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(54) HEPATOCYTE GROWTH FACTOR NUCLEIC ACID SEQUENCE TO ENHANCE MUSCULOCUTANEOUS FLAP SURVIVAL

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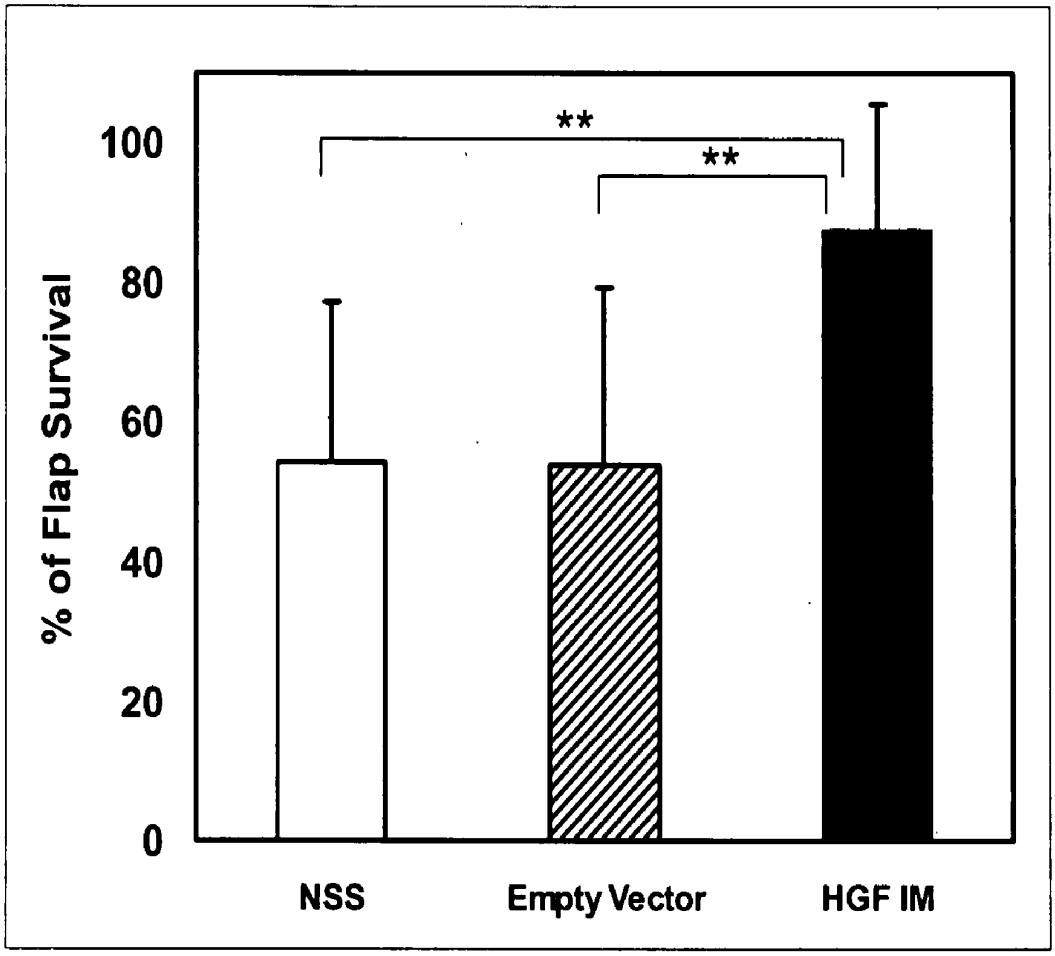
(57) ABSTRACT

The present invention relates to the use of growth factors in improving tissue survival. In particular, the invention describes methods for enhancing organ transplant, musculocutaneous flap or skin graft survival by administering a nucleic acid sequence encoding hepatocyte growth factor.



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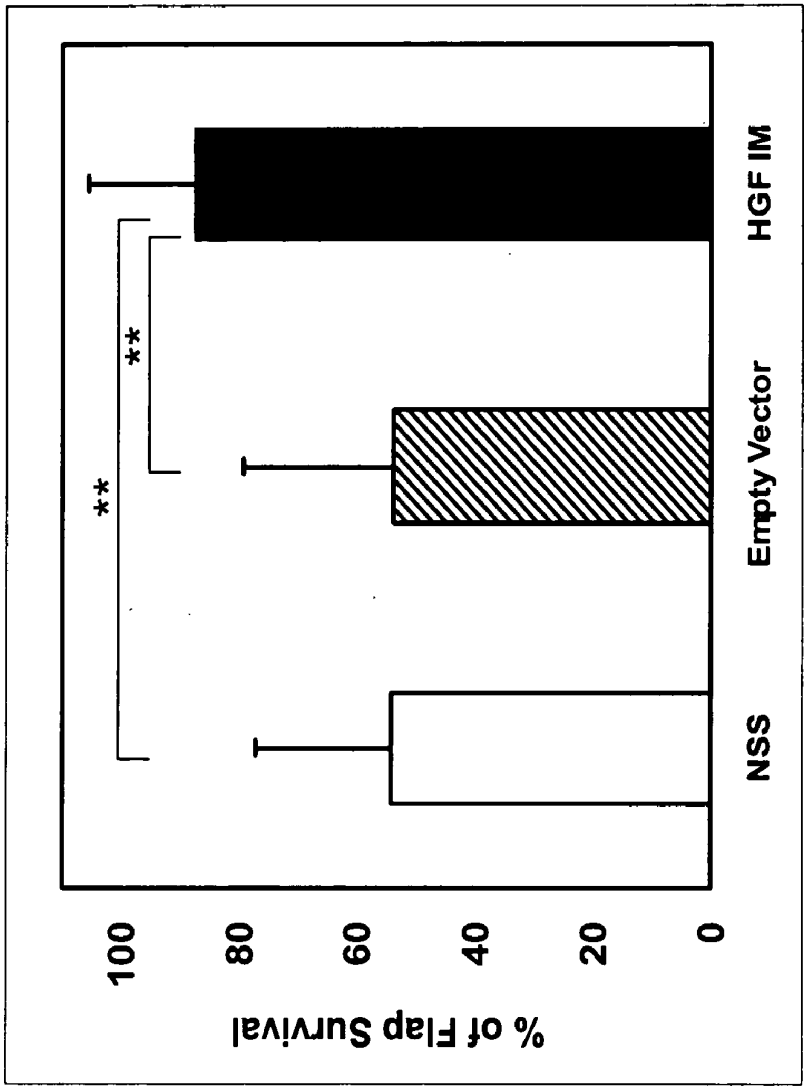

