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(54) Title: METHOD FOR ENHANCING RECOVERY OF COSMETIC LASER-TREATED SKIN

(57) Abstract: Methods of enhancing skin health recovery from a skin procedure comprising laser application to the skin using a siRNA or shRNA directed against a human Fidgetin like-2 nucleic acid.

METHOD FOR ENHANCING RECOVERY OF COSMETIC LASER-TREATED SKIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 62/575,600, filed October 23, 2017, the contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] The disclosures of all publications, patents, patent application publications and books referred to in this application are hereby incorporated by reference in their entirety into the subject application to more fully describe the art to which the subject invention pertains.

[0003] Cosmetic procedures for the skin, such as lasabrasion, often involve an recovery period subsequent to treatment where the skin can be hypersensitive to light and/or touch, as well as redness and demarcation issues, and is uncomfortable for the subject. A method to reduce the recovery time and/or enhance the treatment effects is desirable.

[0004] The present invention addresses this need and identifies a novel target in promoting wound healing and provides therapies and assays based thereon.

SUMMARY OF THE INVENTION

[0005] A method is provided of enhancing skin health recovery from a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to enhance skin health recovery from a skin procedure comprising laser application to the skin.

[0006] Also provided is a method for increasing the rate of recovery of skin from a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase the rate of recovery of skin recovering from a skin procedure comprising laser application to the skin.

[0007] Also provided is a method of promoting skin rejuvenation in skin subsequent to a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to promote skin rejuvenation in skin subsequent to a skin procedure comprising laser application to the skin.

[0008] Also provided is a method comprising:

treating a portion of a subject's skin by applying laser energy to the skin for cosmetic purposes; and

administering, or directing the subject to administer, to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase the rate of recovery of skin recovering from the treatment comprising applying laser energy to the skin.

[0009] Also provided is a method of reducing the visible appearance of a wrinkle in human skin comprising administering to the wrinkle an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to reduce the visible appearance of a wrinkle in human skin.

[0010] Also provided is a composition comprising (i) an amount of siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2 effective to increase the rate of recovery of skin from a skin procedure comprising laser application to the skin contained (ii) in a microneedle array.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig. 1: FL2 siRNA improves wound healing in a mouse skin abrasion model. Age matched female BALB/c mice were shaved on their dorsal surface then treated with Nair to remove hair. Skin abrasions were made within a 1 cm by 1 cm region. After wounding the epidermal surface, mice were treated one time with either control nanoparticles containing scrambled siRNA or nanoparticles containing FL2 siRNA. After 5 days, the mice were sacrificed and their skin excised and sectioned for comparative H&E staining. While controls showed significant wounding within the abrasion area, FL2 siRNA treated mice showed improved restoration of epidermal structure.

DETAILED DESCRIPTION OF THE INVENTION

[0012] Herein is provided a method of enhancing skin health recovery from a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to enhance skin health recovery from a skin procedure comprising laser application to the skin. Also provided is a method for increasing the rate of recovery of skin from a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase the rate of recovery of skin recovering from a skin procedure comprising laser application to the skin.

[0013] Also provided is a method of promoting skin rejuvenation in skin subsequent to a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to promote skin rejuvenation in skin subsequent to a skin procedure comprising laser application to the skin.

[0014] In an embodiment of the methods, the method promotes skin rejuvenation by increasing collagen I density in the skin.

[0015] In an embodiment of the methods, the method enhances skin health recovery by increasing collagen I density in the skin.

[0016] In an embodiment of the methods, the method promotes skin rejuvenation by increasing collagen I organization, or improved linear orientation of the collagen fibers parallel to a dermoepidermal junction of the skin.

[0017] In an embodiment of the methods, the method enhances skin health recovery by increasing collagen I organization in the skin.

[0018] In an embodiment of the methods, the increased rate of recovery is a reduction in the extent of inflammation and/or an increased rate of inflammation reduction.

[0019] In an embodiment of the methods, the procedure is a cosmetic procedure. In an embodiment of the methods, the procedure is laser skin resurfacing. In an embodiment of the methods, the procedure is lasabrasion.

[0020] In an embodiment of the methods, the procedure is a medical procedure.

[0021] In an embodiment of the methods, the laser of the laser application is a non-ablative laser. In an embodiment of the methods, the laser of the laser application is an ablative laser.

[0022] In an embodiment of the methods, the Fidgetin like-2 comprises the amino acid set forth in SEQ ID NO:2

[0023] In an embodiment of the methods, the siRNA is administered. In an embodiment of the methods, the shRNA is administered. In an embodiment of the methods, the siRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

[0024] In an embodiment of the methods, the shRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

[0025] In an embodiment of the methods, the siRNA or shRNA is directed against an mRNA encoding the human Fidgetin-like 2.

[0026] In an embodiment of the methods, the siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2.

[0027] In an embodiment of the methods, the siRNA comprises a sequence set forth in SEQ ID NOS:3, 4, 5, 6, 7, 8, 9, or 10.

[0028] Also provided is a method comprising:

treating a portion of a subject's skin by applying laser energy to the skin for cosmetic purposes; and

administering, or directing the subject to administer, to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase the rate of recovery of skin recovering from the treatment comprising applying laser energy to the skin.

[0029] In an embodiment of the methods, the cosmetic purpose is to reduce the appearance of wrinkles, non-responsive skin after a facelift, aged or sun-damaged skin, skin liver spots, birthmark, wart, enlarged oil glands, port wine stains, hemangiomas, telangiectasias, or to change the appearance of skin complexion. In an embodiment of the methods, the birthmark is a linear epidermal nevus.

[0030] In an embodiment of the methods, the laser is a CO_2 laser.

[0031] In an embodiment of the methods, the laser is an erbium laser.

[0032] In an embodiment of the methods, the laser is a 595-nm PDL laser, 1,320-nm Nd:YAG laser, 1,064-nm Nd:YAG laser with long-pulse or Q-switched.

[0033] Also provided is a method of reducing the visible appearance of a wrinkle in human skin comprising administering to the wrinkle an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to reduce the visible appearance of a wrinkle in human skin.

