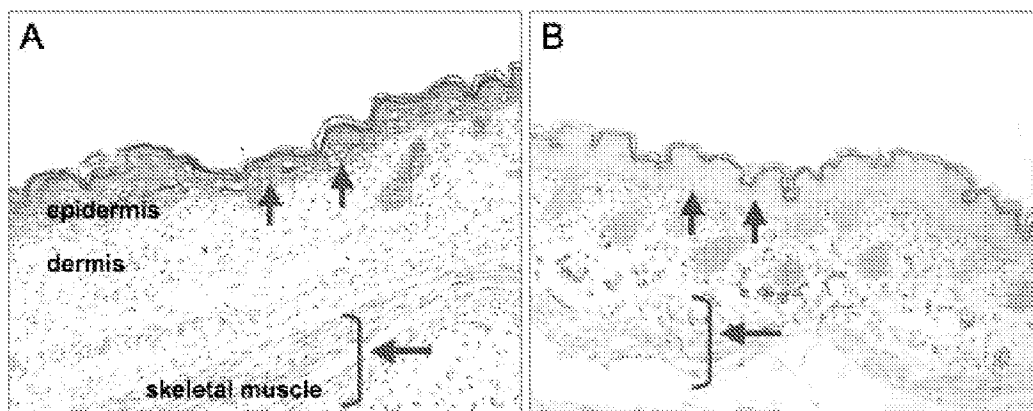




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(54) Title: METHODS FOR PROMOTING WOUND HEALING AND MUSCLE REGENERATION WITH THE CELL
SIGNALING PROTEIN NELL1



(57) Abrégé/Abstract:

The present invention provides methods for promoting wound healing and treating muscle atrophy in a mammal in need. The method comprises administering to the mammal a Nell1 protein or a Nell1 nucleic acid molecule.

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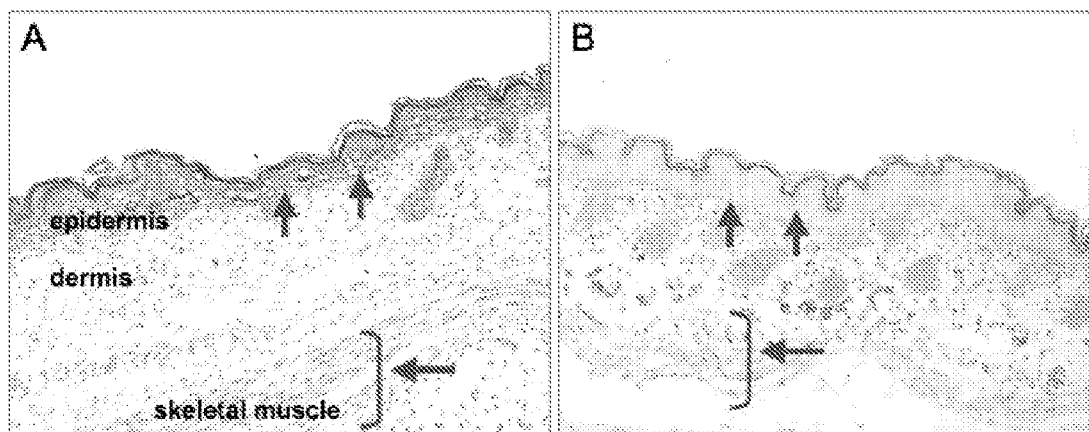


Figure 8

(57) Abstract: The present invention provides methods for promoting wound healing and treating muscle atrophy in a mammal in need. The method comprises administering to the mammal a Nell1 protein or a Nell1 nucleic acid molecule.

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METHODS FOR PROMOTING WOUND HEALING AND MUSCLE REGENERATION WITH THE CELL SIGNALING PROTEIN NELL1

FIELD OF THE INVENTION

[0004]. This invention relates to the field of wound healing and muscle regeneration. In particular, the invention relates to the discovery that Nell1 protein promotes wound healing and muscle regeneration.

BACKGROUND OF THE INVENTION

[0005]. Wound healing involves a series of complex biological processes whereby injured tissue is repaired, specialized tissue is regenerated, and new tissue is

reorganized. The healing of wounds is generally divided into three phases: the inflammatory phase, the proliferative phase, and maturation and remodeling phase.

[0006]. In the inflammatory phase, the clotting cascade is initiated in order to stop blood loss. In addition, various factors, such as chemokines, cytokines, and growth factors, are released to attract and activate cells that phagocytize debris, bacteria, and damaged tissue.

[0007]. The proliferative phase is characterized by angiogenesis and rebuilding of the extracellular matrix architecture which includes collagen deposition, granulation tissue formation, and epithelialization. The formation of new blood vessels, such as capillaries, and the formation of extracellular matrix enable activated satellite cell to proliferate, differentiate, and fuse into new muscle fibers.

[0008]. Typically, the maturation and remodeling phase of wound healing is said to begin when the levels of collagen production and degradation equalize. During maturation, type III collagen, which is prevalent during proliferation, is gradually degraded and the stronger type I collagen is laid down in its place. The originally disorganized collagen fibers are rearranged, cross-linked, and aligned. In addition, the newly regenerated muscle matures and contracts with the reorganization of the scar tissue.

[0009]. An impairment in any of these complex phases leads to complications in wound healing. Therefore, it would be beneficial to provide methods for promoting wound healing and/or muscle regeneration.

SUMMARY OF THE INVENTION

[0010]. These and other objectives have been met by the present invention, which provides, in one aspect, a method for promoting healing of a wound in a mammal in need thereof. The method comprises administering to the mammal an effective amount of Nell1 protein or nucleic acid molecule.

[0011]. In another aspect, the invention provides a method for treating skeletal muscle atrophy in a mammal in need thereof. The method comprises administering to the mammal an effective amount of Nell1 protein or nucleic acid molecule.

[0011.1]. According to one aspect of the invention, there is provided a composition for promoting healing of a skeletal muscle wound in a mammal having said wound, said composition comprising a Nell1 protein or a nucleic acid encoding a Nell1 protein, wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.

[0011.2]. According to another aspect of the invention, there is provided a composition for promoting healing of a skin wound in a mammal having said skin wound, wherein the composition comprises a Nell1 protein or a nucleic acid coding for a Nell1 protein formulated for local administration to the skin wound, and wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.