** indicates P<0.01.

Figure 1

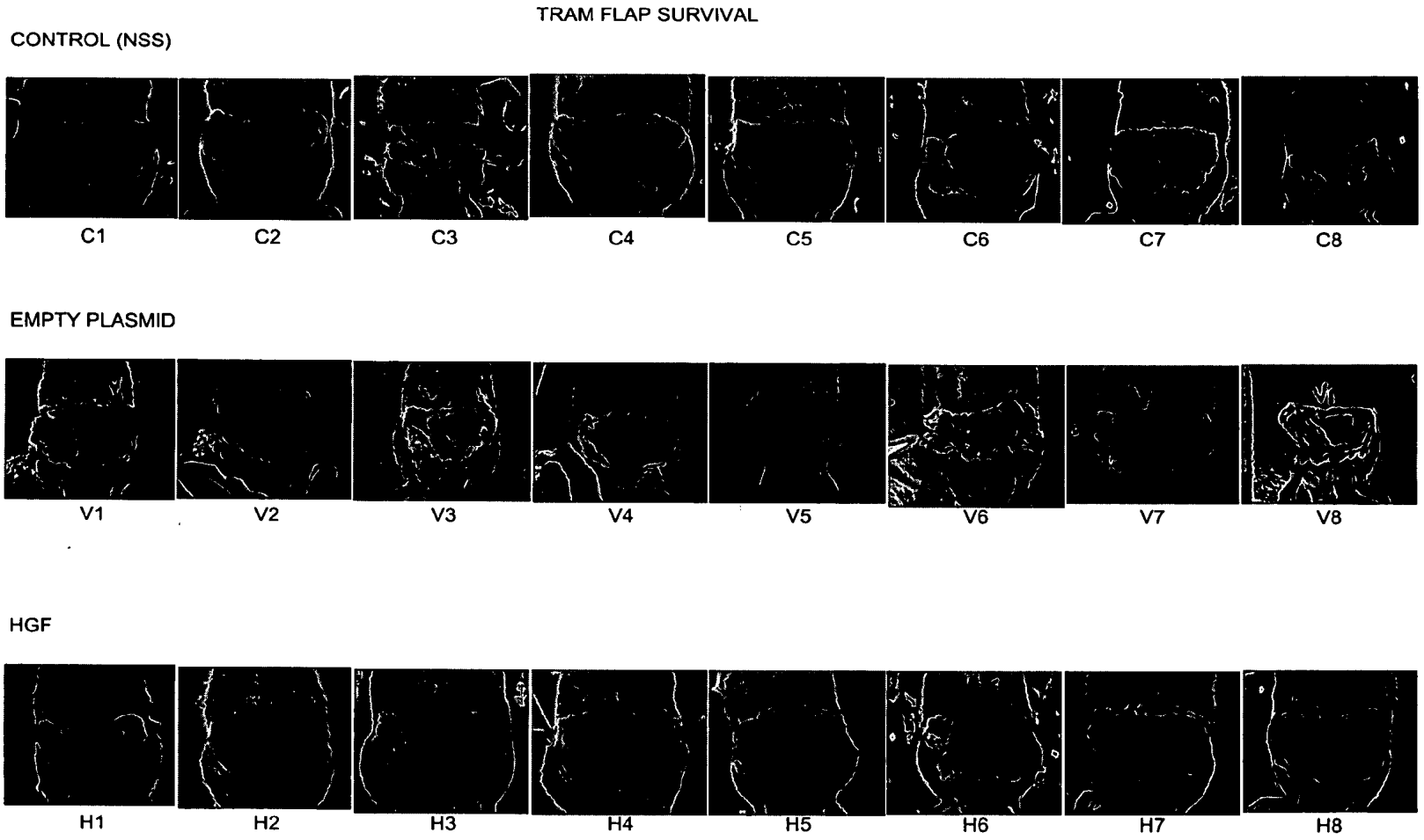
TRAM FLAP SURVIVAL
CONTROL (NSS)
C1
C2
C3
C4
C5
C6
C7
C8
EMPTY PLASMID
V1
V2
V3
V4
V5
V6
V7
V8
HGF
H1
H2
H3
H4
H5
H6
H7
H8

Figure 2

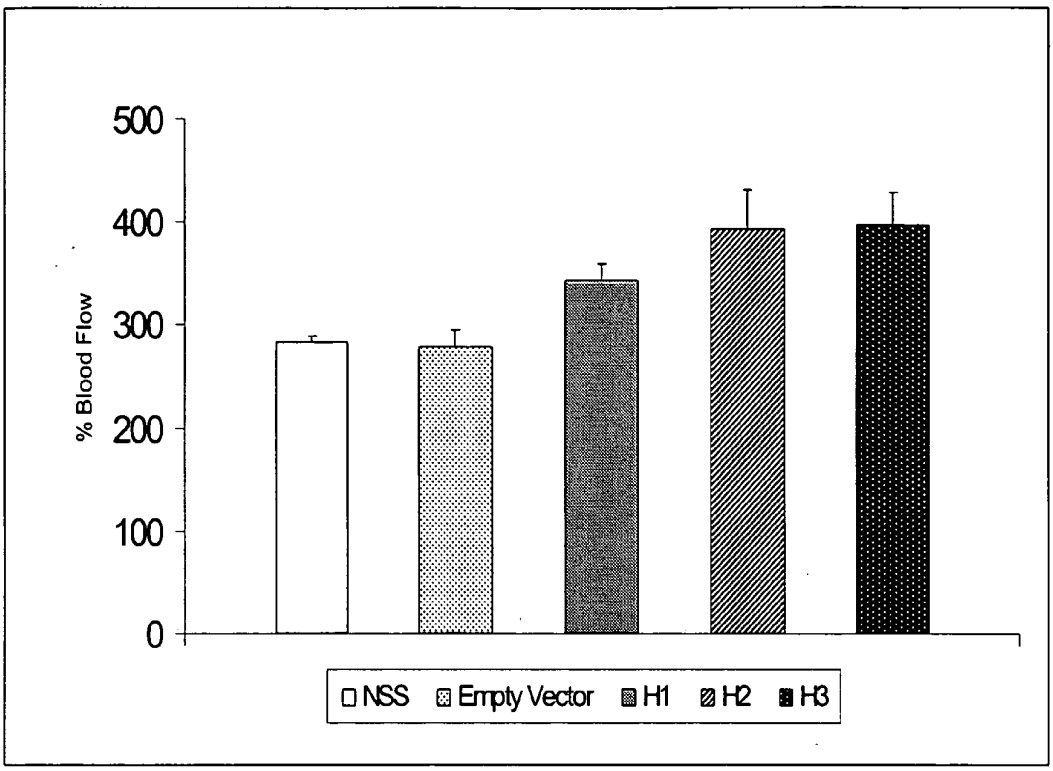

Figure 3

HEPATOCYTE GROWTH FACTOR NUCLEIC ACID SEQUENCE TO ENHANCE MUSCULOCUTANEOUS FLAP SURVIVAL

BACKGROUND

[0001] 1. Field of the Invention

[0002] This invention generally relates to the use of nucleic acid sequences encoding growth factors to promote angiogenesis and wound healing. In particular, described herein are methods and compositions for enhancing flap and skin graft survival by administering a nucleic acid sequence encoding hepatocyte growth factor.