[0034] In an embodiment, the Fidgetin like-2 comprises the amino acid set forth in SEQ ID NO:2. In an embodiment, the siRNA is administered. In an embodiment, the shRNA is administered. In an embodiment, the siRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification. In an embodiment, the shRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification. In an embodiment, the siRNA or shRNA is directed against an mRNA encoding the human Fidgetin-like 2. In an embodiment, the siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like In an embodiment, the siRNA comprises a sequence set forth in SEQ ID NOS:3, 4, 5, 6, 7, 8, 9, or 10.

[0035] In an embodiment, siRNA or shRNA administration is begun on the same day as the laser skin treatment. In an embodiment, siRNA or shRNA administration is then continued every other day until the skin is healed.

[0036] In an embodiment, siRNA or shRNA administration is effected by administering liposomes containing the siRNA or shRNA.

[0037] In an embodiment, the method is used to enhance skin recovery subsequent to a:
chemical peel (e.g. superficial, medium-depth, and deep peels);
visible light device application;
intense pulsed light (IPL) application;
ablative or nonablative laser photo–rejuvenation;
radiofrequency (RF) application;
injectable skin biostimulation and/or rejuvenation procedure.

[0038] In an embodiment, the method is used to prevent dynamic wrinkles. In an embodiment, the method is used to correct static, anatomical wrinkles. In an embodiment, improvement is seen in wrinkle depth as measured using skin profilometry. This involves taking a mold of the face before and after treatment and reading those molds with a three-dimensional camera. (See, for example, Patel et al., *Dermatol. Surg.* (2002) 28:942–945, hereby incorporated by reference.)

[0039] In an embodiment, the method is used to enhance skin recovery subsequent to a restoration (redistribution) of fat and/or volume loss procedure. In an embodiment, the

method is used to enhance skin recovery subsequent to a skin augmentation and/or contouring procedure.

[0040] In an embodiment, the method is used to enhance skin recovery subsequent to a treatment to increase skin elasticity, increase skin smoothness, reduce skin fine lines, reduce signs of skin aging, reduce uneven skin tone, reduce acne, reduce skin hyperpigmentation, reduce skin discoloration, reduce skin sun spots or age spots, or reduce skin discoloration.

[0041] In an embodiment, the inhibitor of Fidgetin-like 2 is administered topically to the skin. In an embodiment, the inhibitor of Fidgetin-like 2 is administered from a reservoir that elutes the inhibitor, for example an eluting skin patch. In an embodiment, the inhibitor of Fidgetin-like 2 is administered from microneedle patch, wherein the microneedles deliver the inhibitor of Fidgetin-like 2, such as the siRNA, into the skin when placed on the skin or adhered onto the skin.

[0042] In an embodiment, the inhibitor of Fidgetin-like 2 is an siRNA or shRNA. In an embodiment, the nucleic acid is directed against a DNA encoding Fidgetin-like 2 or against an mRNA encoding Fidgetin-like 2.

[0043] In an embodiment of the method, the inhibitor of Fidgetin-like 2 is encapsulated in a nanoparticle. In an embodiment the nanoparticle is a liposomal nanoparticle.

[0044] In an embodiment, the Fidgetin-like 2 is human Fidgetin-like 2.

[0045] In an embodiment, the Fidgetin-like 2 comprises consecutive amino acid residues having the sequence set forth in SEQ ID NO:2.

[0046] The dosage of the inhibitor administered in treatment will vary depending upon factors such as the pharmacodynamic characteristics of a specific inhibitor and its mode and route of administration; the age, sex, metabolic rate, absorptive efficiency, health and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment being administered; the frequency of treatment with the inhibitor and the desired therapeutic effect.

[0047] A dosage unit of the inhibitor may comprise a single compound, or a mixture of the compound with one or more anti-infection compound(s) and/or cosmetic compounds.

[0048] In an embodiment, the siRNA (small interfering RNA) as used in the methods or compositions described herein comprises a portion which is complementary to an mRNA sequence encoding a Fidgetin-like 2 protein. In an embodiment, the Fidgetin-like 2 protein is a human Fidgetin-like 2 protein. In an embodiment, the mRNA is encoded by the DNA sequence NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1), and the siRNA is

effective to inhibit expression of Fidgetin-like 2 protein. In an embodiment, the Fidgetin-like 2 protein comprises consecutive amino acid residues having the sequence set forth in SEQ ID NO:2.

[0049] In an embodiment, the siRNA comprises a double-stranded portion (duplex). In an embodiment, the siRNA is 19-25 nucleotides in length. In an embodiment, the siRNA is 20-25 nucleotides in length. In an embodiment the siRNA comprises a 19-21 core RNA duplex with a one or two nucleotide 3' overhang on, independently, either one or both strands. In an embodiment the siRNA comprises a 19-25 RNA duplex with a one or two nucleotide 3' overhang on, independently, either one or both strands. The siRNA can be 5' phosphorylated, or not, and may be modified with any of the known modifications in the art to improve efficacy and/or resistance to nuclease degradation. In an embodiment the siRNA can be administered such that it is transfected into one or more cells. In an embodiment, the siRNA is 5' phosphorylated. In an embodiment, the whole length of the non-overlapping portion of the siRNA is fully complementary to a portion of a mRNA encoding a Fidgetin-like 2 protein.

[0050] In an embodiment, the 5' terminal residue of a strand of the siRNA is phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is phosphorylated. In one embodiment, a siRNA of the invention comprises a double-stranded RNA wherein one strand of the double-stranded RNA is 80, 85, 90, 95 or 100% complementary to a portion of an RNA transcript of a gene encoding Fidgetin-like 2 protein. In an embodiment, the RNA transcript of a gene encoding Fidgetin-like 2 protein is an mRNA. In an embodiment, the Fidgetin-like 2 protein is a human Fidgetin-like 2 protein. In an embodiment, a siRNA of the invention comprises a double-stranded RNA wherein one strand of the RNA comprises a portion having a sequence the same as a portion of 18-25 consecutive nucleotides of an RNA transcript of a gene encoding Fidgetin-like 2 protein . In an embodiment, the Fidgetin-like 2 protein is a human Fidgetin-like 2 protein. In yet another embodiment, a siRNA of the invention comprises a double-stranded RNA wherein both strands of RNA are connected by a non-nucleotide linker. Alternately, a siRNA of the invention can comprise a double-stranded RNA wherein both strands of RNA are connected by a nucleotide linker, such as a loop or stem loop structure. In an embodiment, both of the strands of RNA are not connected by a nucleotide linker, such as a loop or stem loop structure.