[0011.3]. According to yet another aspect of the invention, there is provided a composition for treating skeletal muscle atrophy in a mammal having skeletal muscle atrophy, said composition comprising a Nell1 protein or a nucleic acid encoding a Nell1 protein, wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.

[0011.4]. According to another aspect of the invention, there is provided a Nell1 protein or a nucleic acid encoding a Nell1 protein for use in promoting healing of a skeletal muscle wound in a mammal having said wound, wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to the full length sequence set forth as SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.

[0011.5]. According to another aspect of the invention, there is provided a Nell1 protein or a nucleic acid encoding a Nell1 protein for use in promoting healing of a skin wound in a mammal having said skin wound, wherein the Nell1 protein or the nucleic acid coding for a Nell1 protein is formulated for local administration to the skin wound, and wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to the full length sequence set forth as SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.

[0011.6]. According to yet another aspect of the invention, there is provided a Nell1 protein or a nucleic acid encoding a Nell1 protein for use in treating skeletal muscle atrophy in a mammal having skeletal muscle atrophy, wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to the full length sequence set forth as SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.

[0012]. For a better understanding of the present invention, together with other and further advantages, reference is made to the following detailed description, and its scope will be pointed out in the subsequent claims.

BRIEF DESCRIPTION OF THE FIGURES

[0013]. Figure 1. Amino acid sequence of human Nell1 protein.

[0014]. Figure 2. Nucleotide sequence encoding human Nell1.

[0015]. Figure 3. Amino acid sequence of rat Nell1 protein.

[0016]. Figure 4. Nucleotide sequence encoding rat Nell1.

[0017]. Figure 5. Amino acid sequence of mouse Nell1.

[0018]. Figure 6. Nucleotide sequence encoding mouse Nell1.

[0019]. Figure 7. Amino acid sequence alignment of the human Nell1 protein (SEQ ID NO: 1) and the mouse Nell1 protein (SEQ ID NO: 5). The functional domains of the human Nell1 protein are found in the essentially same regions as those identified in the mouse Nell1 protein

[0020]. Figure 8. Nell1 role in blood vessel and capillary network formation.

DETAILED DESCRIPTION OF THE INVENTION

[0021]. The invention is based on the surprising discovery by the inventor that, Nell1 protein promotes wound healing and muscle regeneration. Throughout this specification, parameters are defined by maximum and minimum amounts. Each

minimum amount can be combined with each maximum amount to define a range.

Method for Promoting Healing of a Wound

[0022]. In one aspect, the present invention provides a method for promoting healing of a wound in a mammal in need thereof. As used herein, the term “promoting healing of a wound” refers to augmenting, improving, increasing, or inducing closure, healing, or repair of a wound. Wound healing is considered to be promoted, for example, if the time of healing a wound treated with Nell1 compared to a wound not treated with Nell1 is decreased by about 10%, preferably decreased by about 25%, more preferably decreased by about 50%, and most preferably decreased by about 75%. Alternatively, wound healing is considered to be promoted if the time and extent of re-acquisition of muscle contractility and function treated with Nell1 compared to a wound not treated with Nell1 is improved by about 10%, preferably improved by about 25%, more preferably improved by about 50%, and most preferably improved by about 75%. Conversely, the degree of scar formation can be used to ascertain whether wound healing is promoted.

[0023]. The wound can be an internal wound or an external wound found in any location of a mammal. A wound is typically caused by physical means, such as mechanical, chemical, bacterial, or thermal means. Wounds can also be caused by accidents, such as a car accident, a fall, injuries sustained in battle (deep lacerations and amputations in soldiers), etc. or by surgical procedures, such as open heart surgery, organ transplants, amputations, and implantations of prosthetics, such as joint and hip replacement, etc. The wound can be an open wound or closed wound.

[0024]. Open wounds refers to wounds in which the skin is broken. Open wounds include, for example, incisions (i.e., wounds in which the skin is broken by, for instance, a cutting instrument (e.g., knife, razor, etc.)), lacerations (i.e., wounds in which the skin is typically broken by a dull or blunt instrument), abrasions (e.g.,

generally a superficial wound in which the topmost layers of the skin are scraped off), puncture wounds (typically caused by an object puncturing the skin, such as nail or needle), penetration wounds (e.g., caused by an object such as a knife), and gunshot wounds.

- [0025]. Closed wounds are typically wounds in which the skin is not broken. An example of a closed wound is a contusion.
- [0026]. Any mammal suffering from a wound, such as those described above, is in need of promoting wound healing in accordance with the method of the present invention.
- [0027]. Mammals also in need of promoting wound healing further include any mammal with a disease or condition associated with impaired neovascularization and/or impaired angiogenesis. Neovascularization typically refers to the formation of functional microvascular networks with red blood cell perfusion. Angiogenesis refers generally to the protrusion and outgrowth of capillary buds and sprouts from pre-existing blood vessels. Examples of diseases or conditions associated with impaired neovascularization and/or impaired angiogenesis include diabetes, vascular diseases and aging
- [0028]. In one embodiment, the wound healing is promoted in the mammal by promoting regeneration of skeletal muscle. Muscle tissue generally regenerate from reserve myoblasts called satellite cells. The satellite cells are typically found distributed throughout muscle tissue. In undamaged muscle, the majority of satellite cells are quiescent in that they neither differentiate nor undergo cell division.
- [0029]. Following muscle injury or during recovery from disease, satellite cells re-enter the cell cycle, proliferate, and enter existing muscle fibers or undergo differentiation into multinucleate myotubes which form new muscle fiber. The myoblasts eventually yield replacement muscle fibers or fuse into existing muscle fibers, thereby increasing fiber girth.