[0003] 2. Background of the Invention

[0004] Hepatocyte growth factor (HGF) functions as a growth factor for particular tissues and cell types. HGF was initially identified as a mitogen for hepatocytes. Michalopoulos et al., *Cancer Res.,* 44:4414-4419 (1984); Russel et al., *J. Cell. Physiol.,* 119:183-192 (1984); Nakamura et al., *Biochem. Biophys. Res. Comm.,* 122:1450-1459 (1984). Nakamura et al. (supra), reported the purification of HGF from the serum of partially hepatectomized rats. Subsequently, the subunit structure of HGF was determined when HGF was purified from rat platelets. Nakamura et al., *Proc. Natl. Acad. Sci. USA,* 83:6489-6493 (1986); Nakamura et al., *FEBS Letters,* 224:311-316 (1987). Human HGF ("huHGF") has also been purified from human plasma. Gohda et al., *J. Clin. Invest.,* 81:414-419 (1988).

[0005] Comparisons of the amino acid sequence of rat HGF and huHGF revealed that the two sequences are highly conserved and have the same characteristic structural features. For example, the length of the four kringle domains in rat HGF is exactly the same as in huHGF, and the location of cysteine residues are in exactly the same positions. This is an indication that the three-dimensional structure of the two proteins is similar. Okajima et al. *Eur. J. Bioch.,* 193:375-81 (1990); Tashiro et al., *Proc. Natl. Acad. Sci., USA,* 87:3200-4 (1990).

[0006] Furthermore, several reports revealed close sequence homology between HGF and scatter factor (SF). Gherardi and Stoker, *Nature,* 346:228 (1990); Weidner et al., *J. Cell Biol.,* 111:2097-2108 (1990); Coffer et al., *Biochem J.,* 278:35-41 (1991). SF is a polypeptide that stimulates dissociation of epithelial cell colonies in monolayer culture. Gherardi et al., *Proc. Natl. Acad. Sci. USA,* 86:5844-5848 (1989). In fact, there now is evidence indicating that the two factors are identical; they are identical in structure and biological activity. Weidner et al., *Proc. Natl. Acad. Sci. USA,* 88:7001-5 (1991); Bhargava et al., *Cell Growth Differ.* 3:11-20 (1992); Naldini et al., *EMBO J.,* 10:2867-78 (1991); Furlong et al., *J. Cell Sci.,* 100:173-7 (1991). HGF and HGF variants are described further in U.S. Pat. Nos. 5,227,158, 5,316,921, and 5,328,837.

[0007] Binding of HGF to its receptor is believed to be conveyed by a functional domain located in the N-terminal portion of the HGF molecule. Matsumoto et al., *Biochem. Biophys. Res. Commun.,* 181:691-699 (1991); Hartmann et al., *Proc. Natl. Acad. Sci. USA.,* 89:11574-11578 (1992); Lokker et al., *EMBO J.,* 11:2503-2510 (1992); Lokker and Godowski, *J. Biol. Chem.,* 268:17145-17150 (1991). The HGF receptor is usually referred to as "c-Met" or "p^{190}MET" and typically comprises, in its native form, a 190-kDa heterodimeric (a disulfide-linked 50-kDa α-chain and a 145-kDa β-chain) membrane-spanning tyrosine kinase protein. Park et al., *Proc. Natl. Acad. Sci. USA,* 84:6379-6383 (1987). The c-Met protein becomes phosphorylated on tyrosine residues of the 145-kDa β-subunit upon HGF binding.

[0008] Various biological activities have been described for HGF and its receptor. See, generally, Chan et al., HEPATOCYTE GROWTH FACTOR—SCATTER FACTOR (HGF-SF) AND THE C-MET RECEPTOR, Goldberg and Rosen, eds., Birkhauser Verlag-Basel (1993), pp. 67-79). For example, HGF has been shown to be a mitogen for certain cell types, including melanocytes, renal tubular cells, keratinocytes, certain endothelial cells and cells of epithelial origin. Matsumoto et al., *Biochem. Biophys. Res. Commun.,* 176:45-51 (1991); Igawa et al., *Biochem. Biophys. Res. Commun.,* 174:831-838 (1991); Han et al., *Biochem.,* 30:9768-9780 (1991); Rubin et al., *Proc. Natl. Acad. Sci. USA,* 88:415-419 (1991). HGF has also been described as an epithelial morphogen, Montesano et al., *Cell,* 67:901-908 (1991), and therefore, HGF has been postulated to be important in tumor invasion, Comoglio, HEPATOCYTE GROWTH FACTOR—SCATTER FACTOR (HGF-SF) AND THE C-MET RECEPTOR, Goldberg and Rosen, eds., Birkhauser Verlag-Basel (1993), pp. 131-165. Until now, the intramuscular delivery of an HGF gene to promote flap and skin graft survival has not been described.

[0009] The use of skin flaps has gained increased acceptance and use in the course of reconstructive surgery, as well as in other forms of surgery. However, these techniques continue to be plagued by problems having to do with survival of the skin flaps which is, at least in part, due to the inefficient revascularization at the surgical site. Indeed, a number of approaches have been considered or evaluated for improving skin flap survival. See, for example, Waters et al., which provides a comparative analysis of the ability of five classes of pharmacological agents to augment skin flap survival in various models and species. *Annals of Plastic Surgery,* 23(2):117-22 (1989). Nevertheless, there still remains a need in the art for compositions and methods for enhancing survival of flap and skin grafts.

SUMMARY OF THE INVENTION

[0010] Therefore, the present invention describes the use of a nucleic acid sequence encoding HGF to enhance the survival of a flap or a skin graft.

[0011] In particular, described herein is a method for enhancing tissue survival, including survival of a flap or a skin graft following flap or skin graft surgery, comprising administering to a subject in need thereof a vector that comprises a nucleic acid sequence encoding hepatocyte growth factor. In specific embodiments, the vector is administered intramuscularly and is administered at least about 3-14 days prior to flap or skin graft surgery, at least about 5-10 days prior to surgery, or at least about 7 days prior to surgery. The instant invention is suitable for enhancing the survival of a flap, such as a skin flap, a muscle flap, a myocutaneous flap, or a cartilocutaneous flap, or a skin graft. Survival of the flap or skin graft may be enhanced by at least about 10-30% as compared to an untreated subject.

[0012] In one embodiment, the vector comprises the HGF nucleic acid sequence represented by SEQ ID NO. 1. The

vector also may comprise the pcDNA3.1(−) plasmid, as set forth in SEQ ID NO. 2, or the pVAX1 plasmid, as set forth in SEQ ID NO. 3.