[0051] In one embodiment, a single strand component of a siRNA of the invention is from 14 to 50 nucleotides in length. In another embodiment, a single strand component of a siRNA of the invention is 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 21 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 22 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 23 nucleotides in length. In one embodiment, a siRNA of the invention is from 28 to 56 nucleotides in length. In another embodiment, a siRNA of the invention is 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 nucleotides in length.

[0052] In another embodiment, an siRNA of the invention comprises at least one 2'-sugar modification. In another embodiment, an siRNA of the invention comprises at least one nucleic acid base modification. In another embodiment, an siRNA of the invention comprises at least one phosphate backbone modification. In embodiments, an siRNA of the invention comprises at least one 2'-O-methyl modification. In embodiments, an siRNA of the invention comprises at least one phosphorodithioate (PS2).

[0053] As used herein, "at least one" means one or more.

[0054] In one embodiment, RNAi inhibition of Fidgetin-like 2 protein is effected by a short hairpin RNA ("shRNA"). The shRNA can be introduced into the appropriate cell by transduction with a vector. In an embodiment, the vector is a lentiviral vector. In an embodiment, the vector comprises a promoter. In an embodiment, the promoter is a U6 or H1 promoter. In an embodiment the shRNA encoded by the vector is a first nucleotide sequence ranging from 19-29 nucleotides complementary to the target gene/mRNA, in the present case the mRNA encodes Fidgetin-like 2 protein. In an embodiment the Fidgetin-like 2 protein is a human Fidgetin-like 2 protein. In an embodiment the shRNA encoded by the vector also comprises a short spacer of 4-15 nucleotides (a loop, which does not hybridize) and a 19-29 nucleotide sequence that is a reverse complement of the first nucleotide sequence. In an embodiment the siRNA resulting from intracellular processing of the shRNA has overhangs of 1 or 2 nucleotides. In an embodiment the siRNA resulting from intracellular processing of the shRNA overhangs has two 3' overhangs. In an embodiment the overhangs are UU.

[0055] In an embodiment, the FL2 is encoded by NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1) (nucleic acid encoding Human Fidgetin-like 2)