- [0030]. Thus, the term “regeneration of skeletal muscle” refers to the process by which new skeletal muscle fibers form from muscle progenitor cells. The new skeletal muscle fibers can be new skeletal muscle fibers that replace injured or damaged muscle fibers or new skeletal fibers that fuse into existing muscle fibers.
- [0031]. Skeletal muscle regeneration is considered to be promoted if the number of new fibers is increased at least about 1%, more preferably at least by about 20%, and most preferably by at least about 50%.
- [0032]. In another embodiment, the wound healing is promoted in the mammal by promoting collagen production. Collagen is a fibrous structural protein and a major component of the extracellular matrix. Any type of collagen can be promoted in accordance with the method of the present invention. Examples of types of collagen include, but are not limited to, collagen types I-XXVIII. Preferably, the collagen is type I, collagen type III, collagen type IV, or collagen type VI.
- [0033]. The term “promoting collagen production” refers to an increase in the amount of collagen produced. Any method known to those skilled in the art can be used to determine whether the production of collagen is increased. For example, an increase in collagen production can be determined by analyzing for increased expression of collagen by using, for example, Northern Blot, real time RTPCR, etc. Typically, collagen production is considered to be promoted if the amount of collagen is increased by at least about 1%, more preferably at least by about 10%, and most preferably by at least about 20%.
- [0034]. In one aspect, the method for promoting healing of a wound comprises administering to the mammal in need thereof, an effective amount of a Nell1 protein. The Nell1 protein useful in the methods of the present invention is described below.
- [0035]. In another aspect, the method for promoting healing of a wound comprises administering to the mammal a nucleic acid molecule encoding a Nell1 protein.

The nucleic acid molecule useful in the methods of the present invention is described below.

Method for Treating Muscle Atrophy

- [0036]. In another aspect, the present invention provides a method for treating skeletal muscle atrophy in a mammal in need thereof. The term “muscle atrophy” refers to loss of skeletal muscle mass and strength. The atrophy can be found in any location of a mammal.
- [0037]. Skeletal muscle atrophy can be caused by, for example, genetic abnormalities (e.g., mutations or combinations of certain single nucleotide polymorphisms), poor nourishment, poor circulation, loss of hormonal support, disuse of the muscle due to lack of exercise (e.g., bedrest, immobilization of a limb in a cast, etc.), and aging.
- [0038]. Alternatively, skeletal muscle atrophy can be caused by loss of nerve supply to a target organ. Examples of such diseases and conditions include CMT (Charcot Marie Tooth syndrome) poliomyelitis, amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease), and Guillain-Barre syndrome.
- [0039]. Conversely, skeletal muscle atrophy can be a disease of the muscle tissue itself. Examples of such diseases include, but are not limited to, muscular dystrophy, myotonia congenita, and myotonic dystrophy.
- [0040]. Similarly, certain diseases and conditions can also induce skeletal muscle atrophy. Examples of such diseases and conditions include congestive heart failure and liver disease.
- [0041]. Any mammal suffering from skeletal muscle atrophy, such as those described above, can be treated in accordance with the method of the present invention. In one aspect, the method for treating skeletal muscle atrophy includes administering to the mammal an effective amount of a Nell1 protein described

below. The Nell1 protein promotes skeletal muscle regeneration, thereby treating the skeletal muscle atrophy.

[0042]. In another aspect, the method for treating skeletal muscle atrophy comprises administering to the mammal a nucleic acid molecule encoding a Nell1 protein. The nucleic acid molecule useful in the methods of the present invention is described below.

Nell1 Protein

[0043]. Nell1 protein is a protein kinase C (PKC) β -binding protein. The Nell1 protein useful in the methods of the present invention can comprise a polypeptide having the same amino acid sequence as Nell1 protein derived from nature, a recombinant Nell1 protein, a homolog thereof, or fragments thereof. Accordingly, a “Nell1 protein” as used herein, also refers to homologs and fragments thereof.

[0044]. The amino acid sequence of Nell1 protein is highly conserved across species. For example, the mouse Nell1 protein shares about 93% sequence identity with the human Nell1 protein, which, in turn, shares about 90% sequence identity with the rat Nell1 protein. Figure 7 shows a sequence alignment between human Nell1 protein and mouse Nell1 protein.

[0045]. Since the amino acid sequence of Nell1 protein is highly conserved, the naturally occurring amino acid sequence of Nell1 protein can be from any animal. For example, the Nell1 protein can be human Nell1, rat Nell1, or mouse Nell1.

[0046]. The amino acid sequence of human Nell1 protein can be found at GenBank Accession No. AAH96102, and is shown in figure 1 (SEQ. ID. NO: 1). Due to the degeneracy of the genetic code, an example of a nucleotide sequence which encodes SEQ. ID. NO: 1 is shown in figure 2 (SEQ. ID. NO:2).

[0047]. The amino acid sequence of rat Nell1 protein can be found at GenBank Accession No. NP_112331, and is shown in figure 3 (SEQ. ID. NO: 3). An

example of a nucleotide sequence which encodes SEQ. ID. NO: 3 is shown in figure 4 (SEQ. ID. NO: 4).

[0048]. The amino acid sequence of mouse Nell1 protein can be found at GenBank Accession No. NP_001032995, and is shown in figure 5 (SEQ. ID. NO: 5). An example of a nucleotide sequence which encodes SEQ. ID. NO: 5 is shown in figure 6 (SEQ. ID. NO: 6).

[0049]. The structure of Nell1 proteins has been characterized (see, e.g., Kuroda et al., 1999a; Kuroda et al., 1999b, Desai et al., 2006). For example, the mouse Nell1 protein (SEQ ID NO: 5) is a protein of 810 amino acids, having a secretion signal peptide (amino acids 1 to 16), an N-terminal TSP-like module (amino acids # 29 to 213), a Laminin G region (amino acids # 86 to 210), von Willebrand factor C domains (amino acids # 273 to 331 and 699 to 749), and a Ca^{2+} -binding EGF-like domains (amino acids # 549 to 586).

[0050]. The secretion signal peptide domain of Nell1 protein is an amino acid sequence in the protein that is generally involved in transport of the protein to cell organelles where it is processed for secretion outside the cell. The N-terminal TSP-like module is generally associated with heparin binding. von Willebrand factor C domains are generally involved with oligomerization of Nell1. Laminin G domains of Nell1 protein are generally involved in adherence of Nell1 protein to specific cell types or other extracellular matrix proteins. The interaction of such domains with their counterparts is generally associated with, for example, processes such as differentiation, adhesion, cell signaling or mediating specific cell-cell interactions in order to promote cell proliferation and differentiation. The Ca^{2+} -binding EGF-like domains of Nell1 binds protein kinase C beta, which is typically involved in cell signaling pathways in growth and differentiation

[0051]. Homologs of Nell1 protein include, for example, a substitution mutant, a mutant having an addition or insertion, or a deletion mutant of the protein. Substitutions in a sequence of amino acids are preferably with equivalent amino

acids. Groups of amino acids known to be of equivalent character are listed below:

- (a) Ala(A), Ser(S), Thr(T), Pro(P), Gly(G);
- (b) Asn(N), Asp(D), Glu(E), Gln(Q);
- (c) His(H), Arg(R), Lys(K);
- (d) Met(M), Leu(L), Ile(I), Val(V); and
- (e) Phe(F), Tyr(Y), Trp(W).