[0013] In another embodiment, the present invention discloses a method for enhancing organ transplant survival in a subject comprising administering to a subject in need thereof a therapeutically effective amount of a composition that comprises a nucleic acid sequence encoding HGF.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **FIG. 1** compares musculocutaneous flap survival in an animal model after pretreatment with normal saline solution, an empty vector, or a vector comprising a nucleic acid sequence encoding HGF.

[0015] **FIG. 2** depicts the survival (1 week after surgery) of a transverse rectus abdominis musculocutaneous (TRAM) flap in rats pre-treated 7 days prior to the surgery with (A) normal saline, (B) empty vector or (C) vector comprising a nucleic acid sequence encoding HGF. (N=8 for each group).

[0016] **FIG. 3** is a histogram representing mean percentage blood flow in animals given normal saline solution (NSS), empty vector, or a vector comprising a nucleic acid sequence encoding HGF one week post-injection. Groups H1, H2 and H3 represent the experimental groups treated with 125 μg, 250 μg, and 500 μg HGF, respectively.

DETAILED DESCRIPTION OF THE INVENTION

[0017] 1. Introduction

[0018] The inventors have surprisingly discovered that pre-treating subjects with a nucleic acid sequence encoding HGF prior to flap, skin graft or organ transplant surgery significantly enhances survival of the tissue as compared to transplanted tissue in control subjects.

[0019] 2. Definitions

[0020] Unless otherwise specified, "a" or "an" means one or more.

[0021] The terms "hepatocyte growth factor" and "HGF" as used herein include hepatocyte growth factor from humans ("huHGF") and any non-human mammalian species of HGF, including rat HGF. The term "HGF" as used herein includes mature, pre, pre-pro, and pro forms, including forms purified from a natural source, and chemically synthesized or recombinantly produced HGF.

[0022] "Sequence identity" is defined herein with reference the Blast 2 algorithm, which is available at the NCBI (http://www.ncbi.nhn.nih.gov/BLAST), using default parameters. References pertaining to this algorithm include those found at http://www.ncbi.nlm.nih.gov/BLAST/blast_references.html; Altschul, et al., J. Mol. Biol. 215: 403-410 (1990); Gish & States, Nature Genet. 3: 266-272 (1993); Madden et al., Meth. Enzymol. 266: 131-141 (1996); Altschul et al., Nucleic Acids Res. 25: 3389-3402 (1997), and Zhang & Madden, Genome Res. 7: 649-656 (1997).

[0023] The terms "alteration,""amino acid alteration,""variant," and "amino acid sequence variant" refer to HGF molecules with some differences in their amino acid sequences as compared to a native human HGF. Ordinarily, the variants will possess at least about 80%, 85%, or 90% homology with the domains of native human HGF, including sequences at least about 95% homologous or at least about 99% homologous to native human HGF.

[0024] 3. Hepatocyte Growth Factor Nucleic Acid Sequence and Variants Thereof

[0025] The nucleic acid sequences encoding HGF for use in the present invention may encode a hepatic parenchymal cell growth factor. The encoded HGF may have a structure with six domains (finger, Kringle 1, Kringle 2, Kringle 3, Kringle 4 and serine protease domains).

[0026] One human HGF nucleic acid sequence suitable for use in the present invention is represented as SEQ ID NO:1 herein. Other human HGF nucleic acids also are suitable for use in the present invention, such as the hepatic parenchymal cell growth factor sequence disclosed in Kitamura et al., U.S. Pat. No. 5,500,354.

[0027] Likewise, non-mammalian HGF nucleic acids are suitable for use in the present invention. Rat HGF, for example, shares the same structural features as human HGF and is described in Tashiro et al., Proc. Nat'l. Acad. Sci. USA, 87(8):3200-4 (1990) and GenBank Accession No. NM_017017.

[0028] In other embodiments, the nucleic acid encoding HGF useful in the present invention has at least about 80% sequence identity, at least about 85% sequence identity, at least about 90% sequence identity, at least about 95% sequence identity, or at least 99% sequence identity with a native mammalian HGF gene. For example, genes having at least about 80% sequence identity, at least about 85% sequence identity, at least about 90% sequence identity, at least about 95% sequence identity, or at least about 99% sequence identity to SEQ ID NO:1 can be used in the present invention. As used herein, two nucleic acid molecules or proteins are said to "share significant sequence identity" if the two contain regions which possess greater than 90% sequence (amino acid or nucleic acid) identity over the entire length of the gene.

[0029] The invention also includes nucleic acid sequences that encode HGF proteins that are variants of a native HGF protein. For example, such HGF variants may have at least about 80% sequence identity, at least about 85% sequence identity, at least about 90% identity, at least 95% identity, or at least about 99% sequence identity to the protein encoded by the nucleic acid sequence represented in SEQ ID NO. 1. Given the known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist readily can construct DNAs encoding the conservative amino acid variants described herein. Examples of suitable variants are described below.

[0030] Fragments of HGF constitute HGF with fewer than all six domains (finger, Kringle 1, Kringle 2, Kringle 3, Kringle 4 and serine protease domains). Variants of HGF may have some of the domains of HGF repeated. Both fragments and variants are included within the scope of the invention if they still retain their respective ability to bind a HGF receptor, as determined by means known in the art.

[0031] Substituted HGF variants are those that have at least one amino acid residue in the corresponding wild-type HGF sequence removed and a different amino acid inserted

in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule. Conservative substitutions are contemplated in the present invention. For example, (a) nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b) polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (c) positively charged (basic) amino acids include arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids include aspartic acid and glutamic acid, and substitutions typically may be made within groups (a)-(d). In addition, glycine and proline may be substituted for one another based on their ability to disrupt α-helices. Similarly, certain amino acids, such as alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine and lysine are more commonly found in α-helices, while valine, isoleucine, phenylalanine, tyrosine, tryptophan and threonine are more commonly found in β-pleated sheets. Glycine, serine, aspartic acid, asparagine, and proline are commonly found in turns. Some preferred substitutions may be made among the following groups: (i) S and T; (ii) P and G; and (iii) A, V, L and I.

[0032] Insertional HGF variants are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in the wild-type HGF molecule. Immediately adjacent to an amino acid means connected to either the α-carboxy or α-amino functional group of the amino acid. The insertion may be of one or more amino acids. Ordinarily, the insertion will consist of one or two conservative amino acids. As stated above, amino acids similar in charge and/or structure to the amino acids adjacent to the site of insertion are defined as conservative. Alternatively, this invention includes insertion of an amino acid with a charge and/or structure that is substantially different from the amino acids adjacent to the site of insertion.

[0033] Deletional variants are those with one or more amino acids in the wild-type HGF molecule removed. Ordinarily, deletional variants will have one or two amino acids deleted in a particular region of the HGF molecule. Such deletional variants are also contemplated in the present invention.

