcers.” Diabetic foot ulcers are sores or wounds, typically, on the feet that typically occur in individuals having diabetes. Oftentimes, these diabetic ulcers occur as a direct or indirect result of nerve damage in the feet of the individual as the prolonged high blood sugar levels associated with diabetes is linked with damage to the nerves in the feet. Such nerve damage in the feet, referred to as peripheral neuropathy, can cause loss of sensation as well as cause deformities of the feet. Due to the loss of sensation, individuals with peripheral neuropathy may hurt their feet by repetitive minor trauma (e.g., by prolonged walking) or a single major trauma (e.g., by scraping skin, stepping on objects, immersing feet in hot water, cutting toenails inappropriately, or wearing ill-fitting shoes), but nevertheless may not notice such injuries. A further complication of diabetes is a reduction in blood flow to the feet due to the arterial blockage or other causes, thereby severely inhibiting the body’s ability to adequately provide complete the proliferative stage of wound regeneration/healing described above. As a result, once the skin of the foot is torn, cut, or punctured, the wound healing process (e.g., the proliferative phase) may be inordinately slow in repairing the wound. Further, once a serious wound develops, the risk of infection is high as the individual’s body is simply unable to heal the wound. Even further, once an infection starts, the infection may be very difficult to reverse, and amputation of the affected limb is common.

RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 13/804, 703 filed Aug. 12, 2009, now abandoned, which is a continuation of U.S. Ser. No. 11/643,057 filed Dec. 20, 2006, now abandoned, which are incorporated herein in their entirety by reference.

REPRESENTATION OF THE DRAWINGS

FIG. 1. Expression of MCPIP on scratch-induced wound healing in HaCaT cells. (A) Cells were cultured to confluence and transfected with GFP or MCPIP vectors for 48 hrs and wounded by scratching. Cells were then incubated for the indicated hours and photographed. (B) Quantification of scratch-induced wound healing during 24 hrs after scratching.

FIG. 2. Knockdown of MCPIP inhibited migration of HaCaT cells. (A) HaCaT cells were cultured and transfected with MCPIP siRNA or non-targeted siRNA for 48 hrs, RT-PCR was performed to confirm that siRNA efficiently knockdown of MCPIP mRNA levels. (B) Cells were cultures to confluence and wounded by scratching. Cells were then incubated for the indicated hours and photographed. (C) Quantification of cell migration during 24 h after scratching.

FIG. 3. Lack of MCPIP impaired wound healing in MCPIP KO mice. (A) Wound sites were photographed at the indicated time. (B) Changes in percentage of wound area at each time point compared with the original wound area.

FIG. 4. Expression of MCPIP enhanced wound healing in diabetic mice. (A) Induction of MCPIP was found in the wound skin but not in the healed skin. (B) Wound sites were photographed at the indicated time. Day 0 pictures were taken immediately after the wound. (C) Changes in percentage of wound area at each time point compared with the original wound area. (D) Expression of cytokines in wound skins at day 12 after wound. (E) Expression of PECAM and VEGF in wound skins at day 12 was examined by RT-PCR.

DESCRIPTION OF THE INVENTION

The inventors have previously identified the novel transcription factor designated as MCPIP (MCP-1-induced protein). MCPIP was initially isolated from human monocytes after stimulation with MCP-1. The nucleotide (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequences of isolated human MCPIP were deposited with GenBank under accession number AY920403 and the nucleotide (SEQ ID NO: 3) and amino acid (SEQ ID NO: 4) sequences of isolated mouse MCPIP were deposited with GenBank under accession number AY920404. The inventors have continued to study the biological relevance of these genes/proteins, and to develop new therapies based on this research. The invention is based on the inventors discovery that maintaining or elevating MCPIP levels can accelerate wound healing in a subject.

According to one embodiment, the invention pertains to a method of treating wounds. MCPIP may be elevated by direct administration of a composition. The composition may comprise a therapeutically effective amount of the MCPIP protein. The MCPIP protein composition may comprise a pharmaceutically acceptable excipient. Alternatively, MCPIP may be elevated by administering a composition comprising a delivery vehicle that results in the in vivo expression of MCPIP. In yet another embodiment, MCPIP is elevated by inducing expression of the endogenous gene.

As used herein, the use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

The term “wound” as used herein refers to any break in the epithelium resulting from a cut, abrasion, adhesion, surgical
incision, thermal, chemical, or friction burn, ulcer, or the like, as a result of an accident, incident, surgical procedure, or the like. Wound can be further defined as acute and/or chronic. Compositions of the present invention have been found to be particularly useful in the treatment of diabetic ulcers, which are a type of wound as defined herein.

The term “pharmaceutical composition” as used herein refers to a wound composition as described herein that is dispersed in a pharmaceutically acceptable carrier.

The term “subject” as used herein refers to any human or nonhuman mammalian animal to which a wound composition is administered according to the methods described herein. In a specific embodiment, the compositions and methods of the present invention are employed to treat a human subject.

The term “effective amount” or “therapeutically effective amount” as used herein refers to an amount effective, at dosages and for periods of time necessary to achieve a desired result. The desired result may be an improvement in the wound healing process (e.g., by reducing a surface area of the wound), remediation of the symptoms of the wound (e.g., pain, infection, and the like), shortening of the duration of any stage in the wound healing process, stabilization of the state of wound (e.g., infection), prevention or slowing of the development of wound progression, prevention of, delay or slowing of wound onset, amelioration or palliation of the wound state, and remission (whether partial or total), whether detectable or undetectable.