[0052]. Any substitutions, additions, and/or deletions in an amino acid sequence are permitted provided that the Nell1 protein is functional. An amino acid sequence that is substantially identical to another sequence, but that differs from the other sequence by means of one or more substitutions, additions, and/or deletions, is considered to be an equivalent sequence.

[0053]. In order to compare a first amino acid to a second amino acid sequence for the purpose of determining homology, the sequences are aligned so as to maximize the number of identical amino acid residues. The sequences of highly homologous proteins can usually be aligned by visual inspection. If visual inspection is insufficient, the amino acid molecules may be aligned in accordance with methods known in the art. Examples of suitable methods include those described by George, D. G. et al., in *Macromolecular Sequencing and Synthesis, Selected Methods and Applications*, pages 127-149, Alan R. Liss, Inc. (1988), such as formula 4 at page 137 using a match score of 1, a mismatch score of 0, and a gap penalty of -1.

[0054]. Preferably, less than 15%, more preferably less than 10%, and still more preferably less than 5% of the number of amino acid residues in the sequence of Nell1 are different (i.e., substituted for, inserted into, or deleted from). More preferably still, less than 3%, yet more preferably less than 2% and optimally less than 1% of the number of amino acid residues in a sequence are different from those in a naturally occurring sequence.

- [0055]. Preferably, the substitutions, additions, and/or deletions are not made in the conserved regions of the protein or in the functional domain of the protein. Examples of conserved regions of Nell1 protein include the secretory signal, Willebrand like domain, thrombospondin-like domains and laminin-like domains. Examples of functional domains of Nell1 protein include the EGF like domains. Thus, substitutions, additions, and/or deletions in the non-conserved and/or non-functional regions of the protein can typically be made without affecting the function of Nell1 protein.
- [0056]. A Nell1 protein further includes Nell1 protein fragments that retain the ability to promote healing of wounds and skeletal muscle regeneration. Preferably, the Nell1 protein fragment contains one or more of the conserved regions and/or functional domains of the protein. For example, the Nell1 protein fragments can comprise the EGF like domains and/or the von Willebrand like domain of Nell1 protein.
- [0057]. The minimum length of a Nell1 functional fragment is typically at least about 10 amino acids residues in length, more typically at least about 20 amino acid residues in length, even more typically at least about 30 amino acid residues in length, and still more typically at least about 40 amino acid residues in length. As stated above, wild type Nell1 protein is approximately about 810 amino acid residues in length. A Nell1 functional derivative can be at most about 810 amino acid residues in length. For example, a Nell1 functional derivative can be at most at most about 820, 805, 800, 790, 780, 750, 600, 650 600, 550, etc. amino acid residues in length
- [0058]. Once a Nell1 functional protein homolog or Nell1 functional protein fragment is made, such protein can be tested to determine whether it retains substantially the activity or function of a wild type Nell1 protein. For example, the ability of a Nell1 homolog or fragment to bind PKC beta can be tested. Suitable assays for assessing the binding of Nell1 to PKC beta is described in e.g., Kuroda et al. (*Biochemical Biophysical Research Comm.* 265: 752-757 (1999b)).

For example, protein-protein interaction can be analyzed by using the yeast two-hybrid system. Briefly, a modified Nell1 protein can be fused with GAL4 activating domain and the regulatory domain of PKC can be fused with the GAL4 DNA-binding domain.

[0059]. In addition, the ability of a Nell1 protein homolog or fragment to stimulate differentiation of precursor cells, such as skeletal satellite cells, to maturity can be tested. Maturity of skeletal muscle cells can be assessed cellularly (histology) and molecularly (expression of skeletal muscle-specific proteins or extracellular matrix materials). Still further, a Nell1 protein homolog or fragment can be tested for its ability to drive osteoblast precursors to mature bone cells, by detecting expression of late molecular bone markers or mineralization (i.e., calcium deposits). By comparing the activity of a Nell1 protein homolog or fragment with that of a wild type Nell1 protein in one or more of the assays such as those described above, one can determine whether such homologs or fragments retain substantially the activity or function of a wild type Nell1 protein.

[0060]. The Nell1 protein, functional homolog or functional fragment may be prepared by methods that are well known in the art. One such method includes isolating or synthesizing DNA encoding the Nell1 protein, and producing the recombinant protein by expressing the DNA, optionally in a recombinant vector, in a suitable host cell. Suitable methods for synthesizing DNA are described by Caruthers et al. 1985. Science 230:281-285 and DNA Structure, Part A: Synthesis and Physical Analysis of DNA, Lilley, D. M. J. and Dahlberg, J. E. (Eds.), Methods Enzymol., 211, Academic Press, Inc., New York (1992). Examples of suitable Nell1 nucleic acid sequences include SEQ. ID. NOs: 2, 4, and 6.

[0061]. The Nell1 protein may also be made synthetically, i.e. from individual amino acids, or semisynthetically, i.e. from oligopeptide units or a combination of oligopeptide units and individual amino acids. Suitable methods for synthesizing proteins are described by Stuart and Young in "Solid Phase Peptide Synthesis," Second Edition, Pierce Chemical Company (1984), Solid Phase Peptide

Synthesis, Methods Enzymol., 289, Academic Press, Inc, New York (1997).

Examples of suitable Nell1 amino acid sequences include SEQ. ID. NOs: 1, 3, 5, homologs thereof, and fragments thereof.

Nell1 Nucleic Acid Molecules

[0062]. Any nucleic acid sequence that encodes for Nell1 protein can be used in the methods of the present invention. Suitable nucleic acid molecules encoding Nell1 protein for use in the methods of the present invention include nucleic acid molecules having a nucleotide sequence as set forth in SEQ. ID. NOs: 2, 4 and 6, as well as degenerate sequences thereof. As used herein, the term “degenerate sequence” refers to a sequence formed by replacing one or more codons in the nucleotide sequence encoding wild type Nell1 protein with degenerate codes which encode the same amino acid residue (e.g., GAU and GAC triplets each encode the amino acid Asp). The nucleic acid molecules can be incorporated into recombinant vectors suitable for use in gene therapy.