1 agtgagctat ggggacacta ctgcactgta gcctgggcaa cagagcaaga ccttgtctca

61 aaaatgtata tatattttgg gctttttttc ctaaaacggg aactacaaca gcatatttgc

121 gagctgatga gagtgaccca gcagagaggg aaatggatca gctctgttga agatgcactg

181 gacaccagaa cacgcccagc ccctcaacca gtggccagag cagcacctgg acgtctcctc

241 caccaccccg tcgccggccc acaagttgga gttgcccccc gggggtcgcc aacgctgcca

301 ctacgcttgg gcacacgacg acatctcagc cctcactgcc tccaacctcc taaagcgcta

361 tgcagagaag tactctgggg tcttggattc tccctacgag cgtccggccc tgggcgggta

421 cagcgacgcc tccttcctca acggcgccaa aggggatccc gagccctggc cagggccgga

481 gccaccctac cccttggcct cactccacga aggcctccca ggaaccaaat cgggcggtgg

541 cggcggttcc ggggccctgg ggggctcccc agttttagcc gggaacctcc ctgaacccct

601 ctacgccggc aatgcgtgcg ggggcccatc ggcggcgccc gagtacgcgg ccggctacgg

661 cgggggggtac ctggcgccgg gttactgcgc gcagacgggc gccgcgctgc ccccgccgcc

721 cccggccgcg ctcctgcagc ccccaccgcc tccggggtac gggccctcag cgccgctgta

781 caactatccc gcaggggggct acgcagcgca gcccggctat ggcgcgctcc cgccgccccc

841 aggcccaccc ccggcccccct acctgacccc gggcctgccc gcgcccacgc ccctgcccgc

901 gccggcaccg cccaccgcct atggcttccc cacggccgcg ccgggtgccg aatccgggct

961 gtcgctgaag cgcaaggccg ccgacgaggg gcccgagggc cgctaccgca agtacgcgta

1021 cgagcccgcc aaggcccccg tggctgacgg agcctcctac cccgccgcgg acaacggcga

1081 atgtcggggc aacgggttcc gggccaagcc gccaggagcc gcggaggagg cgtcgggcaa

1141 gtacggtggc ggcgtccccc tcaaggtcct gggctccccc gtctacggcc cgcaactgga

1201 gcccttgaa aagttcccgg agcgggcccc ggctcctcgt gggggggttcg ccgtgccgtc

1261 gggggagact cccaaaggcg tggaccctgg ggccctggag ctggtgacga gcaagatggt

1321 ggactgcggg cccccggtgc agtgggcgga tgtggcgggc cagggcgcgc tcaaggcggc

1381 gctggaggag gagctggtgt ggcccctgct caggccgccc gcctacccgg gcagcctgcg

1441 cccgccgcgg accgtcctgc tctttgggcc gcggggcgcg ggcaaagcgc tgctgggccg

1501 ctgcctcgcc acgcagctgg gcgccacgct gttgcgcctg cgcggcgcga ccctggctgc

1561 gcccggcgcc gccgagggcg cgcgcctcct ccaggccgcc ttcgcggccg cgcgctgccg

1621 cccaccctcc gtactcctca tcagcgagct agaggcgctg ctccccgccc gggacgacgg

1681 cgcggcggca gggggcgcgc tgcaggtgcc gctcctggcc tgcctggacg ggggctgcgg

1741 cgcgggggct gacggcgtgc tggttgtggg caccacctcg cggcccgcgg ctctggacga

1801 ggcgacccgc cggcgcttct ctctccgctt ctacgtggcg ctgcccgaca gcccggcccg
1861 cgggcagatc ctgcagcggg cgctggccca gcagggctgc gcgctcagtg agcgggaact
1921 ggcggcgctg gtgcagggca cgcagggctt ctctgggggc gagctggggc agctgtgcca
1981 gcaggcggcg gccggggcgg gcctcccggg gctgcagcgc cccctctcct acaaggacct
2041 ggaggcggcg ctggccaagg tgggccctag ggcctctgcc aaggaactgg actcgttcgt
2101 ggagtgggac aaaatgtacg gctccggaca ctgacggcgc gcgggggagg ccgcgggagc
2161 cgcagtccct ccgtccccgc cgcctccgcg tgggagggat gtcactgact aaacccggct
2221 ggcaggggct ggagtggtga atgtgggatc ggggacagga ggggtctgcc ggtggatatt
2281 tttttttcg tgggaaggaa aatgcttctg ccaggcagat gccatatgcg ccgtgtactc
2341 aggtttttcc tatttattgt ggactggaag ctcgccatct ccgcccggca gaccgggcag
2401 atccggcatg ggctggcacc cggggcctta agaactcctg ctctcttgcc acaacgcttt
2461 tgtctcctcg ctatctgaat ggcaccctcc ttctccctca ctctctccat cccattctct
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2701 ttccctgcct tctgttctta tttggactgc agtggcccct tgcaggagct ctggaggccc
2761 aggggctgag gaggagggtt acccctctac ccatctgaaa cctagggtct agggggatca
2821 aggaaaaaaa gtccccaaag aaggggaatt ttttgtttgt ttttgagggg agatcccaga
2881 aatgtagctt gtttcatatt ttagtcttct tatttttgta aaatgtgtag aatttgctgt
2941 ttttcttttt cttttgacaa ctcaggaaga aactgacctc agaaagaatg ttagactttg
3001 gctgctctcc tgtgtgcccc tcacacctgc cccctccccc ccactccatc caggggacca
3061 aattctccca gacactcaaa aaatgagact tacggggaag gggagaggaa gacccagagg
3121 cctcagtgaa accccagcta ttcctggtca gaagcagaat gtattcctaa gggcttcctc
3181 cccagggccg aggcctaggc atgaatgtgg ggagtgggct gtggggtttg agagaaggga
3241 ggccttattc ctctcctgct gctccccacc ccctgcccca cccaaccccc ccgctgagtg
3301 ttttctgtga agggctatcc agagttagga tgcccttgcc caattccttc ctgagaccca
3361 gaaggtaggg tgggagggcc caaatgggaa ggtgacctaa gcagaaagtc tccagaaagg
3421 tcatgtcccc tggccctgcc ttggcagagg tccccagtga cttatgctag gaggattcca
3481 tctgggtaga cagtctggcc acaaaatcag ctactggacc tcagccatct ctgctggagg
3541 ctctgaggag gagtgagcat ccctcacttg tgggggctct gtgaggaaat gtgccttccc
3601 cattcccccg gagtcctagg tctggagctc cagggctggg agagggtgag ggagatgggc
3661 aggggtgttt tctctgacct tgggggctta gtctcagtcc tgcctgaact ttccactagg
3721 cttggaaccc ttccaagaac catatttctc tccttcccac caattttccc ttgatgaggc

3781 tttagcagtt tgctcccacc acccccagcc catttcacaa ctctgatctt agtccaaagc
3841 aggggacacg ccccccccacc accacttttt ctctctccca tctcagcctc ctgtgcagtt
3901 ccttgcctgc ccgtgcattt cctagagtct actgcctccc ccctggctgg gagggtgtct
3961 gggggggatc tttcaggggc cctggcaccc agggcctgtg ctggcctagg agtgctgacc
4021 agaaggctgc tctgttcccc cccacccccg ttgctttctg gccccctctt tggagccagc
4081 cacccacagg gctttggtgc ctcagaagca gtgggctgcc gggtcacagc cgcaggctgc
4141 aaaagaccct cggagggagc atggagtgag gggttctctc tcaggtgtgt atgtattggg
4201 gggtgggggt gggtggaggg tgtcagggaa gttggggtgg gatcccagcc ttcccttcaa
4261 gaggcaggga gctctgggag gtggagtccc caccgctttc tctactaggc tcctcctgtt
4321 ccccaggctt ggggagcttt gcacaaggag actgccccca gcctagtggc acctacctca
4381 tgggctctgg ggcaggtagg ggaagggcca gtccagctct ggtaatgctg gggggaggca
4441 taccaaagaa tccaggggca gggagtgggg agggtgactt ccgagctggc ctctcccctt
4501 cctctaccca gactggggct gggatcctct cctcccgctg taaccatttc tacctcattt
4561 tgctgcgtgt tgtacatgga cgtatttatc tcctgtctga cgatgctctg cagttgtggt
4621 ctgtctacct cagaagagac tgtattttaa aagaaagtat tacacagtat taaagcgatg
4681 acatgtggtt tgcaaaaaaa aaaaaaaaaa a.

[0056] In an embodiment, the FL2 protein sequence comprises:

MHWTPEHAQPLNQWPEQHLDVSSTTPSPAHKLELPPGGRQRCHYAWAHDDISALT
ASNLLKRYAEKYSGVLDSPYERPALGGYSDASFLNGAKGDPEPWPGPEPPYPLASL
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LPGLQRPLSYKDLEAALAKVGPRASAKELDSFVEWDKMYGSGH (SEQ ID NO:2).

[0057] In an embodiment, the FL2 is naturally occurring variant having 95% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1). In an embodiment, the FL2 is naturally occurring variant having 96% or greater identity with

NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1). In an embodiment, the FL2 is naturally occurring variant having 97% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1). In an embodiment, the FL2 is naturally occurring variant having 98% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1). In an embodiment, the FL2 is naturally occurring variant having 99% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1).