The term “topical administration” as used herein includes the contact of the wound compositions of the present invention with tissue on or about the wound area.

The term “parenteral administration” as used herein includes any form of administration in which a composition is delivered or absorbed into the patient without involving absorption via the intestines. Exemplary parenteral administrations that are used in the present invention include, but are not limited to intradermal or subcutaneous administration.

The term “treatment” and “treating” as used herein refers to an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, improvement in the wound healing process (e.g., by reducing a surface area of the wound), remediation of the symptoms of the wound (e.g., pain, infection, and the like), shortening of the duration of any stage in the wound healing process or the overall wound healing process, stabilization of infection (if any), and remission (whether partial or total), whether detectable or undetectable.

Compounds and Compositions

The compounds described herein may be provided in any suitable form for administration to a wound of a patient. In one embodiment, the compounds are provided as a pharmaceutical composition comprising a pharmaceutically acceptable carrier. In a typical embodiment, the compound is MCPIP or a variant or derivative thereof.

In one aspect, the pharmaceutically acceptable carrier may comprise a substrate, such as a natural or synthetic non-woven or woven material, having the Compounds disposed on and/or therein to define a dressing that can be applied to, about, or adjacent to a wound. The substrate defining the dressing may be selected from the group consisting of a transdermal patch, a pad, a powder, a bandage, a host matrix, or any other suitable substrate configured to be applied to, over, and/or adjacent the wound. In certain embodiments, the dressing comprising the Compounds may be a hemostatic dressing as is known in the art. Exemplary hemostatic dressings are described in US Published Patent Application No. 2012028320, the entirety of which is hereby incorporated by reference. The Compounds may be impregnated within, otherwise dispersed within, and/or applied to a surface of the dressing using any suitable technique known in the art, such as by coating, spraying or dipping. It is appreciated that the dressing may include a polymeric coating or surface to be placed in contact with the wound, such as Teflon®, to avoid sticking of the dressing to the wound.

In addition, the wound compositions of the present invention may be provided and/or manufactured as a pharmaceutical composition for topical administration, parenteral administration, and/or any other suitable administration. It is understood that the active ingredient(s) and pharmaceutically acceptable carrier may be provided individually (with or without instructions for future mixing) or together (mixed, non-mixed, or with instructions for future mixing) in the respective formulation. Further, the wound composition may be prepared by known methods for the preparation of pharmaceutically acceptable compositions suitable for administration to a patient, such that an effective quantity of the active ingredients is combined in a mixture with a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers are described, for example, in Remington’s Pharmaceutical Sciences (Remington’s Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985).

In one embodiment, the wound composition is provided in the form of a topical composition that can be applied to, over, or adjacent the wound area. Without limitation, the topical composition may be in the form of a solution, a suspension, a spray, a cream, a gel, a foam, an ointment, a lotion, or a powder. The wound composition may be formulated as a controlled release, delayed release, or a sustained release formulation as is known in the art.

When a wound composition of the present invention is provided in the form of a topical composition, the wound composition may further include other cosmetic ingredients and pharmaceutically acceptable topical carriers which have substantially non-irritating, skin compatible components. Such suitable cosmetic and pharmaceutical agents include, but are not limited to, moisturizers, stabilizers, preservatives, antiseptics, lubricants, humectants, gelling agents, chelating agents, skin penetration enhancers, emollients, colorants, solvents, fatty bodies, thickening agents, emulsifiers, and/or any other excipient(s), which do not alter the therapeutic effect of the active ingredient(s) therein, e.g., Compounds.

In certain embodiments, the pharmaceutically acceptable carrier may include one or more of emulsifying agents, inert carriers including hydrocarbon bases, emulsifying bases, non-toxic solvents or water-soluble bases. Particularly suitable examples include phlorhizin, HPMC, CMC and other cellulose-based ingredients, lanolin, hard paraffin, liquid paraffin, soft yellow paraffin or soft white paraffin, white beeswax, yellow beeswax, cetostearyl alcohol, cetyl alcohol, dimethicones, emulsifying waxes, isopropyl myristate, microcrystalline wax, oleyl alcohol and stearyl alcohol.

Exemplary topical formulations to which the Nanoparticles may be included are set forth in U.S. Published Patent Application No. 20120289579, the entirety of which is hereby incorporated by reference. The Compounds may be impregnated within, otherwise dispersed within, and/or applied to a surface of the dressing using any suitable technique known in the art, such as by coating, spraying or dipping. It is appreciated that the dressing may include a polymeric coating or surface to be placed in contact with the wound, such as Teflon®, to avoid sticking of the dressing to the wound.

The concentration of active compound in the pharmaceutical composition will depend on absorption, inactivation and excretion rates of the active compound, the physicochemical
exemplary chronic wounds may include arterial ulcers, burn ulcers, decubitus ulcers, diabetic ulcers, diabetic foot ulcers, infectious ulcers, pressure ulcers, trauma-induced ulcers, ulcerations associated with pyoderma gangrenosum, venous ulcers, venous stasis ulcers, and vascular ulcers. It is appreciated that the wound may be associated with a particular disorder or condition, which renders the subject susceptible to, indirectly causes, or directly causes a non-healing wound. In certain embodiments, the subject is a diabetic, for example.