[0034] All variants suitable for use in the present invention retain HGF activity. Such activity can be assayed by one of skill in the art according to known methods.

[0035] 4. Recombinant Vector Production

[0036] Recombinant vector production is well known in the art and is outlined in a brief exemplary fashion below.

[0037] Generally speaking, the constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a genomic DNA or DNA fragment or cDNA bearing an open reading frame is inserted, in either orientation. The invention further contemplates cells containing these vectors.

[0038] Bacterial Expression

[0039] Useful vectors for bacterial expression may be constructed by inserting a structural DNA sequence encoding a desired protein, together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector may comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and, if desirable, to provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera *Pseudomonas, Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice. In one embodiment, the prokaryotic host is *E. coli.*

[0040] Bacterial vectors may be, for example, bacteriophage-, plasmid- or cosmid-based. These vectors can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids typically containing elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pGEM 1 (Promega Biotec, Madison, Wis., USA), pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pKK232-8, pDR540, and pRIT5 (Pharmacia).

[0041] These "backbone" sections may be combined with an appropriate promoter and the structural sequence to be expressed. Bacterial promoters include lac, T3, T7, lambda P_R or P_L, trp, and ara.

[0042] These vectors optionally may be used for recombinant protein production. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter may be derepressed/induced by appropriate means (e.g., temperature shift or chemical induction) and cells may be cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification and isolation of the recombinant protein.

[0043] Eukaryotic Expression Vectors

[0044] In one embodiment, HGF cDNA is subcloned into a mammalian expression vector. Various mammalian cell culture systems can be employed to express recombinant protein. Examples of mammalian expression systems include selected mouse L cells, such as thymidine kinase-negative (TK) and adenine phosphoribosyl transferase-negative (APRT) cells. Other examples include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell* 23: 175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors may comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

[0045] Mammalian promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. In one embodiment, the HGF cDNA is subcloned into any expression vector in which the expression is driven by a CMV promoter. Exemplary mammalian vectors include pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV,

pMSG, and pSVL (Pharmacia). In one specific embodiment, the mammalian expression vector is pcDNA3.1 (Invitrogen) or pVAX1 (Invitrogen). pVAX1 is a 3.0 kb plasmid vector designed for use in the development of DNA vaccines. HGF cDNA can be inserted, for example, into a multiple cloning site of the pVAX1 vector. pcDNA3.1(−) has the same multiple cloning site as pcDNA3.1(+) but in a reverse orientation.

[0046] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a target protein in infected hosts. (See, e.g., Logan et al., 1984, *Proc. Natl. Acad. Sci. USA* 81: 3655-3659). If a viral vector is chosen as the delivery vehicle it may be one which is capable of integrating into the host genome so that the gene can be expressed permanently. In cases where the vector does not integrate into the host genome, the expression of the gene may be transient rather than permanent.

[0047] Adenoviral vectors ("Ad") are currently among the most efficient gene transfer vehicles for both in vitro and in vivo delivery, but the utilization of a first generation Ad for many gene therapy applications is limited due to the transient nature of transgene expression obtained by these vectors. Several factors have been shown to contribute to and modulate the duration of Ad-mediated gene expression as well as the immunogenicity of these vectors, including "leaky" viral protein expression and the transgene delivered. The development of Ad vectors, deleted in all viral protein coding sequences offers the prospects of a potentially safer, less immunogenic vector with an insert capacity of up to approximately 37 kb. This vector requires supplementation of viral regulatory and structural proteins in trans for packaging and rescue and is therefore helper dependent (HD). This is further described in Parks et al., Proc. Natl. Acad. Sci. USA, 93:13565-13570 (1996).

[0048] Use of retroviral vectors for protein expression are also known in the art. See, for example, Veres, et al., *J. Virol.,* 72:1894-1901 (1998); Agarwal et al., *J. Virol.,* 72:3720-3728 (1998); Forestell et al., *Gene Therapy,* 4:600-610 (1997); Plavec et al., *Gene Therapy,* 4:128-139, 1997; Forestell et al., *Gene Therapy,* 2:723-730 (1995); and Rigg et al., *J. Virol.,* 218:290-295, 1996. The genome of a recombinant retroviral vector is comprised of long terminal repeat (LTR) sequences at both ends which serve as a viral promoter/enhancer and a transcription initiation site, and a Psi site which serves as a virion packaging signal and a selectable marker gene. In one embodiment, the HGF polynucleotide sequences disclosed herein can be cloned into a suitable cloning site in the retroviral genome. Expression is under the transcriptional control of the retroviral LTR. Tissue selectivity is determined by both the origin of the viral genome (e.g., sarcoma virus, leukemia virus, or mammary tumor virus) and the cell line used to package the virus.

[0049] The recombinant vector useful in the present invention may include the exogenous DNA and regulatory sequences necessary and sufficient for expression of the encoded product (e.g., HGF) upon entry into the target cell. In one embodiment of the present invention, the vector includes exogenous DNA encoding the desired product (i.e., HGF), and, optionally, DNA encoding a selectable marker, along with additional sequences necessary for expression of the exogenous DNA in a target cell. In one specific embodiment, the vector comprises the nucleic acid sequence of SEQ ID NO. 1 and a pcDNA3.1(−) plasmid or a pVAX1 plasmid is used. See, e.g., SEQ ID NOs. 2 and 3. In yet another embodiment, the vector does not comprise an enhancer element. Additionally, infectious vectors can be used in the present invention, such as adenoviral, retroviral, and adenovirus-associated viral vectors, to express the exogenous DNA sequence in a target cell.

[0050] In accordance with the invention, a vector encoding HGF may be administered by intramuscular injection, or by intravenous, intraperitoneal, oral or subcutaneous means, or by other means of delivery. Suitable titers will depend on a number of factors, such as the particular vector chosen, the host, and the strength of promoter used.

[0051] In accordance with the invention, the vector encoding HGF is administered (for example, by intramuscular injection) prior to organ transplant, skin flap or skin graft surgery, up to a week or more prior to surgery. Administration at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, or at least about 10 days prior to organ transplant or flap or skin graft surgery is expressly contemplated and included within the invention. Administration up to about 14 days prior to organ transplant or flap or skin graft surgery is also contemplated and included within the invention. The term "about" in this context connotes up to one day before the specified number of days. For example, the phrase "at least about 3 days" means that the construct encoding HGF is administered between 2-3 days prior to organ transplant or flap or skin graft surgery. Thus, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24 hours prior to precisely 3 days before the surgery is within the meaning of the term "about."

[0052] Administration of the vector encoding HGF may be by direct injection, i.e., at or near the site of the flap, graft or organ transplant. In one embodiment, the construct encoding HGF is administered intramuscularly, such into the rectus abdominus muscle.