[0058] In embodiments, the siRNA comprise one of the following pairs of sense/antisense sequences:

Sense: UUACACAGUAUUAAAGCGAUU (SEQ ID NO:3)
Antisense: 5' UCGCUUUAAUACUGUGUAAUU(SEQ ID NO:4); or

Sense: CAUCUGAAACCUAGGGUCUUU(SEQ ID NO:5)
Antisense: 5' AGACCCUAGGUUUCAGAUGUU(SEQ ID NO:6); or

Sense: GUGACUUAUGCUAGGAGGAUU (SEQ ID NO:7)
Antisense: 5' UCCUCCUAGCAUAAGUCACUU (SEQ ID NO:8); or

Sense: GGUCAGAAGCAGAAUGUAUUU(SEQ ID NO:9)
Antisense: 5' AUACAUUCUGCUUCUGACCUU (SEQ ID NO:10).

[0059] In an embodiment, the siRNA is double-stranded and comprises SEQ ID NO:3 and 4; SEQ ID NO:5 and 6; SEQ ID NO:7 and 8; or SEQ ID NO:9 and 10.

[0060] In an embodiment, the 5' terminal residue of a strand of the siRNA is phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is phosphorylated. In an embodiment, the 5' terminal residue of a strand of the siRNA is not phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is not phosphorylated.

[0061] In an embodiment the inhibitor of Fidgetin-like 2 is provided in a bulk-eroding system such as polylactic acid and glycolic acid (PLGA) copolymer based microspheres or microcapsules systems containing the inhibitor of Fidgetin-like 2. In an embodiment, blends of PLGA:ethylcellulose systems may be used as an appropriate carrier. A further medicament in accordance with this aspect of the invention may be formulated in a surface-eroding system wherein the inhibitor of Fidgetin-like 2 is embedded in an erodible matrix such as the poly(ortho) ester and polyanhydride matrices wherein the hydrolysis of the polymer is rapid. A medicament in accordance with this aspect of the invention may also be

formulated by combining a pulsatile delivery system as described above and an immediate release system such as a lyophilized injectable composition described above.

[0062] In an embodiment, the inhibitor of FL2 is administered in a dissolving microneedle. In an embodiment, the dissolving microneedle comprises one or more of dextran, hyaluronic acid, and Polyvinylpyrrolidone/PVP.

[0063] In an embodiment, the inhibitor of FL2 is administered in a composition with polyethylenimine. In a non-limiting example the polyethylenimine is 25KDa PEI.

[0064] The inhibitor may be used in a composition with additives. Examples of suitable additives are sodium alginate, as a gelatinizing agent for preparing a suitable base, or cellulose derivatives, such as guar or xanthan gum, inorganic gelatinizing agents, such as aluminum hydroxide or bentonites (termed thixotropic gel-formers), polyacrylic acid derivatives, such as Carbopol®, polyvinylpyrrolidone, microcrystalline cellulose and carboxymethylcellulose. Amphiphilic low molecular weight and higher molecular weight compounds, and also phospholipids, are also suitable. The gels can be present either as water-based hydrogels or as hydrophobic organogels, for example based on mixtures of low and high molecular weight paraffin hydrocarbons and vaseline. The hydrophilic organogels can be prepared, for example, on the basis of high molecular weight polyethylene glycols. These gelatinous forms are washable. Hydrophobic organogels are also suitable. Hydrophobic additives, such as petroleum jelly, wax, oleyl alcohol, propylene glycol monostearate and/or propylene glycol monopalmitostearate, in particular isopropyl myristate can be included. In an embodiment the inhibitor is in a composition comprising one or more dyes, for example yellow and/or red iron oxide and/or titanium dioxide for the purpose of matching as regards color. Compositions may be in any suitable form including gels, lotions, balms, pastes, sprays, powders, bandages, wound dressing, emulsions, creams and ointments of the mixed-phase or amphiphilic emulsion systems (oil/water-water/oil mixed phase), liposomes and transfersomes or plasters/band aid-type coverings. Emulsifiers which can be employed in compositions comprising the inhibitor of Fidgetin-like 2 include anionic, cationic or neutral surfactants, for example alkali metal soaps, metal soaps, amine soaps, sulphurated and sulphonated compounds, invert soaps, higher fatty alcohols, partial fatty acid esters of sorbitan and polyoxyethylene sorbitan, e.g. lanette types, wool wax, lanolin or other synthetic products for preparing the oil/water and/or water/oil emulsions.

[0065] Compositions comprising the inhibitor of Fidgetin-like 2 can also comprise vaseline, natural or synthetic waxes, fatty acids, fatty alcohols, fatty acid esters, for example

as monoglycerides, diglycerides or triglycerides, paraffin oil or vegetable oils, hydrogenated castor oil or coconut oil, hog fat, synthetic fats (for example based on caprylic acid, capric acid, lauric acid or stearic acid, such as Softisan®), or triglyceride mixtures, such as Miglyol®, can be used as lipids, in the form of fatty and/or oleaginous and/or waxy components for preparing the ointments, creams or emulsions of the compositions comprising the inhibitor of fidgetin-like 2 used in the methods described herein.

[0066] Osmotically active acids and alkaline solutions, for example hydrochloric acid, citric acid, sodium hydroxide solution, potassium hydroxide solution, sodium hydrogen carbonate, may also be ingredients of the compositions and, in addition, buffer systems, such as citrate, phosphate, tris buffer or triethanolamine, for adjusting the pH. It is possible to add preservatives as well, such as methyl benzoate or propyl benzoate (parabens) or sorbic acid, for increasing the stability.

[0067] Pastes, powders and solutions are additional forms of compositions comprising the inhibitor of Fidgetin-like 2 which can be applied topically. As consistency-imparting bases, the pastes frequently contain hydrophobic and hydrophilic auxiliary substances, preferably, however, hydrophobic auxiliary substances containing a very high proportion of solids. In order to increase dispersity, and also flowability and slipperiness, and also to prevent agglomerates, the powders or topically applicable powders can, for example, contain starch species, such as wheat or rice starch, flame-dispersed silicon dioxide or siliceous earth, which also serve as diluent.

