The seven human granulin modules have been expressed individually and the structures analyzed by NMR spectroscopy[9]. Three contain relatively well-defined three-dimensional structures in solution (A, C and F), whereas the others are mainly mixtures of poorly structured disulfide isomers[9]. The structure of human granulin A includes a β-hairpin structure similar to carp granulin-1 but there is significant structural disorder in the C-terminal region. Of the well folded human granulin modules, granulin A demonstrates potent inhibition of proliferation of a breast cancer cell line, while by contrast, human granulin F stimulates cell proliferation[9]. The poorly folded peptides exhibit weak or no inhibitory or activity. It should be noted, however, that the limited activity may be due to the absence of key signaling pathways in the target cells, and/or that the production of the recombinant peptides in bacteria induced incomplete/incorrect folding. To date, the range of granulin activities and binding partners is broad, and seemingly organ- and co-factor-dependent[10-13].

Structural analysis with NMR spectroscopy has shown that the N-terminal regions of carp granulin-1 and human granulin A can fold independently of the C-terminal regions[14, 15]. Truncated analogues of these two granulins containing only two disulfide bonds, have β-hairpin structures, as shown for a 30-residue N-terminal domain of carp granulin-1 (Figure 1).

SUMMARY

Surprisingly, peptides derived from the N-terminal region of granulin have activity in promoting cell proliferation, migration and/or wound healing. Furthermore, an additional disulphide bond may be engineered into such peptides to improve or otherwise modify the folding of the peptide.

A broad form of the disclosure describes an isolated peptide comprising an amino acid sequence derived from, or based on, the amino acid sequence of an N-terminal region of a granulin protein.

In one aspect, the present disclosure refers to an isolated peptide comprising, or consisting of the amino acid sequence: 1Xn C 2XD 3XVYTCR 4XGQTC C/A RGLHGYGC 5Xm (SEQ ID NO: 1), wherein *n* is 0-10 and *m* is 1-4, wherein ^{1}X and ^{5}X are any amino acid but at least one is a cysteine (C), wherein ^{2}X, ^{3}X, ^{4}X = P or A, and wherein the isolated peptide has 6 cysteine residues that form 3 intrachain disulfide bonds. In one example, there is disclosed an isolated peptide comprising, consisting essentially of, or consisting of the amino acid sequence:

$^{1}X_n$ C ^{2}XD ^{3}XVYTCR ^{4}XGQTC C/A RGLHGYGC $^{5}X_m$ (SEQ ID NO:1) or an amino acid sequence at least 70% identical thereto.

Preferably *n* is 0-10.

When *n* is 1-10, ^{1}X is any same or different amino acid or amino acids.

In one example, when *n* is 3, ^{1}X = SPS

In one example, when *n* is 4, ^{1}X = RSPS

In one example, when *n* is 11, ^{1}X = MDTLQPIRSPS

Preferably, *m* is 0-4.

When *m* is 1-4, ^{5}X may be any same or different amino acid or amino acids.

In one example, when *m* is 1, ^{5}X = C or A

In one example, when *m* is 4, ^{5}X = APMD

In another example, when *m* is 4, ^{5}X = CPMD.

Each of ^{2}X ^{3}X ^{4}X may be, respectively, any same or different amino acid or amino acids.

Preferably, each of ^{2}X ^{3}X ^{4}X is independently P or A.

In some preferred examples, at least one, at least two, or each of ^{2}X ^{3}X ^{4}X = P.

In some preferred examples, one of ^{2}X ^{3}X ^{4}X = A.

In one example, the disclosure refers to an isolated polypeptide comprising the isolated peptide as disclosed herein. In one example, there is described an isolated peptide that comprises, consists essentially of, or consists of the amino acid sequence set forth in any one of FIGS 1-13 or Table 3 other than SEQ ID NO:11, or an amino acid sequence at least 70% identical thereto.

In a particular example, the isolated peptide comprises an amino acid sequence set forth in any one of SEQ ID NOS:1-10, or an amino acid sequence at least 70% identical thereto.

In some examples, the isolated peptide comprises, or is capable of forming, two or three intrachain disulphide bonds.

In some examples, the isolated peptide is capable of forming proline *cis/trans* isomers.

In yet another example, the disclosure describes a method of modifying a peptide with an amino acid sequence set forth in any one of SEQ ID NOS: 1-11, including the step of incorporating one or more amino acid insertions, deletions, or substitutions into the amino acid sequence of the peptide. In one example, there is disclosed a method of modifying a peptide comprising an amino acid sequence set forth in any one of SEQ ID NOS:1-11, or an amino acid sequence at least 70% identical thereto, including the step of incorporating one or more amino acid insertions, deletions, or substitutions into the amino acid sequence of the peptide.

Preferably, modifying the peptide results one or more increased or enhanced biological activities of the peptide.

In another example, the disclosure refers to an isolated nucleic acid encoding the isolated peptide as disclosed herein, or the isolated polypeptide as disclosed herein. A further example refers to an isolated peptide modified according to the method disclosed herein.

The disclosure also refers to an antibody or antibody fragment that specifically binds the isolated peptide as disclosed herein.

In another aspect, the disclosure refers to an isolated nucleic acid encoding the isolated peptide as disclosed herein.

In yet another aspect, the disclosure refers to a genetic construct comprising the isolated nucleic acid as disclosed herein.