[0053] The inventive methods described herein enhance flap or skin graft survival by at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30% or more compared to an untreated control subject. The term "about" in this context connotes a range of up to 5% before or after the specified percentage. Thus, the phrase "about 10%" refers to a range of 5%-15%, but also specifically includes 10%.

[0054] 5. Pharmaceutically Acceptable Formulations

[0055] The HGF vector or HGF nucleic acid sequence compositions as described herein can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the inventive compositions, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human

proteins, e.g., human serum albumin, are described, for example, in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton Pa. (1980)). To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the vectors of the present invention, together with a suitable amount of carrier vehicle.

[0056] Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Thus, the HGF vector or HGF nucleic acid sequence compositions described herein, and their physiologically acceptable salts and solvate, may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, rectal, subcutaneous or intramuscular administration. In one embodiment, the HGF nucleic acid sequence or HGF vector compositions are formulated for intramuscular administration.

[0057] For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they maybe presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound. For buccal administration the composition may take the form of tablets or lozenges formulated in conventional manner.

[0058] For administration by inhalation, the HGF vector or HGF nucleic acid sequence compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0059] The HGF vector or HGF nucleic acid sequence compositions may be formulated for intravenous, subcutaneous or intramuscular administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampules or in multi-dose containers, with an added preservative. The HGF compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0060] The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0061] In addition to the formulations described previously, the HGF vector or HGF nucleic acid sequence compositions may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0062] The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

[0063] The present invention describes therapy with HGF nucleic acid sequences to enhance organ transplant, flap and skin graft survival. In particular, a nucleic acid sequence encoding HGF can be administered prior to organ transplant, flap or skin graft surgery. The invention is useful in any type of organ transplant, flap or skin graft surgery. For example, the invention is useful in conjunction with transplantation of a wide variety of organs, including skin, kidney, heart, liver, spleen, bone marrow, pancreas, lung, and islet of langerhans. There are many different kinds of flaps that can be used to address cutaneous defects, defects of muscle, defects of subcutaneous tissues, and defects in bone. For example, the HGF methods and compositions of the present invention can be used in conjunction with (including prior to) a cutaneous flap, muscle flap, myocutaneous flap, or cartilocutaneous flap surgery. Likewise, the HGF methods and compositions of the present invention can be used in conjunction with (including prior to) skin graft surgery.

[0064] The therapeutic methods of the present invention involve administering to a subject in need of treatment a therapeutically effective amount of the HGF vector or HGF nucleic acid sequence compositions described herein. "Therapeutically effective" is employed here to denote the amount of the HGF composition that is of sufficient quantity to promote angiogenesis and accelerate wound healing. In particular, it is desirable to administer a therapeutically effective amount of an HGF composition that will enhance organ transplant, musculocutaneous flap or skin graft survival. Some methods contemplate combination therapy with known medicaments or therapies that also promote angiogenesis or organ or flap survival.

[0065] The therapeutically effective amount of the HGF composition for use in this invention largely will depend on particular patient characteristics, the route of administration, and the nature of the disorder being treated (such as the type of organ transplant or the size, location, and thickness of the flap or skin graft at issue). General guidance can be found, for example, in the publications of the International Conference on Harmonisation and in REMINGTON'S PHARMACEUTICAL SCIENCES, chapters 27 and 28, pp. 484-528 (Mack Publishing Company 1990). In addition, the therapeutically effective amount may depend on such factors as toxicity and efficacy of the medicament. Toxicity may be determined using methods well known in the art and found in the foregoing references. Efficacy may be determined utilizing the same guidance in conjunction with the methods described below in the Examples. A pharmaceutically effective amount, therefore, is an amount that is deemed by the clinician to be toxicologically tolerable, yet efficacious. Efficacy, for example, can be measured by the decrease in necrosis, or the increase in angiogenesis or organ transplant or flap or graft survival. As illustrated by the foregoing references, the determination of a therapeutically effective amount can be determined by those skilled in the art, as guided by this disclosure.

[0066] In one embodiment, the therapeutically effective amount is from about 0.1 to about 50 mg per treatment, such as from about 0.5 to about 25 mg per treatment, and from about 1 to about 10 mg per treatment. In a specific embodiment, the plasmid is administered in several injections to the same area of the body as part of a single treatment.

[0067] The patient may be a human or non-human mammal, or another animal. A patient typically will be in need of treatment when scheduled to receive flap or skin graft surgery.

[0068] The invention is further described by reference to the following examples, which are provided for illustration only. The invention is not limited to the examples but rather includes all variations that are evident from the teachings provided herein.

EXAMPLE 1

Plasmid Constructs

[0069] Human HGF is subcloned into the pcDNA3.1(−) (Invitrogen) into the NotI site, which produces the human HGF protein under the control of the CMV promoter. See, e.g., SEQ ID NO. 2. The HGF gene plasmid concentrate is reconstituted with normal saline solution (NSS) to obtain a 1 μg/μl concentration.

EXAMPLE 2

HGF Nucleic Acid Sequence Enhances Flap Survival

[0070] The experiment is conducted following the guidelines set by the animal laboratory of the Institute of Animal and Experimental Sciences, Osaka University. Twenty four male Sprague-Dawley rats weighing 300-350 grams are used and equally divided into three groups (N=8). Animals are anesthetized with an intraperitoneal injection of pentobarbital (2 mg/100 g). In group 1, 250 μl of NSS is injected into the left rectus abdominus muscle, while 250 μg (1 μg/μl) of empty plasmid (pcDNA3.1(−)) and 250 μg (1 μg/μl) of HGF gene containing plasmid is injected into the same site in Group 2 and Group 3, respectively. After 7 days, a simulated transverse rectus abdominus musculocutaneous (TRAM) flap measuring 4.5 cm×9 cm is elevated. TRAM flap based on the left rectus abdominus muscle as the carrier and the superior epigastric vessels as the vascular pedicle are designed on the lower half of the abdomen. The flap is sutured to its original location and monitored for 1 week for infection, necrosis and flap survival. All the animals are sacrificed using an overdose of pentobarbital given intraperitonally. Direct measurement is made on the area of necrosis and flap survival using a transparent metric template.

[0071] The data indicate the following with regard to flap survival one week post-surgery:

TABLE 1

	NSS	Empty Vector	HGF IM
% Flap Survival	54.125	53.875	87.75
Standard Deviation	22.96853438	25.41336825	17.88654722
Sample Size	8	8	8

[0072] The superiority of pre-treating a TRAM flap with a gene encoding HGF is also exemplified in **FIG. 2**. **FIG. 2** demonstrates that flap survival is markedly enhanced one week post-surgery in animals receiving a gene encoding HGF (designated as "H" animals; n=8) at least 7 days prior to flap surgery, compared to control animals (designated as "C" animals; n=8) and animals treated with the empty vector (designated as "V" animals; n=8). (The number following the letters "H,""C" or "V" indicate the animal number.)