[0068] In an embodiment, the compositions comprise further active ingredients suitable for accelerating recovery from a skin cosmetic procedure, for example one or more antibiotics, antiseptics, vitamins, anesthetics, antihistamines, anti-inflammatory agents, moisturizers, penetration-enhancing agents and/or anti-irritants. In an embodiment, the compositions do not comprise further active ingredients suitable for accelerating recovery from a skin cosmetic procedure, for example one or more antibiotics, antiseptics, vitamins, anesthetics, antihistamines, anti-inflammatory agents, moisturizers, penetration-enhancing agents and/or anti-irritants.

[0069] In an embodiment of the methods and compositions described herein the subject is a mammal. In an embodiment the subject is human.

[0070] In one embodiment, excluded from the present invention is a method performed on skin which has a wound in the area of the skin being treated, i.e. a gross break or

discontinuity in the structure of the skin tissue. Examples of wounds include ulcerations, bedsores, grazes, tears, cuts, and punctures.

[0071] Preferably the inhibitor is biomembrane-permeable or is conjugated or otherwise attached to a moiety which renders the inhibitor biomembrane-permeable.

[0072] A composition is provided comprising (i) an amount of siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2 as described herein effective to increase the rate of recovery of skin from a skin procedure comprising laser application to the skin contained (ii) in a microneedle array.

[0073] In an embodiment, the microneedle array comprises a structure made of one or more of dextran, hyaluronic acid and PVP. In an embodiment, the composition comprises a polyethylenimine.

[0074] All combinations of the various elements described herein are within the scope of the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0075] This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

EXPERIMENTAL DETAILS

[0076] Introduction

Cosmetic skin procedures are popular and widespread. However, patients can be self-conscious about, and/or in discomfort from, the subsequent recovery process. Methods to enhance the recovery process, and to reduce the visible aspects of the recovering skin, are desirable. Patients who want a quick recovery time can choose non-ablative or fractional resurfacing, although repeat treatments may be necessary to attain optimum results. The methods disclose herein reduce recovery times after treatment, which enables, for example, subjects to return to public appearance quicker or to choose more intense non-ablative (thereby reducing the overall number of treatments needed to meet a predetermined endpoint compared to those without applying the methods disclosed herein), or to chose an ablative treatment where previously only non-ablative was considered due to long recovery times needed for the former.

Example 1

[0077] Initially, inhibition of FL2 is performed *in vivo* in a Kunming type mouse model to determine its effect of collagen remodeling (e.g. see Liu et al., *Lasers in Surgery and Medicine*, 40:13-19 (2006), incorporated by reference herein). Depilated skin is treated with laser, e.g. 595-nm PDL (pulsed dye laser) (10 ms), 1,320-nm Nd:YAG (neodymium–yttrium–aluminum garnet) laser (0.35 ms), 1,064-nm Nd:YAG lasers with long-pulsed (0.3ms), and Q-switched (5 ns). Laser-treated skin is subsequently treated at one location with siRNA or shRNA directed to FL2 and at a second location with control. The skin is then examined at the FL2 treatment site and control site at 1 hour, 1 day, 1 week, 3 weeks, 4 weeks, and 8 weeks after laser treatment. Skin treated with siRNA or shRNA directed to FL2 shows increased rate and extent of re-epithelialization of the skin compared to control. In addition, the topically applied siRNA or shRNA is effective to increase collagen density and organization in the skin compared to control.

Example 2

[0078] A portion of skin on a human, for example facial skin, that has been treated with a lasabrasion procedure is subsequently treated with a topically applied siRNA or shRNA which inhibits Fidgetin-like 2. The topically applied siRNA or shRNA is effective to increase the rate and extent of re-epithelialization of the skin compared to control. In addition, the topically applied siRNA or shRNA is effective to increase collagen density and organization in the skin compared to control. Moreover, the topically applied siRNA or shRNA is effective to accelerate the rate of visual healing of the skin relative to controls. Visual healing can be assessed as evaluation of tactile roughness, visual texture, wrinkles, blotchiness, skin tone evenness, radiance, and translucence, e.g. on a 5-point scale. Other suitable methods are set forth, for example, in Hillebrand et al. (*British Journal of Dermatology* (2010) 162:647–654, hereby incorporated by reference, see: "improvement in the appearance of fine lines and wrinkles was measured by expert visual grading of high-resolution digital images using the rapid evaluation of anti-aging leads (REAL 3.0) system taken at baseline and at 8 and (in the cohort) 24 weeks").

[0079] In addition, the topically applied siRNA or shRNA is effective to reduce inflammation of the skin relative to controls. This results in an improved quality of recovery for the subject since visible redness and inflammation is a primary cause of the effective

recovery time for such cosmetic procedures. This treatment manifests itself in improved outcomes as measured by shorter healing times and/or reduced wrinkling, permitting subjects to return to work and public life more quickly than otherwise.

Example 3

[0080] A portion of skin on a human, which portion has one or more fine lines and/or wrinkles, is treated with a topically applied siRNA or shRNA which inhibits Fidgetin-like 2. The topically applied siRNA or shRNA is effective to improve the appearance of fine lines and/or wrinkles (see also, for example, methods used in Hillebrand et al., *British Journal of Dermatology* (2010) 162:647–654, hereby incorporated by reference).

Example 4

[0081] FL2 siRNA improves wound healing in a mouse skin abrasion model. Age matched female BALB/c mice were shaved on their dorsal surface then treated with Nair to remove hair. Skin abrasions were made within a 1 cm by 1 cm region. After wounding the epidermal surface, mice were treated one time with either control nanoparticles containing scrambled siRNA or nanoparticles containing FL2 siRNA. After 5 days, the mice were sacrificed and their skin excised and sectioned for comparative H&E staining. While controls showed significant wounding within the abrasion area, FL2 siRNA treated mouse showed improved restoration of epidermal structure as shown in Fig. 1.

What is claimed is:

1. A method of enhancing skin health recovery from a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to enhance skin health recovery from a skin procedure comprising laser application to the skin.

2. A method for increasing the rate of recovery of skin from a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase the rate of recovery of skin recovering from a skin procedure comprising laser application to the skin.