[0063]. Examples of vectors suitable for use in gene therapy may be any vector that comprises a nucleic acid sequence capable of expressing the Nell1 protein in a mammal, especially a human, in need of such therapy. The suitable vector may be for example a viral vector (e.g., such as an adenovirus vector, adeno-associated virus (AAV) vector, retroviral vector, herpes simplex viral vector, polio viruses and vaccinia vectors), nonviral vectors (e.g., plasmid vectors), etc. See for example: Ledley 1996. Pharmaceutical Research 13:1595-1614 and Verma et al. Nature 1997. 387:239-242.

[0064]. Examples of retroviral vectors include, but are not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV)-derived recombinant vectors. A Nell1-coding nucleotide sequence can be placed in an operable linkage to a promoter in the expression vector, wherein the promoter directs the expression of the Nell1 protein in the targeted tissue or cells, and includes both a constitutive promoter and a tissue or cell-specific promoter

Administration

[0065]. The Nell1 protein or nucleic acid molecule is administered to a mammal in need thereof. The mammal may be a farm animal, such as a goat, horse, pig, or cow; a pet animal, such as a dog or cat; a laboratory animal, such as a mouse, rat, or guinea pig; or a primate, such as a monkey, orangutan, ape, chimpanzee, or human. In a preferred embodiment, the mammal is a human.

[0066]. The Nell1 protein or nucleic acid molecule can be incorporated in a pharmaceutical composition suitable for use as a medicament, for human or animal use. The pharmaceutical compositions may be for instance, in an injectable formulation, a liquid, cream or lotion for topical application, an aerosol, a powder, granules, tablets, suppositories or capsules, such as for instance, enteric coated capsules etc. The pharmaceutical compositions may also be delivered in or on a lipid formulation, such as for instance an emulsion or a liposome preparation. The pharmaceutical compositions are preferably sterile, non-pyrogenic and isotonic preparations, optionally with one or more of the pharmaceutically acceptable additives listed below.

[0067]. Pharmaceutical compositions of Nell1 protein or nucleic acid molecule are preferably stable compositions which may comprise one or more of the following: a stabilizer, a surfactant, preferably a nonionic surfactant, and optionally a salt and/or a buffering agent. The pharmaceutical composition may be in the form of an aqueous solution, or in a lyophilized form.

[0068]. The stabilizer may, for example, be an amino acid, such as for instance, glycine; or an oligosaccharide, such as for example, sucrose, tetralose, lactose or a dextran. Alternatively, the stabilizer may be a sugar alcohol, such as for instance, mannitol; or a combination thereof. Preferably the stabilizer or combination of stabilizers constitutes from about 0.1% to about 10% weight for weight of the Nell1 protein.

- [0069]. The surfactant is preferably a nonionic surfactant, such as a polysorbate. Some examples of suitable surfactants include Tween20, Tween80; a polyethylene glycol or a polyoxyethylene polyoxypropylene glycol, such as Pluronic F-68 at from about 0.001% (w/v) to about 10% (w/v).
- [0070]. The salt or buffering agent may be any salt or buffering agent, such as for example, sodium chloride, or sodium/potassium phosphate, respectively. Preferably, the buffering agent maintains the pH of the pharmaceutical composition in the range of about 5.5 to about 7.5. The salt and/or buffering agent is also useful to maintain the osmolality at a level suitable for administration to a human or an animal. Preferably the salt or buffering agent is present at a roughly isotonic concentration of about 150 mM to about 300 mM.
- [0071]. The pharmaceutical composition comprising Nell1 protein or nucleic acid molecule may additionally contain one or more conventional additive. Some examples of such additives include a solubilizer such as for example, glycerol; an antioxidant such as for example, benzalkonium chloride (a mixture of quaternary ammonium compounds, known as "quats"), benzyl alcohol, chlorctone or chlorobutanol; anaesthetic agent such as for example a morphine derivative; or an isotonic agent etc., such as described above. As a further precaution against oxidation or other spoilage, the pharmaceutical compositions may be stored under nitrogen gas in vials sealed with impermeable stoppers.
- [0072]. An effective amount of the Nell1 protein or nucleic acid molecule, preferably in a pharmaceutical composition, may be administered to a human or an animal in need thereof by any of a number of well-known methods. For example, the Nell1 protein or nucleic acid molecule may be administered systemically or locally, for example by injection.
- [0073]. The systemic administration of the Nell1 protein or nucleic acid molecule may be by intravenous, subcutaneous, intraperitoneal, intramuscular, intrathecal or oral administration. Alternatively, the Nel-1 protein or nucleic acid molecule

may be applied topically in appropriate situations. Such situations include, for example, skin abrasions and surface wounds.

[0074]. The Nell1 protein can be administered by a cell based gene therapy. For example, allogeneic or xenogenic donor cells are genetically modified *in vitro* to express and secrete Nell1 protein. The genetically modified donor cells are then subsequently implanted into the mammal in need for delivery of Nell1 protein *in vivo*. Examples of suitable cells include, but are not limited to, endothelial cells, epithelial cells, fibroblasts, myoblasts, satellite cells, and skeletal muscle cells, stem cells, such as adult stem cells, embryonic stem cells, and cord blood stem cells.

[0075]. Alternatively, the genetically modified donor cells can be incorporated into a matrix containing an appropriate microenvironment to maintain, for a given time, the viability and growth of the genetically modified donor cells. The matrix can be applied to, for example, a surface wound. Expression and secretion of Nell1 by the genetically modified donor cells promotes healing of the wound. After the wound is healed, the matrix can be removed. Examples of suitable matrices include, but are not limited to, wound dressings, collagen matrix, patches, and hydrogels.

[0076]. An effective amount of a pharmaceutical composition of the invention is any amount that is effective to achieve its purpose. The effective amount, usually expressed in mg/kg can be determined by routine methods during pre-clinical and clinical trials by those of skill in the art.

EXAMPLES

Example 1. Expression of the Nell1 Protein in the Skin and Underlying Muscle Cells .