Kits

The compositions described herein may be provided in suitable packaging for maintaining the integrity of the article. In addition, the article may be provided as a kit along with suitable instructions that direct a user to use of a wound composition, e.g., pharmaceutical composition or dressing, comprising the Compounds in the treatment of a wound in a subject. The instructions may comprise user-readable and/or computer-readable information embodied on any suitable medium, such as a paper insert or a computer-readable disc. Alternatively, the instructions may direct the user to an external source, such as to a website, where such instructions may be found.

Dosing

One skilled in the art would readily appreciate that the administration, duration, and dosing of the wound compositions may be determined or adjusted based on the age, body weight, general condition, sex, diet, the severity of the wound, and/or degree of inflammation and/or infection associated with the wound. Effective amounts of the wound composition can be given repeatedly, depending upon the effect of the initial treatment regimen. Administrations are typically given periodically, while monitoring any response. It will be recognized by a skilled person that lower or higher dosages or number of applications other than those indicated herein may be given, according to the administration schedules and routes selected. In certain embodiments, the compounds may be provided in a composition, such as a topical composition or an injectable composition, in a concentration of at least 1 µM, and in particular embodiments, at least 10 µM.

In an embodiment, the wound compositions may be administered at least once daily for at least a first week's time. Thereafter, the wound compositions may be applied to the wound at greater intervals, such as once a week, until such time as the wound is satisfactorily healed. In one embodiment, a wound composition is applied once a week (after the initial daily application) for a period of at least seven weeks. It is understood that prior to each application, the wound may be rinsed with a sterile solution, such as saline, and allowed to dry. Thereafter, a wound composition may be applied.

Conjunctive Agents

In accordance with another aspect, the wound compositions comprising Compounds described herein may be employed alone in the treatment, or may be combined with a conjunctive agent, which is administered to the patient independently of the wound composition or as part of the same formulation. Such conjunctive agents may be delivered simultaneously, before and/or after the administration of the wound composition. Suitable conjunctive agents may include, by way of example only, vasodilators, vasoconstrictors, hypertensive agents, antibacterial agents, antibiotics, antioxidants, antifungal agents, non-steroidal antiinflammatory agents, steroid agents, anesthetics, and/or diabetes agents.

Suitable vasodilators include, but are not limited to, mandipine, nicardipine, nilvadipine, nisoldipine, nitrendipine, benidipine, amlopidine, arandipine, budralazine, cadralazine, ecarazine, hydralazine, toeralazine, oxyphedrine, diltiazem, tolazoline, hexobendine, bamethan, clonidine, methyldopa, guanabenz, and the like.

Suitable vasoconstrictors include, but are not limited to, dopamine, dobutamine denopamine and the like.
Suitable hypertensive agents include, but are not limited to,
dopamine, dobutamine, denopamine, digitoxin, digoxin,
methylidyminoglycin, lanatoside C, G-strophantin, and the like.

Suitable antibacterial agents include, but are not limited to,
sulfonamides, such as sulfamethizole, sulfisoxazole, sulfami-
methoxazole, sulfamethoxazole, sulfoisalfapyridine, silver
sulfadiazine, and the like, and quinolones, such as nalidixic
acid, pipemidic acid trihydrate, enoxacin, norfloxacin,
ofloxacin, tosufloxacin tosylate, ciprofloxacin hydrochloride,
lomefloxacin hydrochloride, sparflloxacin, fleroxacin, and the like.

Suitable antibiotics include, but are not limited to, tetracy-
clin hydrochloride, ampicillin, piperacillin, gentamycin,
dibeakcin, kanamycin, liviodmycin, tobramycic, amikacin,
fradiomycin, sisomicin, tetracyclin, oxytetracyclin, rol-
tetracyclin, doxycyclin, ampicillin, piperacillin, ticarcillin,
celafloxin, cefotaxin hexetil, cefuroxime axetil, cefdinir, cef-
dinor, cefprozil, cefadroxil, cefadroxil, cefamoxin, cefmenox-
ete, cefuroxime proxetil, ceftriaxone, ceftizoxime, cef-
sulodin, cefotaxime, cefmenoxime, cefinetazole, cefminox, cef-
volazone, latamoxef, flomoxef, cefizolin, cefotaxime, cef-
operazon, cefotaxime, moxalactam, thiamycin, sul-
fazecin, aztreonam and its salts, griseofulvin, lankacitin.

Suitable non-steroidal antiinflammatory agents include,
but are not limited to, aceatinopen, fenacetin, ethen-
azaide, sulpyrine, paracetamol, mefenamic acid, acetylsalicylic acid, diclofenac sodium, loxoprofen sodium,
enoxacin hydrochloride, indomethacin, ibuprofen, ketoprofen,
naproxen, oxaprozin, flurbiprofen, fenbufen, pranoprofen,
flencfenac sodium, eptizol, tiaramide hydrochloride, raloxifen,
gabexate mesilate, camostat mesilate, ulatantin, colchicine,
probencid, sulfipyrazone, benzbromaron, allopurinol,
sodium glutathione, sodium hyaluronate, sodium salicy-
late, morphine hydrochloride, salicylic acid, atropine, sco-
plamine, morphine, pethidine, levorphanol, ketoprofen,
naproxen, oxymorphone, and their salts.