3. A method of promoting skin rejuvenation in skin subsequent to a skin procedure comprising laser application to the skin, the method comprising directly administering to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to promote skin rejuvenation in skin subsequent to a skin procedure comprising laser application to the skin.

4. The method of Claim 3, wherein the method promotes skin rejuvenation by increasing collagen I density in the skin.

5. The method of Claim 1, wherein the method enhances skin health recovery by increasing collagen I density in the skin.

6. The method of Claim 3, wherein the method promotes skin rejuvenation by increasing collagen I organization, or improved linear orientation of the collagen fibers parallel to a dermoepidermal junction of the skin.

7. The method of Claim 1, wherein the method enhances skin health recovery by increasing collagen I organization in the skin.

8. The method of Claim 2, wherein the increased rate of recovery is a reduction in the extent of inflammation and/or an increased rate of inflammation reduction.

9. The method of any of Claims 1-8, wherein the procedure is a cosmetic procedure

10. The method of Claim 9, wherein the procedure is laser skin resurfacing.

11. The method of Claim 9, wherein the procedure is lasabrasion.

12. The method of any of Claims 1-8, wherein the procedure is a medical procedure.

13. The method of any of Claims 1-12, wherein the laser of the laser application is a non-ablative laser.

14. The method of any of Claims 1-13, wherein the laser of the laser application is an ablative laser.

15. The method of any of Claims 1-14, wherein the Fidgetin like-2 comprises the amino acid set forth in SEQ ID NO:2

16. The method of any of Claims 1-15, wherein the siRNA is administered.

17. The method of any of Claims 1-15, wherein the shRNA is administered.

18. The method of Claim 16, wherein the siRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

19. The method of Claim 17, wherein the shRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

20. The method of any of Claims 1-19, wherein the siRNA or shRNA is directed against an mRNA encoding the human Fidgetin-like 2.

21. The method of any of Claims 1-20, wherein the siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2.

22. The method of any of Claims 1-18, 20 or 21, wherein the siRNA comprises a sequence set forth in SEQ ID NOS:3, 4, 5, 6, 7, 8, 9, or 10.

23. A method comprising:
treating a portion of a subject's skin by applying laser energy to the skin for cosmetic purposes; and
administering, or directing the subject to administer, to the skin that has undergone the procedure an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase the rate of recovery of skin recovering from the treatment comprising applying laser energy to the skin.

24. The method of Claim 23, wherein the cosmetic purpose is to reduce the appearance of wrinkles, non-responsive skin after a facelift, aged or sun-damaged skin, skin liver spots, birthmark, wart, enlarged oil glands, port wine stains, hemangiomas, telangiectasias, or to change the appearance of skin complexion.

25. The method of Claim 24, wherein the birthmark is a linear epidermal nevus.

26. The method of any of Claims 1-25, wherein the laser is a CO_2 laser.

27. The method of any of Claims 1-25, wherein the laser is an erbium laser.

28. The method of any of Claims 1-25, wherein the laser is a 595-nm PDL laser, 1,320-nm Nd:YAG laser, 1,064-nm Nd:YAG laser with long-pulse or Q-switched.

29. A method of reducing the visible appearance of a wrinkle in human skin comprising administering to the wrinkle an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to reduce the visible appearance of a wrinkle in human skin.

30. The method of Claim 29, wherein the Fidgetin like-2 comprises the amino acid set forth in SEQ ID NO:2

31. The method of Claim 29 or 30, wherein the siRNA is administered.

32. The method of Claim 29 or 30, wherein the shRNA is administered.

33. The method of Claim 31, wherein the siRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

34. The method of Claim 32, wherein the shRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

35. The method of any of Claims 29-34, wherein the siRNA or shRNA is directed against an mRNA encoding the human Fidgetin-like 2.

36. The method of any of Claims 29-34, wherein the siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2.

37. The method of any of Claims 29-31, 33, 35 or 36, wherein the siRNA comprises a sequence set forth in SEQ ID NOS:3, 4, 5, 6, 7, 8, 9, or 10.

38. A composition comprising (i) an amount of siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2 effective to increase the rate of recovery of skin from a skin procedure comprising laser application to the skin contained (ii) in a microneedle array.

39. The composition of Claim 38, wherein the microneedle array comprises a structure made of one or more of dextran, hyaluronic acid and PVP.