[0073] In a separate study, 20 Sprague Dawley rats were randomized into 5 groups, 4 rats per group, and were directly injected with 125 μg, 250 μg, or 500 μg of a plasmid encoding HGF, NSS, or an empty vector into the left rectus abdominus muscle, (the likely TRAM flap cite). One week post-injection, skin blood flow measurements were taken by a laser color Doppler (Laser Doppler Imager, Moor Instruments), which determines blood flow velocity and correlates with capillary density. The results indicated that blood flow was higher in the treated area for the HGF treated groups compared to the groups given NSS or the empty vector (**FIG. 3** and Table 2).

TABLE 2

	NSS	Empty Vector	HGF IM (125 μg)	HGF IM (250 μg)	HGF IM (500 μg)
Mean % Blood Flow	282.675	278.175	343.333	392.900	396.725
Standard Error	5.923593	16.7246	15.59202	37.70972	33.55604
Sample Size	4	4	4	4	4

[0074] **FIG. 3** was generated by quantitatively converting the laser images from the laser Doppler, creating a histogram with the amount of blood flow provided on the y-axis and each experimental group, i.e., animals given NSS, the empty vector, H1(125 μg HGF), H2 (250 μg HGF) or H3 (500 μg

HGF), on the x-axis. Use of the laser Doppler to visualize blood vessels and measure blood flow is described in Wong et al., Gastrointest. Endosc., 55(1): 88-95 (2002).

[0075] Additional embodiments are within the scope of the invention. For example, the invention is further illustrated by the following numbered embodiments:

[0076] 1. A method for enhancing tissue survival, including survival of a flap or a skin graft following flap or skin graft surgery, comprising administering to a subject in need thereof a vector that comprises a nucleic acid sequence encoding hepatocyte growth factor.

[0077] 2. The method of embodiment 1, wherein said flap is selected from the group consisting of a skin flap, a muscle flap, a myocutaneous flap and a cartilocutaneous flap.

[0078] 3. The method of embodiment 1, wherein said vector is administered intramuscularly.

[0079] 4. The method of embodiment 1, wherein said nucleic acid encoding HGF is represented by SEQ ID NO. 1.

[0080] 5. The method of embodiment 4, wherein said vector is administered at least 3-14 days prior to flap or skin graft surgery.

[0081] 6. The method of embodiment 4, wherein said vector is administered at least 5-10 days prior to flap or skin graft surgery.

[0082] 7. The method of embodiment 4, wherein said vector is administered at least 7 days prior to flap or skin graft surgery.

[0083] 8. The method of embodiment 5, wherein said vector is a plasmid selected from the group consisting of pcDNA3.1(−) and pVAX1.

[0084] 9. The method of embodiment 5, wherein said vector comprises the sequence of SEQ ID NO. 2.

[0085] 10. The method of embodiment 5, wherein said vector comprises sequence of SEQ ID NO. 3.

[0086] 11. The method of embodiment 1, wherein the survival of the flap or skin graft is enhanced by at least 10-30% as compared to an untreated subject.

[0087] 12. A method for enhancing organ transplant survival in a subject comprising administering to a subject in need thereof a therapeutically effective amount of a composition that comprises a nucleic acid encoding HGF.

[0088] 13. The method of embodiment 3, wherein said vector is administered in the rectus abdominus muscle.

[0089] 14. A method for enhancing tissue survival, including survival of a flap or a skin graft following flap or skin graft surgery, comprising administering to a subject in need thereof a vector that comprises a nucleic acid sequence encoding hepatocyte growth factor, wherein administration of the vector results in enhanced blood flow.

[0090] Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention. All of the publications, patent applications and patents cited in this specification are incorporated herein by reference in their entirety.