Suitable steroidal agents include, but are not limited to,
dexamethasone, hexestrol, meethazone, betamethasone, tri-
amicinolone, triamcinolone acetonide, fluorocination, fluo-
rocicolone acetonide, prednisolone, methylprednisolone,
cortisone acetate, hydrocortison, fluorometholone, beclom-
etusone dipropionate, estril, and the like.

Suitable anesthetics include, but are not limited to, cocaine
hydrochloride, procaaine hydrochloride, lidocaine, dibucaine
hydrochloride, tetracaine hydrochloride, meipavacaine hydro-
chloride, bupivacaine hydrochloride, oxybuprocaine hydro-
chloride, ethyl aminozenoate, oxethazine, and the like, or
other systemic, inhalation, or intravenous anesthetics.

Suitable diabetes agents include, but are not limited to,
actos, lodiglitazon, kinedak, penfill, humalin, euglucon,
glimicroc, daonil, novolin, monotard, insulins, glucobay,
dimeln, rastinon, baclecin, deamelin S, lizilins; hypothy-
roidism treating agent [dried thyroid gland (thrycoid),
levothyroxine sodium (thyradin S), lithiumrionin sodium
(thyronine, thyromim), and the like.

The ratio between a compound of the present invention and
a conjugative agent in a concomitant formulation may be
selected appropriately on the basis of the target, route, and
disease/condition of the patient and may be readily deter-
dined by one skilled in the art. In a particular embodiment,
the present invention includes a method of treating a wound
comprising administering to a patient in need thereof a
wound composition comprising an effective amount of Compounds
with an effective amount of an antibiotic to aid in combating
wound infection.

Alternative Modes of Administration

The compounds for use in the invention are typically
formulated for topical or local administration but
also can be formulated for oral, transdermal, sublingual, buc-
cal, parenteral, rectal, intranasal, intracranial, intrabronchial
or intrapulmonary administration. For oral administration
the compounds can be of the form of tablets or capsules prepared
by conventional means with pharmaceutically acceptable
excipients such as lactose, dibasic calcium phosphate); fillers
(e.g., cornstarch, lactose, microcrystalline cellulose or calcium
phosphate); lubricants (e.g., magnesium stearate, talc, or silica);
dispersants (e.g., sodium starch glycolate); or wetting agents (e.g., sodium laurel sulphate). If desired, the tablets can be coated using suitable methods and coating
materials such as OPAADRY film coating systems available
from Colorcon, West Point, Pa. (e.g., OPAADRY, OY Type,
OY-C Type, Organic Enteric OY-P Type, Aqueous Enteric
OY-A Type, OY-PM Type and OPAADRY® White,
32K18400).

In a particular embodiment, the oral form is a tablet con-
taining MCPPI and a pharmaceutically acceptable excipient,
such as, but not limited to, mannitol, corn starch, microcryst-
talline cellulose, colloidal silicon dioxide, polyvinyl pyrroli-
done, talc, magnesium stearate, and the like which are option-
ally coated with an OPAADRY film coating.

Liquid preparation for oral administration can be in the
form of solutions, syrups or suspensions. The liquid prepara-
tions can be prepared by conventional means with pharmace-
etically acceptable additives such as suspending agents
(e.g., sorbitol syrup, methyl cellulose or hydrogenerated edible
fats); emulsifying agent (e.g., lecithin or accacia); non-aque-
ous vehicles (e.g., almond oil, oily esters or ethyl alcohol);
and preservatives (e.g., methyl or propyl p-hydroxy ben-
zoates or sorbic acid).

For buccal administration, the compounds for use in
the method of the invention can be in the form of tablets or
lozenges formulated in a conventional manner.

For parenteral administration, the compounds for use in
the method of the invention can be formulated for injection or
infusion, for example, intravenous, intramuscular or subcuta-
aneous injection or infusion, or for administration in a bolus
dose and/or continuous infusion. Suspensions, solutions or
emulsions in an oily or aqueous vehicle, optionally contain-
ing other formulation agents such as suspending, stabilizing
and/or dispersing agents can be used.
For rectal administration, the compounds for use in the method of the invention can be in the form of suppositories or enemas.

For sublingual administration, tablets can be formulated in conventional manner.

For intranasal, intraocular, intrabronchial or intrapulmonary administration, conventional formulations can be employed.

Further, the compounds (e.g. protein or delivery vehicle) for use in the method of the invention can be formulated in a sustained release preparation. For example, the compounds can be formulated with a suitable polymer or hydrophobic material which provides sustained and/or controlled release properties to the active agent compound. As such, the compounds for use in the method of the invention can be administered in the form of microparticles for example, by injection or in the form of wafers or discs by implantation.

Delivery Vehicles

1) Viral Vectors

In accordance with the method of the invention, an expression vector is a viral or a non-viral expression vector. Viral expression vectors which may be used advantageously in the method of the invention include, but are not limited to, an adenovirus vector, a lentivirus vector, an adeno-associated virus vector, and a herpes simplex virus (HSV) vector.

In a preferred embodiment, the compositions of the invention can be tailored to include a nucleic acid sequence (e.g., SEQ ID NO: 1, derivatives, fragments and variants thereof) in an expression vector. Viral vectors for use in the invention are those that exhibit low toxicity to a host cell. Viral vector methods and protocols that may be used in the invention are reviewed in Kay et al. Nature Medicine 7:33–40, 2001. The use of specific vectors, including those based on adenoviruses, adeno-associated viruses, herpes viruses, and retroviruses are described in more detail below.