[0077]. Sagittal sections of whole fetal bodies collected a day before birth were analyzed by immunohistochemical methods using an antibody for the Nell1 protein. The red/pink staining in the epidermis, dermis and underlying skeletal muscle of normal fetal mice (Figure 8, Panel A) indicates the abundant presence of the Nell1 protein. Note the absence of the protein in the *Nell1*^{6R} mutant (Figure 8, Panel B) and the resulting disordered architecture of dermis and underlying muscle.

Example 2. Genes in the Nell1 Pathway

[0078]. Genes that are part of the Nell1 pathway during musculoskeletal development were determined by quantitative real time PCR (qRT-PCR) assays and microarray analyses of fetal bodies (15 and 18 days of gestation). The role of Nell1 in muscle formation was revealed by the immunohistochemistry and microarray data. The genes in the Nell1 pathway associated with wound healing and muscle regeneration include Tenascin b (Tnxb), Tenascin C (Tnc), osteoblast specific factor (Osf2), periostin, Matrilin 2 (Matn2), Collagen VI a1 (Col6a1), protein kinase C (PKC), Notch 3, TAL/SCL, Bcap31, Collagen IV a1 (Col4a1).

Example 3. Nell1 Promotes Wound Healing and Muscle Regeneration in a Poor Wound Healing Mouse Strain

[0079]. Severe muscle injury is induced in adult SJL/J mice a strain, known to be a genetically poor wound healer. The ability of purified recombinant human Nell1 protein is tested in the wound healing of severely lacerated leg muscles of SJL/J mice. Wounding is induced by surgically removing a sliver of muscle (approximately 5 mm long X 1 mm skin wound is sutured. On the third day after wounding, 5 mice are given phosphate buffered saline (PBS) solution to serve as controls, 5 mice are treated with 312 ng Nell1 protein (Dose 1) and another 5 mice with 624 ng protein (Dose II). Nell1 Protein

diluted in PBS (8 microliters) is administered directly along the entire length of the gaping muscle wound by dripping from a microinjector with a fine gauge needle. Wound healing is assessed one week post-treatment. Observations are made under a dissecting microscope.

Example 4. Nell1 Promotes Muscle Regeneration in Chemically-Induced Type I Diabetic Mice

[0080]. Type I diabetes is induced in mice by streptozotocin (STZ), an alkylating agent that destroys the pancreatic islet cells.. Commercially produced diabetic mice are purchased from The Jackson Laboratory. Diabetic mice are generated by the following method: At 8 weeks of age, one daily intraperitoneal injection of STZ for five consecutive days. Two weeks after the last STZ injection, mice are weighed and blood glucose levels are measured. Mice with at least 300-400 mg/dL blood glucose levels are considered diabetic. Wounding surgeries are performed as previously described in Example 3, but are done on younger adult mice (three months old) due to the severity of the induced diabetes (diabetic mice are already at 400-600 mg/dL at this stage). Four diabetic mice are given PBS as controls and four are given 312 ng of purified recombinant human Nell1 protein diluted in PBS. Protein is given two days after wounding and treatment effects are examined after one week.

Example 5. Nell1 Promotes Muscle Regeneration in Aged Mice

[0081]. Ten to twelve month old C57BL/6 mice are lacerated in the leg muscle as described Examples 3 and 4. Nell1 protein is injected or administered as described in Examples 3 and 4 directly into the wound site. The mice are euthanized at different time points, wounds are evaluated and their tissue is collected for histological analysis.

Example 6. Nell1 Promotes Wound Healing of Chemically and Heat Damaged Muscle Tissue

[0082]. Mammals with chemically or heat-induced muscle damage are treated with Nell1 protein in the wound and areas immediately bordering the damaged tissue. This can be a single administration of the appropriate Nell1 protein level or can be incorporated into wound dressings, bandages or ointments to provide a lower dose but continuous/time release introduction of Nell1. In addition, tissue grafts can be implanted into the damaged area along with the Nell1 protein introduced in the boundaries of the graft in order to promote vascularization and success of the tissue grafts onto the damaged areas.

Claims:

1. A Nell1 protein or a nucleic acid encoding a Nell1 protein for use in promoting healing of a skeletal muscle wound in a mammal having said wound, wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to the full length sequence set forth as SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.
2. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 1, wherein the Nell1 protein or the nucleic acid encoding a Nell1 protein is formulated for systemic administration.
3. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 1, wherein the Nell1 protein or the nucleic acid encoding a Nell1 protein is formulated for local administration.
4. A Nell1 protein or a nucleic acid encoding a Nell1 protein for use in promoting healing of a skin wound in a mammal having said skin wound, wherein the Nell1 protein or the nucleic acid coding for a Nell1 protein is formulated for local administration to the skin wound, and wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to the full length sequence set forth as SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.
5. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 4, wherein said skin wound is an open wound.
6. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 5, wherein said open wound is selected from the group consisting of an incision, a laceration, an abrasion, a puncture wound, a penetration wound, and a gunshot wound.

7. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 5 or 6, wherein promoting healing of the open wound comprises closure of the open wound.
8. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 3 to 7, wherein local administration is by injection.
9. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 3 to 7, wherein the local administration is topical.
10. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 9, wherein the skeletal muscle wound or the skin wound is caused by chemical or thermal means.
11. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 10, wherein said Nell1 protein or nucleic acid encoding a Nell1 protein is formulated for administration to said wound about 2 to about 3 days after wounding.
12. A Nell1 protein or a nucleic acid encoding a Nell1 protein for use in treating skeletal muscle atrophy in a mammal having skeletal muscle atrophy, wherein said Nell1 protein has an amino acid sequence having at least 85% sequence identity to the full length sequence set forth as SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.
13. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 12, wherein the Nell1 protein or the nucleic acid encoding a Nell1 protein is formulated for systemic administration.

14. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 12, wherein the Nell1 protein or the nucleic acid encoding a Nell1 protein is formulated for local administration.
15. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 14, wherein local administration is by injection.
16. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 14, wherein the local administration is topical.
17. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 16, wherein the Nell1 protein has an amino acid sequence having at least 95% sequence identity to the full length sequence set forth as SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.
18. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 17, wherein the Nell1 protein has an amino acid sequence having at least 99% sequence identity to the full length sequence set forth as SEQ ID NO: 1, 3, or 5 and stimulates differentiation of precursor cells to maturity.
19. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 18, wherein the Nell1 protein comprises SEQ. ID. NO: 1.
20. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 18, wherein the Nell1 protein comprises SEQ. ID. NO: 3.
21. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 18, wherein the Nell1 protein comprises SEQ. ID. NO: 5.
22. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 16, wherein the Nell1 protein is a human Nell1 protein.

23. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1, 4, and 12, wherein the Nell1 protein is expressed by a cell.
24. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 23, wherein the cell is an autologous or allogeneic cell.
25. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 23 or 24, wherein the cell comprises an endothelial cell, epithelial cell, fibroblast, myoblast, satellite cell, skeletal muscle cell, or stem cell.
26. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 25, wherein the cell is an adult stem cell.
27. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 23 to 26, wherein the cell is incorporated into a matrix.
28. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to claim 27, wherein said matrix comprises a wound dressing.
29. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 28, wherein said nucleic acid is operably linked to a promoter in an expression vector.
30. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 29, wherein the mammal is a human.
31. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 29, wherein the mammal is a goat, pig, cow, dog, cat, mouse, rat, guinea pig, primate or horse.

32. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 31, wherein the mammal suffers from impaired neovascularization.

33. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 31, wherein the mammal suffers from impaired angiogenesis.

34. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 31, wherein the mammal suffers from diabetes, a vascular disease, or is aging.

35. The Nell1 protein or the nucleic acid encoding a Nell1 protein for use according to any one of claims 1 to 34, wherein said Nell1 protein or the nucleic acid encoding a Nell1 protein is formulated with a pharmaceutically acceptable additive.

Figure 1

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Figure 2

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Figure 3

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Figure 4

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Figure 5

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181  nlppgsnlwl ggrnqkhgff kgiiqdgkii fmpngfitqc pnlnrtcptc sdfslslvqgi
241  mdlqellakm taklnyaetr lgqlenchce ktcqvsglyy rdqdswwdgd ncrnctcksg
301  avecrmscp plncspdslp vhisgqcckv crpkciyggk vlaegqrilt kcrecrggv
361  lvkiteacpp lncsekdhil penqccrvcr ghnfcaeapk cgensecknw ntkatceckn
421  gyisvqgnsa ycedidecaa kmhychantv cvnlpglyrc dcipgyirvd dfsctehddc
481  gsgqhncdkn aictntvqgh sctcqpgyvg ngtvckafce egcryggtcv apnkcvcpsg
541  ftgshcekti decaegfvec hnhsrvcnlp gwyhcecrsg fhddgtysls gescididec
601  alrthtcwnd sacinlaggf dclcpsgpvc sgdcphegg1 khngqvwlr edrcsvcsck
661  dgkifcrrta cdcqnpnvd1 fccpecdtrv tsqcldqsgq klyrsgdnwt hscqqqcrcle
721  geadcwplac ps1scey1tai fegeccprcv sdpcladnia ydirktclds sgisrlsgav
781  wtmagspctt cqckngrvcc svdlvclenn

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Figure 6

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1 gcgttggtgc gccctgcttg gcgggggggc tccggagcga tgccgatgga tgtgatttta
61 gttttgtggt tctgtgtgtg caccgccagg acagtgtctg gctttgggat ggaccctgac
121 cttcagatgg acatcatcac tgaacttgac cttgtgaaca ccacctggg cgtcactcag
181 gtggctggac tacacaatgc cagtaaggca tttctgtttc aagatgtaca gagagagatc
241 cactcagccc ctcatgtgag tgagaagctg atccagctat tccggaataa gagtgagttt
301 acctttttgg ctacagtgca gcagaagccg tccacctcag gggtgatact gtcgatccgg
361 gagctggaac acagctattt tgaactggag agcagtggcc caagagaaga gatacgctat
421 cattacatcc atggcggcaa gccaggact gagggccttc cctaccgcat ggccgatgga
481 cagtggcaca aggtcgcgct gtctgtgagc gcctctcacc tcctactcca tgtcgactgc
541 aataggattt atgagcgtgt gatagatcct cggagacca accttctctc aggaagcaat
661 aagatcatct tcatgccgaa cggcttcac acacagtgcc ccaacctaaa tcgcacttgc
721 ccaacatgca gtgatttcct gagcctgggt caaggaataa tggatttgca agagcttttg
781 gccaatgata ctgcaaaact gaattatgca gagacgagac ttgggtcaact ggaaaattgc
841 cactgtgaga agacctgcca agtgagtggg ctgctctaca gggaccaaga ctctgggta
901 gatggtgaca actgcaggaa ctgcacatgc aaaagtgggt ctgtggagtg ccgaaggatg
961 tcctgtcccc cactcaactg ttccccagac tcacttctct tgcatatttc tggccaatgt
1021 tgtaaagttt gcagaccaa atgtatctat ggaggaaaag ttcttgctga gggccagcgg
1081 attttaacca agacctgcc ggaatgtcga ggtggagtct tggtaaaat cacagaagct
1141 tgccctcctt tgaactgctc agagaaggat catattcttc cggagaacca gtgctgcagg
1201 gtctgccgag gtcataactt ctgtgcagaa gcacctaat gtggagaaaa ctcggaatgc
1261 aaaaattgga atacaaaagc gacttgtgag tgcaagaatg gatacatctc tgtccagggc
1321 aactctgcat actgtgaaga tatcgatgag tgtgcagcaa agatgcacta ctgtcatgcc
1381 aacacgggtg gtgtcaactt gccgggggta tatcgctgtg actgcatccc aggatacatc
1441 cgtgtggatg acttctcttg tacggagcat gatgattgtg gcagcggaca acacaactgt
1501 gacaaaaatg ccatctgtac caacacagtc cagggacaca gctgtacctg ccagccaggc
1561 tacgtgggaa atggtactgt ctgcaaagca ttctgtgaag agggttgcag atacggaggt
1621 acctgtgtgg cccctaacaa atgtgtctgt ccttctggat tcacaggaag ccactgtgag
1681 aaagatatgt atgaatgtgc agagggattc gttgagtgcc acaaccactc ccgctgcgtt
1741 aaccttccag ggtggtacca ctgtgagtgc agaagcgggt tccatgacga tgggacctat
1801 tcaactgtcc gggagtcctg cattgatatt gatgaatgtg ccttaagaac tcacacttgt
1861 tggaatgact ctgcctgcat caacttagca ggaggatttg actgcctgtg tccctctggg
1921 ccctcctgct ctggtgactg tccccacgaa ggggggctga agcataatgg gcaggtgtgg
1981 attctgagag aagacagggt ttcagtctgt tcctgtaagg atgggaagat attctgccgg
2041 cggacagctt gtgattgcca gaatccaaat gttgaccttt tctgctgccc agagtgtgac
2101 accaggttca ctagccaatg tttagatcaa agcggacaga agctctatcg aagtggagac
2161 aactggaccc acagctgcca gcagtgcga tgtctggaag gagaggcaga ctgctggcct
2221 ctactgtgcc ctagtgtgag ctgtgaatac acagccatct ttgaaggaga gtgtgtgccc
2281 cgctgtgtca gtgacctctg cctggctgat aatattgcct atgacatcag aaaaacttgc
2341 ctggacagct ctggtatttc gaggtgagc ggcgcagtgt ggacaatggc tggatctccc
2401 gtacaacct gtcaatgcaa gaatgggaga gtctgctgct ctgtggatct ggtgtgtctt
2461 gagaataact gaagatttta aatggactga tcacatgaga aaatggacaa aatgaccatc
2521 caacctgagg aagaggagg gctgatttct ttttcttttt aaccacagtc aattacaaa
2581 gtctccatca gaggaaggcg tttgggttgc ctttaccact ttgctcatcc ttgctgacct
2641 agtctagatg cctgcagtac cgtgtatttc ggtcgatggg tgttgagtct ccgtgctgta
2701 aatcacatth cccttgtcag atcatttaca gatacattha aaggattcca tgataaatgt
2761 taaagtacct tttgtttatt ttgtgtacca acataataga gacttggcac ca