Hepatocyte Growth Factor Nucleic Acid Sequence
SEQ ID NO. 1

ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCAGCATGTCCTCCT

GCATCTCCTCCTGCTCCCCATCGCCATCCCCTATGCAGAGGGACAAAGGA

AAAGAAGAAATACAATTCATGAATTCAAAAAATCAGCAAAGACTACCCTA

ATCAAAATAGATCCAGCACTGAAGATAAAAACCAAAAAAGTGAATACTGC

AGACCAATGTGCTAATAGATGTACTAGGAATAAAGGACTTCCATTCACTT

GCAAGGCTTTTGTTTTTGATAAAGCAAGAAAACAATGCCTCTGGTTCCCC

TTCAATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCATGAATTTGA

CCTCTATGAAAACAAAGACTACATTAGAAACTGCATCATTGGTAAAGGAC

GCAGCTACAAGGGAACAGTATCTATCACTAAGAGTGGCATCAAATGTCAG

CCCTGGAGTTCCATGATACCACACGAACACAGCTTTTTGCCTTCGAGCTA

TCGGGGTAAAGACCTACAGGAAAACTACTGTCGAAATCCTCGAGGGGAAG

AAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAGGTACGCTACGAAGTC

TGTGACATTCCTCAGTGTTCAGAAGTTGAATGCATGACCTGCAATGGGGA

GAGTTATCGAGGTCTCATGGATCATACAGAATCAGGCAAGATTTGTCAGC

GCTGGGATCATCAGACACCACACCGGCACAAATTCTTGCCTGAAAGATAT

CCCGACAAGGGCTTTGATGATAATTATTGCCGCAATCCCGATGGCCAGCC

GAGGCCATGGTGCTATACTCTTGACCCTCACACCCGCTGGGAGTACTGTG

CAATTAAAACATGCGCTGACAATACTATGAATGACACTGATGTTCCTTTG

GAAACAACTGAATGCATCCAAGGTCAAGGAGAAGGCTACAGGGGCACTGT

CAATACCATTTGGAATGGAATTCCATGTCAGCGTTGGGATTCTCAGTATC

CTCACGAGCATGACATGACTCCTGAAAATTTCAAGTGCAAGGACCTACGA

GAAAATTACTGCCGAAATCCAGATGGGTCTGAATCACCCTGGTGTTTTAC

CACTGATCCAAACATCCGAGTTGGCTACTGCTCCCAAATTCCAAACTGTG

ATATGTCACATGGACAAGATTGTTATCGTGGGAATGGCAAAAATTATATG

GGCAACTTATCCCAAACAAGATCTGGACTAACATGTTCAATGTGGGACAA

GAACATGGAAGACTTACATCGTCATATCTTCTGGGAACCAGATGCAAGTA

AGCTGAATGAGAATTACTGCCGAAATCCAGATGATGATGCTCATGGACCC

TGGTGCTACACGGGAAATCCACTCATTCCTTGGGATTATTGCCCTATTTC

TCGTTGTGAAGGTGATACCACACCTACAATAGTCAATTTAGACCATCCCG

TAATATCTTGTGCCAAAACGAAACAATTGCGAGTTGTAAATGGGATTCCA

ACACGAACAAACATAGGATGGATGGTTAGTTTGAGATACAGAAATAAACA

TATCTGCGGAGGATCATTGATAAAGGAGAGTTGGGTTCTTACTGCACGAC

AGTGTTTCCCTTCTCGAGACTTGAAAGATTATGAAGCTTGGCTTGGAATT

CATGATGTCCACGGAAGAGGAGATGAGAAATGCAAACAGGTTCTCAATGT

TTCCCAGCTGGTATATGGCCCTGAAGGATCAGATCTGGTTTTAATGAAGC

TTGCCAGGCCTGCTGTCCTGGATGATTTTGTTAGTACGATTGATTTACCT

-continued

AATTATGGATGCACAATTCCTGAAAAGACCAGTTGCAGTGTTTATGGCTG

GGGCTACACTGGATTGATCAACTATGATGGCCTATTACGAGTGGCACATC

TCTATATAATGGGAAATGAGAAATGCAGCCAGCATCATCGAGGGAAGGTG

ACTCTGAATGAGTCTGAAATATGTGCTGGGGCTGAAAAGATTGGATCAGG

-continued

ACCATGTGAGGGGGATTATGGTGGCCCACTTGTTTGTGAGCAACATAAAA

TGAGAATGGTTCTTGGTGTCATTGTTCCTGGTCGTGGATGTGCCATTCCA

AATCGTCCTGGTATTTTTGTCCGAGTAGCATATTATGCAAAATGGATACA

CAAAATTATTTTAACATATAAGGTACCACAGTCATAG

[0091]

pcDNA3.1(-)HGF Sequence

SEQ ID NO. 2

GACGGATCGG GAGATCTCCC GATCCCCTAT GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT
GGAGGTCGCT GAGTAGTGCG CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC TTAGGGTTAG GCGTTTTGCG
CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT GATTATTGAC TACTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG CTGCTTATCG AATTAATACG CTCACTATAG GGAGACCCAA GCTGGCTAGC
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CACAAATTCT TGCCTGAAAG ATATCCCGAC AAGGGCTTTG ATGATAATTA TTGCCGCAAT CCCGATGGCC AGCCGAGGCC ATGGTGCTAT ACTCTTGACC
CTCACACCCG CTGGGAGTAC AAACATGCGC TGACAATACT ATGAATGACA CTGATGTTCC TTTGGAAACA ACTGAATGCA TCCAAGGTCA
AGGAGAAGGC TACAGGGGCA CATTTGGAAT GGAATTCCAT GTCAGCGTTG GGATTCTCAG CAGGACCATG ACTCCTCAGA TATGGTGCCC
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ACCGCTTCCT	CGTGCTTTAC	GGTATCGCCG	CTCCCGATTC	GCAGCGCATC	GCCTTCTTGA	CGAGTTCTTC	CGAGTTCTTC	TGAGCGGGAC	TCTGGGGTTC
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GCGGTATCAG	CTCACTCAAA	GGCGGTAATA	CGGTTATCCA	CAGAATCAGG	GGATAACGCA	GGAAGAACA	TGTGAGCAAA	AGGCCAGCAA	AAGGCCAGGA
ACCGTAAAAA	GGCCGCGTTG	CTGGCGTTTT	TCCATAGGCT	CCGCCCCCCT	GACGAGCATC	ACAAAAATCG	ACGCTCAAGT	CAGAGGTGGC	GAAACCCGAC
AGGACTATAA	AGATACCAGG	CGTTTCCCCC	CTGGAAGCTC	CTCGTGCGCT	CTCCTGTTCC	GACCCTGCCG	CTTACCGGAT	ACCTGTCCGC	CTTTCTCCCT
TCGGGAAGCG	TGGCGCTTTC	TCATAGCTCA	CGCTGTAGGT	ATCTCAGTTC	GTCGTGAGGT	ATTCGCTCCA	AGCTGGGCTG	TGTGCACGAA	CCCCCCGTTC
AGCCCGACCG	CTGCGCCTTA	TCCGGTAACT	ATCGTCTTGA	GTCCAACCCG	GTAAGACACG	ACTTATCGCC	ACTGGCAGCA	GCCACTGGTA	ACAGGATTAG
CAGAGCGAGG	TATGTAGGCG	GTGCTACAGA	GTTCTTGAAG	TGGTGGCCTA	ACTACGGCTA	CACTAGAAGA	ACAGTATTTG	GTATCTGCGC	TCTGCTGAAG
CCAGTTACCT	TCGGAAAAAG	AGTTGGTAGC	TCTTGATCCG	GCAAACAAAC	CACCGCTGGT	AGCGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA
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AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTCAT	CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG
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[0093]

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What is claimed:

1. A method for enhancing tissue survival, comprising administering to a subject in need thereof a vector that comprises a nucleic acid sequence encoding hepatocyte growth factor.

2. The method of claim 1, wherein said tissue is selected from the group consisting of a flap and a skin graft.

3. The method of claim 2, wherein said flap is selected from the group consisting of a skin flap, a muscle flap, a myocutaneous flap and a cartilocutaneous flap.

4. The method of claim 2, wherein said vector is administered at least 3-14 days prior to flap or skin graft surgery.

5. The method of claim 2, wherein said vector is administered at least 5-10 days prior to flap or skin graft surgery.

6. The method of claim 2, wherein said vector is administered at least 7 days prior to flap or skin graft surgery.

7. The method of claim 2, wherein the survival of the flap or skin graft is enhanced by at least 10-30% as compared to an untreated subject.

8. The method of claim 2, wherein said vector is administered intramuscularly, at or near the planned cite of flap or skin graft.

9. The method of claim 8, wherein said vector is administered in the rectus abdominus muscle.

10. The method of claim 1, wherein said vector comprises the nucleic acid sequence of SEQ ID NO. 1.

11. The method of claim 10, wherein said vector is a plasmid selected from the group consisting of pcDNA3.1(−) and pVAX1.

12. The method of claim 11, wherein said vector comprises the sequence of SEQ ID NO. 2.

13. The method of claim 11, wherein said vector comprises sequence of SEQ ID NO. 3.

14. A method for enhancing organ transplant survival in a subject comprising administering to a subject in need thereof a therapeutically effective amount of a composition that comprises a nucleic acid sequence encoding HGF.

15. The method of claim 14, wherein said composition further comprises a pharmaceutically acceptable excipient.

16. A method for enhancing tissue survival, comprising administering to a subject in need thereof a vector that comprises a nucleic acid sequence encoding hepatocyte growth factor, wherein administration of the vector results in enhanced blood flow.

17. The method of claim 16, wherein said tissue is selected from the group consisting of a flap and a skin graft.

18. The method of claim 16, wherein said tissue is a transplanted organ.

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