The use of recombinant adenoviruses as vectors is discussed in W. C. Russell, Journal of General Virology 81:2573–2604, 2000; and Bramson et al., Curr. Opin. Biotechnol. 6:590–595, 1995. Adenoviruses are preferred for use in the invention because they (1) are capable of highly efficient gene expression in target cells and (2) can accommodate a relatively large amount of heterologous (non-viral) DNA. A preferred form of recombinant adenovirus is a “gutless”, “high-capacity”, or “helper-dependent” adenovirus vector. Such a vector features, for example, (1) the deletion of all or most viral-coding sequences (those sequences encoding viral proteins), (2) the viral inverted terminal repeats (ITRs) which are sequences required for viral DNA replication, (3) up to 28–32 kb of “exogenous” or “heterologous” sequences (e.g., sequences encoding an ammonia producing enzyme), and (4) the viral DNA packaging sequence which is required for packaging of the viral genomes into infectious capsids. For specifically targeting liver, preferred variants of such recombinant adenoviral vectors contain tissue-specific enhancers and promoters operably linked to for example, SEQ ID NO: 1.

Other viral vectors that might be used in the invention are adeno-associated virus (AAV)-based vectors. AAV-based vectors are advantageous because they exhibit high transduction efficiency of target cells and can integrate into the host genome in a site-specific manner. Use of recombinant AAV vectors is discussed in detail in Tal, J., J. Biomed. Sci. 7:279–291, 2000 and Mounahan and Samulski, Gene Therapy 7:24–30, 2000. A preferred AAV vector comprises a pair of AAV inverted terminal repeats which flank at least one cassette containing a tissue (e.g., gum) or cell-specific promoter operably linked to a urease nucleic acid. The DNA sequence of the AAV vector, including the ITRs, the promoter and, for example, urease gene may be integrated into the host genome.

The use of herpes simplex virus (HSV)-based vectors is discussed in detail in Cotter and Robertson, Curr. Opin. Mol. Ther. 1:633–644, 1999. HSV vectors deleted of one or more immediate early genes (IE) are advantageous because they are generally non-cytotoxic, persist in a state similar to latency in the host cell, and afford efficient host cell transduction. Recombinant HSV vectors can incorporate approximately 30 kb of heterologous nucleic acid. A preferred HSV vector is one that: (1) is engineered from HSV type 1, (2) has its IE genes deleted, and (3) contains a tissue-specific promoter operably linked to a urease nucleic acid. HSV amplicon vectors may also be useful in various methods of the invention.

Typically, HSV amplicon vectors are approximately 15 kb in length, and possess a viral origin of replication and packaging sequences.

Retroviruses such as C-type retroviruses and lentiviruses might also be used in the invention. For example, retroviral vectors may be based on murine leukemia virus (MLV). See, e.g., Hu and Pathak, Pharmacol. Rev. 52:493–511, 2000 and Feng et al., Crit. Rev. Ther. Drug Carrier Syst. 17:1–60, 2000. MLV-based vectors may contain up to 8 kb of heterologous (therapeutic) DNA in place of the viral genes.

Additional retroviral vectors that might be used are replication-defective lentivirus-based vectors, including human immunodeficiency (HIV)-based vectors. See, e.g., Vigna and Naldini, J. Gene Med. 5:308–316, 2000 and Miyoshi et al., J. Virol. 72:8150–8157, 1998. Lentiviral vectors are advantageous in that they are capable of infecting both actively dividing and non-dividing cells. They are also highly efficient at transducing human epithelial cells. Lentiviral vectors for use in the invention may be derived from human and non-human (including SIV) lentiviruses. Preferred lentiviral vectors include nucleic acid sequences required for vector propagation as well as a tissue-specific promoter operably linked to, for example, SEQ ID NO: 1, derivatives, variants and fragments thereof. These former may include the viral ITRs, a primer binding site, a polypurine tract, att sites, and an encapsidation site.

A lentiviral vector may be packaged into any suitable lentiviral capsid. The substitution of one particle protein with another from a different virus is referred to as "pseudotyping". The vector capsid may contain viral envelope proteins from other viruses, including murine leukemia virus (MLV) or vesicular stomatitis virus (VSV). The use of the VSV G-protein yields a high vector titer and results in greater stability of the vector virus particles.

Alphavirus-based vectors, such as those made from semliki forest virus (SFV) and sindbis virus (SIN), might also be used in the invention. Use of alphaviruses is described in Lundstrom, K., Intervirology 43:247–257, 2000 and Perri et al., Journal of Virology 74:9802–9807, 2000. Alphavirus vectors typically are constructed in a format known as a replicon. A replicon may contain (1) alphavirus genetic elements required for RNA replication, and (2) a heterologous nucleic acid such as one encoding SEQ ID NO: 1. Within an alphavirus replicon, the heterologous nucleic acid may be operably linked to a tissue-specific promoter or enhancer.

Recombinant, replication-defective alphavirus vectors are advantageous because they are capable of high-level heterologous (therapeutic) gene expression, and can infect a wide host cell range. Alphavirus replicons may be targeted to specific cell types by displaying on their virion surface a functional heterologous ligand or binding domain that would allow selective binding to target cells expressing a cognate binding partner. Alphavirus replicons may establish latency,
and therefore long-term heterologous nucleic acid expression in a host cell. The replicons may also exhibit transient heterologous nucleic acid expression in the host cell. A preferred alphavirus vector or replicon is non-cytopathic.