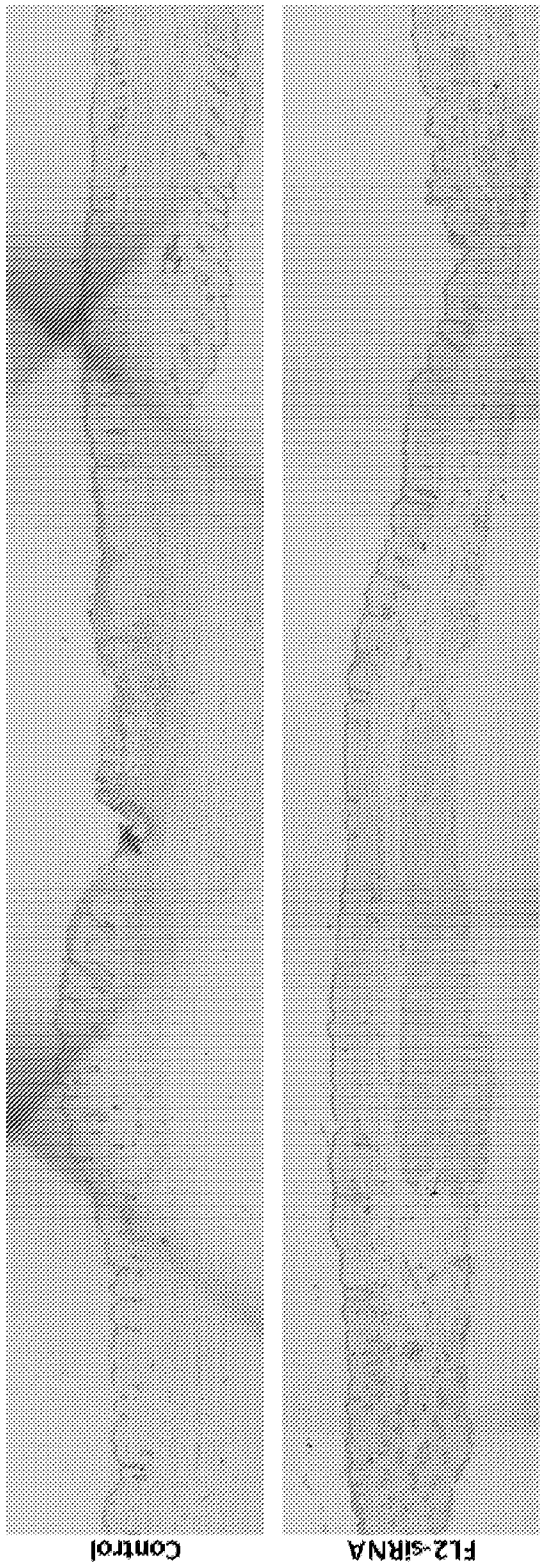

Fig. 1

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<120> METHOD FOR ENHANCING RECOVERY OF COSMETIC LASER-TREATED SKIN

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Gln His Leu Asp Val Ser Ser Thr Thr Pro Ser Pro Ala His Lys Leu

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Glu Leu Pro Pro Gly Gly Arg Gln Arg Cys His Tyr Ala Trp Ala His
        35                  40                  45

Asp Asp Ile Ser Ala Leu Thr Ala Ser Asn Leu Leu Lys Arg Tyr Ala
    50                  55                  60

Glu Lys Tyr Ser Gly Val Leu Asp Ser Pro Tyr Glu Arg Pro Ala Leu
65                  70                  75                  80

Gly Gly Tyr Ser Asp Ala Ser Phe Leu Asn Gly Ala Lys Gly Asp Pro
                85                  90                  95

Glu Pro Trp Pro Gly Pro Glu Pro Pro Tyr Pro Leu Ala Ser Leu His
            100                 105                 110

Glu Gly Leu Pro Gly Thr Lys Ser Gly Gly Gly Gly Gly Ser Gly Ala
        115                 120                 125

Leu Gly Gly Ser Pro Val Leu Ala Gly Asn Leu Pro Glu Pro Leu Tyr
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Ala Gly Asn Ala Cys Gly Gly Pro Ser Ala Ala Pro Glu Tyr Ala Ala
145                 150                 155                 160

Gly Tyr Gly Gly Gly Tyr Leu Ala Pro Gly Tyr Cys Ala Gln Thr Gly
                165                 170                 175

Ala Ala Leu Pro Pro Pro Pro Pro Ala Ala Leu Leu Gln Pro Pro Pro
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Pro Pro Gly Tyr Gly Pro Ser Ala Pro Leu Tyr Asn Tyr Pro Ala Gly
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Gly Tyr Ala Ala Gln Pro Gly Tyr Gly Ala Leu Pro Pro Pro Pro Gly
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Leu Pro Ala Pro Ala Pro Pro Thr Ala Tyr Gly Phe Pro Thr Ala Ala
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Pro Gly Ala Glu Ser Gly Leu Ser Leu Lys Arg Lys Ala Ala Asp Glu
            260                 265                 270

Gly Pro Glu Gly Arg Tyr Arg Lys Tyr Ala Tyr Glu Pro Ala Lys Ala
        275                 280                 285

Pro Val Ala Asp Gly Ala Ser Tyr Pro Ala Ala Asp Asn Gly Glu Cys
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Arg Gly Asn Gly Phe Arg Ala Lys Pro Pro Gly Ala Ala Glu Glu Ala
305                 310                 315                 320

Ser Gly Lys Tyr Gly Gly Gly Val Pro Leu Lys Val Leu Gly Ser Pro
                325                 330                 335

Val Tyr Gly Pro Gln Leu Glu Pro Phe Glu Lys Phe Pro Glu Arg Ala
            340                 345                 350

Pro Ala Pro Arg Gly Gly Phe Ala Val Pro Ser Gly Glu Thr Pro Lys
        355                 360                 365

Gly Val Asp Pro Gly Ala Leu Glu Leu Val Thr Ser Lys Met Val Asp
    370                 375                 380

Cys Gly Pro Pro Val Gln Trp Ala Asp Val Ala Gly Gln Gly Ala Leu
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Lys Ala Ala Leu Glu Glu Glu Leu Val Trp Pro Leu Leu Arg Pro Pro
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Ala Tyr Pro Gly Ser Leu Arg Pro Pro Arg Thr Val Leu Leu Phe Gly
420 425 430

Pro Arg Gly Ala Gly Lys Ala Leu Leu Gly Arg Cys Leu Ala Thr Gln
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Leu Gly Ala Thr Leu Leu Arg Leu Arg Gly Ala Thr Leu Ala Ala Pro
450 455 460

Gly Ala Ala Glu Gly Ala Arg Leu Leu Gln Ala Ala Phe Ala Ala Ala
465 470 475 480

Arg Cys Arg Pro Pro Ser Val Leu Leu Ile Ser Glu Leu Glu Ala Leu
485 490 495

Leu Pro Ala Arg Asp Asp Gly Ala Ala Ala Gly Gly Ala Leu Gln Val
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Pro Leu Leu Ala Cys Leu Asp Gly Gly Cys Gly Ala Gly Ala Asp Gly
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Val Leu Val Val Gly Thr Thr Ser Arg Pro Ala Ala Leu Asp Glu Ala
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Thr Arg Arg Arg Phe Ser Leu Arg Phe Tyr Val Ala Leu Pro Asp Ser
545 550 555 560

Pro Ala Arg Gly Gln Ile Leu Gln Arg Ala Leu Ala Gln Gln Gly Cys
565 570 575

Ala Leu Ser Glu Arg Glu Leu Ala Ala Leu Val Gln Gly Thr Gln Gly
580 585 590

Phe Ser Gly Gly Glu Leu Gly Gln Leu Cys Gln Gln Ala Ala Ala Gly

595 600 605

Ala Gly Leu Pro Gly Leu Gln Arg Pro Leu Ser Tyr Lys Asp Leu Glu
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