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Figure 7

Query	1	MPMDLILVWFVCTARTVVGFGMDPDLQMDI+TELDLVNTTTLGVAQVSGMHNASKAFLE	6
Sbjct	1	MPMD+ILV+WFCVCTARTV+GFGMDPDLQMDI+TELDLVNTTTLGV QV+G+HNASKAFLE	6
Query	61	QDIEREIHAAAPHVSEKLIQLFONKSEFTILATVQOKPSTSGVILSIRELEHSYFELESSG	1
Sbjct	61	QD++REIH+APHVSEKLIQLF+HKSEFT LATVQOKPSTSGVILSIRELEHSYFELESSG	1
Query	121	LRDEIRYHYIHNKGPRTALPYRMADGQWHKVALSVSASHLLHVDCHRIYERVIDPPDT	1
Sbjct	121	R+RIYHYIH GKPRTEALPYRMADGQWHKVALSVSASHLLHVDCHRIYERVIDPP+T	1
Query	181	NLPPGIMLWLGQRNQKHGFKGLIQDGKIIFMPNGYITQCPNLNHTCPTCSDFLSLVQGI	2
Sbjct	181	NLPPG NLWLGQRNQKHG FKGLIQDGKIIFMPNG+ITQCPNLN TCPTCSDFLSLVQGI	2
Query	241	MDLQELLAKMTAKLNYAETRLQLENCHCEKTCQVSGLLYRDQDSWVDGDHCRNCTCKSG	3
Sbjct	241	MDLQELLAKMTAKLNYAETRL QLENCHCEKTCQVSGLLYRDQDSWVDGD+CRNCTCKSG	3
Query	301	AVECRMSPPLNCSFDSLPHIAGQCKVCRPKCIYGGKVLAEQRIILTKSCRECRGGV	3
Sbjct	301	AVECRMSPPLNCSFDSLPHI+GQCKVCRPKCIYGGKVLAEQRIILTK+CRECRGGV	3
Query	361	LVKITEMCPPLNCSERDHLIPENQCCRVCRGHNFCAEGPKCGENSECKNWNKATCECKS	4
Sbjct	361	LVKITE CPPLNCSERDHLIPENQCCRVCRGHNFCAEAPKCGENSECKNWNKATCECKN	4
Query	421	GYISVQGSAYCEDIDECAAKMHYCHANTVCVNLPLGLYRCDCVPGYIRVDDFSCTEHDEC	4
Sbjct	421	GYISVQG+SAYCEDIDECAAKMHYCHANTVCVNLPLGLYRCDC+PGYIRVDDFSCTEHDC	4
Query	481	GSGQHNCDENAICTNTVOGHSCTCKPGYVGNGTICRAFCEEGCRYGGTCVAPNKCVCPSG	5
Sbjct	481	GSGQHNC+NAICTNTVOGHSCTC+PGYVGNGT+C+AFCEEGCRYGGTCVAPNKCVCPSG	5
Query	541	FTGSHCEKDIDECSEGIIECHNHSRCVNLPGWYHCECRSGFHDDGTYSLSGESCIDIDEC	6
Sbjct	541	FTGSHCEKDIDEC+EG +ECHNHSRCVNLPGWYHCECRSGFHDDGTYSLSGESCIDIDEC	6
Query	601	ALRTHTCWNDSACINLAGGFDCLCPSGPSCSGDCPHEGGLKHNGQVWTLKEDRCSVCSCCK	6
Sbjct	601	ALRTHTCWNDSACINLAGGFDCLCPSGPSCSGDCPHEGGLKHNGQVW L+EDRCSVCSCCK	6
Query	661	DGKIFCRRTACDCQNP+DLFCCPECDTRVTSQCLDQNGHKLKLYRSGDNWTHSCQQCRCL	7
Sbjct	661	DGKIFCRRTACDCQNPVNDLFCCPECDTRVTSQCLDQSGQKLKLYRSGDNWTHSCQQCRCL	7
Query	721	GEVDCWPLTCPNLSCRYTAILEGECCPRCVSDPCLADNIYDIRKTCCLDSYGVSRLSGSV	7
Sbjct	721	GEADCWPLACPSLSCEYTAILEGECCPRCVSDPCLADNIAYDIRKTCCLDSYGVSRLSGAV	7
Query	781	WTMAGSPCTTCCKNGRVCCSVDFECLQNN 810	
Sbjct	781	WTMAGSPCTTCCKNGRVCCSVDFECLQNN CL+NN 810	

Figure 8