In many of the viral vectors compatible with methods of the invention, more than one promoter can be included in the vector to allow more than one heterologous gene to be expressed by the vector. Further, the vector can comprise a sequence which encodes a signal peptide or other moiety which facilitates the secretion of a gene product from the host cell.

To combine advantageous properties of two viral vector systems, hybrid viral vectors may be used to deliver a nucleic acid to a target tissue. Standard techniques for the construction of hybrid vectors are well-known to those skilled in the art. Such techniques can be found, for example, in Sambrook, et al., In Molecular Cloning: A laboratory manual. Cold Spring Harbor, N.Y. or any number of laboratory manuals that discuss recombinant DNA technology. Double-stranded AAV genomes in adenoviral capsids containing a combination of AAV and adenoviral Ilt5s may be used to transduce cells. In another variation, an AAV vector may be placed into a "gutless", "helper-dependent" or "high-capacity" adenoviral vector. Adenovirus/AAV hybrid vectors are discussed in Lieber et al., J. Virol. 73:9314-9324, 1999. Retrovirus/adenovirus hybrid vectors are discussed in Zheng et al., Nature Biotechnol. 18:176-186, 2000. Retroviral genomes contained within an adenovirus may integrate within the host cell genome and effect stable urease gene expression.

Other nucleotide sequence elements which facilitate expression of SEQ ID NO: 1, derivatives, variants and fragments thereof and cloning of the vector are further contemplated. For example, the presence of enhancers upstream of the promoter or terminators downstream of the coding region, for example, can facilitate expression.

In an aspect of the method wherein the viral expression vector is an AAV vector capable of transducing the target cell, the AAV vector is free of both wildtype and helper virus. Exemplary types of AAV vectors useful in the present invention include serotype 2 AAV vectors and chimeric serotype 1/2 AAV vectors.

2) Other Delivery Vehicles

Many viral techniques for the delivery of a nucleic acid sequence into a cell can be used, including direct naked DNA uptake (e.g., Wolff et al., Science 247: 1465-1468, 1990), receptor-mediated DNA uptake, e.g., using DNA coupled to asialoorosomucoid which is taken up by the asialoglycoprotein receptor in the liver (Wu and Wu, J. Biol. Chem. 262: 4429-4432, 1987), Wu et al., J. Biol. Chem. 266: 14338-14342, 1991), and liposome-mediated delivery (e.g., Kaneda et al., Exptl. Cell Res. 173: 56-69, 1987; Kaneda et al., Science 243: 375-378, 1989; Zhu et al., Science 261: 209-211, 1993). Many of these physical methods can be combined with one another and with viral techniques; enhancement of receptor-mediated DNA uptake can be effected, for example, by combining its use with adenovirus (Curiel et al., Proc. Natl. Acad. Sci. USA 88: 8850-8854, 1991; Cristiano et al., Proc. Natl. Acad. Sci. USA 90: 2122-2126, 1993). Other examples include stem cells such as mesenchymal stem cells, hematopoietic stem cells, cardiac stem cells or neural stem cells, embryonic stem cells that have been engineered to express MCPiPiP. Such stem cells can be administered in such a way to be incorporated in to tissues of a patient in need. In a particular embodiment, stem cells are administered to myocardial tissue. Bi L., et al. "Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages" Nature 2009 460:113-117.

Polynucleotides and Polypeptides

Aspects of the invention therefore include polynucleotides encoding at least one mammalian MCPiPiP and amino acid sequences representing at least one MCPiPiP protein. Aspects of the invention also include subunits or variants of polynucleotides or MCPiPiP proteins or peptides encoded by those polynucleotides. The terms “protein” and “polypeptide” are used interchangeably herein, unless specified otherwise.

It is well known in the art that a single amino acid may be encoded by more than one nucleotide codon—and that the nucleotide sequence may be easily modified to produce an alternate nucleotide sequence that encodes the same peptide. Therefore, alternate embodiments of the present invention include alternate DNA sequences encoding peptides containing the amino acid sequences described for MCPiPiP DNA sequences encoding peptides containing the claimed amino acid sequence include DNA sequences which encode any combination of the claimed sequence and any other amino acids located N-terminal or C-terminal to the claimed amino acid sequence.

It is to be understood that amino acid and nucleic acid sequences may include additional residues, particularly N- or C-terminal amino acids or 5' or 3' nucleotide sequences, and still be essentially as set forth in the sequences disclosed herein, as long as the sequence produces a functionally similar polypeptide or protein. A nucleic acid fragment of almost any length may be employed, and may be combined with other DNA sequences, such as promotors, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like. Therefore, overall length may vary considerably.

MCPiPiP polypeptides, as used herein, may comprise short fragments of proteins often referred to as peptides, as well as longer fragments generally referred to as polypeptides, and full-length proteins. These polypeptides can be prepared by standard peptide synthesis methods known to those of skill in the art, but may also be produced using an expression vector having a polynucleotide sequence encoding the polypeptide(s) of choice operably linked to appropriate promoter, terminator, and other functional sequences (such as a sequence encoding a purification tag) to facilitate expression and purification of the peptides.

It is to be understood that amino acid and nucleic acid sequences may include additional residues, particularly N- or C-terminal amino acids or 5' or 3' nucleotide sequences, and still be essentially as set forth in the sequences disclosed herein, as long as the sequence confers MCP-1 inducible transcription factor activity upon the polypeptide or protein moiety of the expressed protein. Nucleic acids which hybridize with a nucleic acid encoding the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4 under stringent conditions and encode a polypeptide having a similar MCP-1 inducible transcription factor activity to that of a polypeptide comprising SEQ ID NO: 2 or SEQ ID NO: 4 are also included as embodiments of the present invention.

The term "moderately stringent conditions", as used herein, means conditions in which non-specific hybridization will not generally occur. Hybridization under such conditions can be performed based on the description provided in Molecular Cloning: A Laboratory Manual 2nd ed., published by cold Spring Harbor Laboratory in 1989, edited by T. Maniatis et al. Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated Tm of the hybrid under study. The Tm of a hybrid between a polynucleotide of interest or the complement thereof and a polynucleotide sequence which is at least about
50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, Proc. Natl. Acad. Sci. U.S.A. 48, 1390 (1962): $
abla \frac{T_m}{C} = 81.5 - 16.6 \log_{10}(\% G+C) + 0.63 (\% G+C) - 6.02$.

where $l$—the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4xSSC at 65°C, or 50% formamide, 4xSSC at 42°C, or 0.5xSSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2xSSC at 65°C. According to another example, stringent conditions include incubation with a probe in 6xSSC containing 0.5% SDS, 5xDenhardt’s solution and 100 micrograms/ml salmon sperm DNA at 60 degrees C.

Additional nucleic acid bases may be added either 5' or 3' to the MCPIP ORF, and may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like. Therefore, overall length of such a polynucleotide may vary considerably. In a method described by the present invention, a nucleotide sequence of SEQ ID NO: 1 is inserted into a protein expression vector to produce a protein which can be used to synthesize a DNA copy of an RNA molecule. The DNA can then be amplified to form multiple copies.

“Control sequences” are those DNA sequences that are necessary for the expression of a protein from a polynucleotide sequence containing such a sequence, operably linked to the polynucleotide sequence encoding the protein. These sequences include prokaryotic sequences such as, for example, promoters, operators, and ribosome binding sites, and eukaryotic sequences such as, for example, promoters, enhancers, and polyadenylation signals. “Expression systems” are DNA sequences (such as, for example, plasmids) appropriate for expression of a target protein in a particular host cell, these sequences comprising appropriate control sequences for protein expression in the host cell operably linked to the polynucleotide sequence encoding the target protein.

It is to be understood that a “variant” of a polypeptide is not completely identical to the native protein. A variant MCPIP protein, for example, can be obtained by altering the amino acid sequence by insertion, deletion or substitution of one or more amino acids. The amino acid sequence of the protein can be modified, for example, by substitution to create a polypeptide having substantially the same or improved qualities as compared to the native polypeptide. The substitution may be a conserved substitution. A “conserved substitution” is a substitution of an amino acid with another amino acid having a side chain that is similar in polar/nonpolar nature, charge, or size. The 20 essential amino acids can be grouped as those having nonpolar side chains (alanine, valine, leucine, isoleucine, proline, phenylalanine, and tryptophan), uncharged polar side chains (methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamate), acidic side chains (aspartate and glutamate), and basic side chains (lysine, arginine, and histidine). Conserved substitutions might include, for example, Asp to Glu, Asn, or Gln; His to Lys, Arg or Phe; Asn to Gln, Asp or Glu; and Ser to Cys, Thr or Gly. Alanine, for example, is often used to make conserved substitutions.

To those of skill in the art, variant polypeptides can be obtained by substituting a first amino acid for a second amino acid at one or more positions in the polypeptide structure in order to affect biological activity. Amino acid substitutions may, for example, induce conformational changes in a polypeptide that result in increased biological activity.
A variant of the peptide is not completely identical to a disclosed MCPIP polypeptide sequence. A variant, given the disclosure of the present invention, can be obtained by altering amino acid sequence by insertion, deletion or substitution of one or more amino acid. The amino acid sequence of a disclosed peptide can be modified, for example, by substitution to create a peptide having substantially the same or improved qualities. The substitution may be a conserved substitution. A “conserved substitution” is a substitution of an amino acid with another amino acid having a side chain that is similar in polar/nonpolar nature, charge, or size. The 20 essential amino acids can be grouped as those having nonpolar side chains (alanine, valine, leucine, isoleucine, proline, phenylalanine, and tryptophan), uncharged polar side chains (methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine), acidic side chains (aspartate and glutamate) and basic side chains (lysine, arginine, and histidine). Conserved substitutions might include, for example, Asp to Glu, Asn or Gln; His to Lys, Arg or Phe; Asn to Gln, Asp or Glu; Leu to Ile or Val, and Ser to Cys, Thr or Gly. Alanine is commonly used to make conserved substitutions.

To those of skill in the art, variant polypeptides can be obtained by substituting a first amino acid for a second amino acid at one or more positions in the polypeptide structure in order to affect biological activity. Amino acid substitutions may, for example, induce conformational changes in a polypeptide that result in increased biological activity. Those of skill in the art may also make substitutions in the amino acid sequence based on the hydrophilicity index or hydropathic index of the amino acids.

A variant polypeptide of the present invention has less than 100%, but at least about 50%, and more preferably at least about 80% to about 90% amino acid sequence homology or identity to the amino acid sequence of a corresponding native nucleic acid molecule or polypeptide comprising SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3, or SEQ ID NO 4. The amino acid sequence of a variant MCPIP polypeptide therefore corresponds essentially to the disclosed amino acid sequences. As used herein, “corresponds essentially to” refers to a polypeptide sequence that will elicit a similar biological activity as that generated by a disclosed MCPIP, such activity being from at least about 70 percent of that of disclosed MCPIP polypeptide, to greater than 100 percent of the activity of a disclosed MCPIP peptide.

The human MCPIP polypeptide sequence comprises a Zc3h12a Ribonuclease domain at residues 130-280 and zinc finger domain at 284-309. It is to be understood that non-conserved substitutions at these regions will likely result in negating activity of the protein. Mizgalska et al., FEBS Journal 276:7386-7399 (2009). Conserved substitutions in these domains will likely result in preserving activity of the protein. Most mutations, whether conservative or non-conservative, outside the two domains will not likely affect activity of the protein to any great extent. Accordingly, the disclosure of SEQ ID NO: 2 combined with the knowledge in the art regarding the genetic code establishes possession of using the amino acid sequence or a fragment thereof. Percent identity between a putative polypeptide variant and a full length amino acid sequence is determined using the Blast2 alignment program (Blosum62, Expect 10, standard genetic codes).

**Examples**

**Example 1**

**Expression of MCPIP on Scratch-Induced Wound Healing in HaCaT Cells**

Cells were cultured to confluence and transfected with GFP or MCPIP vectors for 48 hrs and wounded by scratching. Cells were then incubated for the indicated hours and photographed. See FIG. 1A. The scratch induced wound healing during 24 hrs after scratching was then quantified. The wound distance was quantified and it was shown enhanced expression of MCPIP caused a significant decrease in wound distance. This was correlated with HaCaT cell migration. see FIG. 1B.

**Example 2**

**Knockdown of MCPIP Inhibited Migration of HaCaT Cells**

HaCaT cells were cultured and transfected with MCPIP siRNA or non-targeted siRNA for 48 hrs. RT-PCR was performed to confirm that siRNA efficiently knockdown of MCPIP mRNA levels. FIG. 2A is a photograph of a gel of RT-PCR samples showing that MCPIP siRNA efficiently knocks down MCPIP. HaCaT Cells were cultured to confluence and wounded by scratching. Cells were then incubated for 0, 12, and 24 hours and then photographed (FIG. 2B). Migration of the cells proximate to the scratch wound was measured at 24 hours. FIG. 2C shows that HaCaT cells transfected with MCPIP siRNA show a significant decrease in migration.

**Example 3**

**Lack of MCPIP Impaired Wound Healing in MCPIP KO Mice**

(A) Wounds were formed on the epidermis of MCPIP KO mice and wild type mice. Wound sites were photographed at day 7 and day 12. FIG. 3A clearly shows that the level of wound healing in the MCPIP knockout mice is significantly less in as compared to wild-type. Changes in percentage of wound area at the day 7 and day 12 time points of the knockout and wild-type mice were compared with the original wound area. The percentage of the wound area at day 7 and 12 is dramatically greater in the knockout mice as compared to wild-type (FIG. 3C).
Expression of MCPIP Enhanced Wound Healing in Diabetic Mice

Induction of MCPIP was found in the wound skin but not in the healed skin (see FIG. 4). As shown in FIG. 4(B), wound sites were photographed at day 0, 3, 7 and 12. Day 0 pictures were taken immediately after the wound. Changes in percentage of wound area at each time point compared with the original wound area, which is shown in FIG. 4C. The level of healing in the MCPIP enhanced animals is clearly greater than the control. FIG. 4D shows the expression of cytokines in wound skins at day 12 after wound. Expression of PECAM and VEGF in wound skins at day 12 was examined by RT-PCR (see FIG. 4F).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of molecular biology. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

What is claimed is:

1. A topical composition for application to a wound in a subject to promote healing of the wound, the wound composition comprising:
   an effective amount of MCPIP polypeptide for treatment of the wound; and
   a pharmaceutically acceptable carrier in a form configured for topical administration to the subject.

2. The topical composition of claim 1, which is in a form selected from the group consisting of a solution, a suspension, a spray, a cream, a gel, a foam, an ointment, a lotion, and a powder.

3. The topical composition of claim 1, wherein the pharmaceutically acceptable carrier comprises a dressing, the dressing selected from the group consisting of a transdermal patch, a pad, a powder, a matrix, and a bandage.

4. A kit comprising:
   a pharmaceutical composition comprising an effective amount of MCPIP for treating a wound and a pharmaceutically acceptable carrier; and
   instructions directing a user to use of the pharmaceutical composition in the treatment of a wound in a subject.

5. The kit of claim 4, wherein the wound is at least one of a wound selected from the group consisting of an incomplete healing wound, a chronic wound, a burn, and a wound caused by trauma.

6. The kit of claim 4, wherein the pharmaceutical composition is in a form from the group consisting of a solution, a suspension, a spray, a cream, a gel, a foam, an ointment, a lotion, or a powder.

7. The kit of claim 4, wherein the pharmaceutically acceptable carrier comprises a dressing, the dressing selected from the group consisting of a transdermal patch, a pad, a powder, a matrix, and a bandage.

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