In still yet another aspect, the disclosure refers to a host cell comprising the genetic construct as disclosed herein.

In a further aspect, the disclosure refers to a pharmaceutical composition comprising the isolated peptide or the isolated nucleic acid as disclosed herein together with a pharmaceutically acceptable carrier, diluent or excipient.

In another further aspect, the disclosure refers to use of a composition in the manufacture of a medicament for wound healing, said composition comprising an isolated peptide as disclosed herein, the isolated nucleic acid disclosed herein, or the pharmaceutical composition as disclosed herein, to thereby initiate, stimulate or facilitate proliferation and/or migration of the one or more cells. In one example, there is disclosed a method of promoting cell proliferation and/or migration, said method including the step of contacting one or more cells with the isolated peptide, isolated nucleic acid or the pharmaceutical composition of the aforementioned aspects to thereby initiate, stimulate or facilitate proliferation and/or migration of the one or more cells.

In yet another further aspect, the disclosure refers to use of a composition in the manufacture of a medicament for healing a wound, said composition comprising an isolated peptide as disclosed herein, the agent as disclosed herein or the pharmaceutical composition of as disclosed herein to thereby at least partly heal the wound. In one example describes a method of healing a wound, said method including the step of contacting the wound with the isolated peptide, isolated nucleic acid or the pharmaceutical composition of the aforementioned aspects to thereby at least partly heal the wound.

In still yet another further example, there is described a method of producing an agent that promotes cell proliferation, migration and/or heals wounds, said method including the step of identifying, engineering, screening or designing an analogue or agonist of the isolated peptide of the aforementioned aspects that promotes cell proliferation and/or migration and/or heals wounds.

This disclosure also describes an agent that promotes cell proliferation, migration and/or heals wounds produced by the method of this aspect.

Suitably, the agent may be used according to the methods of the aforementioned aspects.

Throughout this specification, unless otherwise indicated, "*comprise*", "*comprises*" and "*comprising*" are used inclusively rather than exclusively, so that a stated integer or group of integers may include one or more other non-stated integers or groups of integers.

By "*consist essentially of*" is meant in this context that the isolated protein or each immunogenic fragment has one, two or no more than three amino acid residues in addition to the recited amino acid sequence. The additional amino acid residues may occur at the N- and/or C-termini of the recited amino acid sequence, although without limitation thereto.

It will also be appreciated that the indefinite articles "*a*" and "*an*" are not to be read as singular indefinite articles or as otherwise excluding more than one or more than a single subject to which the indefinite article refers. For example, "a" protein includes one protein, one or more proteins or a plurality of proteins.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Three-dimensional structure of a 30 residue N-terminal domain of carp granulin-1. (A) PDB code 1QGM, the β-strands are shown as purple arrows and the disulfide bonds in yellow ball and stick format. The image was generated using MolMol. (B) The disulfide connectivity in the full length carp granulin-1 protein[7]. Cysteines are designated with sequential roman numerals I-XII. The two bonds in the truncated carp granulin-1 are highlighted in yellow.

Figure 2. Sequences and secondary shifts of the *Ov*-GRN-1 truncated analogues. (A) Sequences show CysIV and CysVI were replaced with alanine residues; the cysteines are highlighted in red and the substitutions are shown in blue. The N-terminal 30 residues of carp granulin-1 is also provided, with CysIV and VI replaced with alanine residues. Sequence identifiers are as follows: *Ov*-GRN(1-35) = SEQ ID NO:2; *Ov*-GRN(8-38) = SEQ ID NO:3; *Ov*-GRN(12-34) = SEQ ID NO:4; AND *Ov*-GRN(12-35) = SEQ ID NO:5. (B) Secondary shifts of *Ov*-GRN-1 peptides with four cysteine residues (*Ov*-GRN$_{1-35}$, *Ov*-GRN$_{8-38}$ and *Ov*-GRN$_{12-34}$). The secondary shifts were derived by subtracting random coil shifts[16] from the αH shifts. The similarity in the secondary shifts for the conserved residues indicates that the overall fold is the same in the three peptides. Color scheme is retained in following figures. Both panels: black connecting lines represent disulfide bond connectivity.

Figure 3: Structural analysis of *Ov*-GRN$_{12-34}$ and *Ov*-GRN$_{12-35_3s}$. (A) Secondary shifts of *Ov*-GRN$_{12-34}$ and *Ov*-GRN$_{12-35_3s}$ compared to carp granulin$_{1-30}$. The

CLAIMS

1. An isolated peptide consisting of the amino acid sequence:
1Xn C 2XD 3XVYTCR 4XGQTC C/A RGLHGYGC 5Xm (SEQ ID NO: 1), wherein *n* is 0-10 and *m* is 1-4, wherein ^{1}X and ^{5}X are any amino acid but at least one is a cysteine (C), wherein ^{2}X, ^{3}X, ^{4}X = P or A, and wherein the isolated peptide has 6 cysteine residues that form 3 intrachain disulfide bonds.

2. The isolated peptide of Claim 1, wherein *n* is 1-10 and $^{1}X_{n}$ is any amino acid; or *n* = 4 and $^{1}X_{n}$ = RSPS; or *n* = 11 and $^{1}X_{n}$ = MDTLQPIRSPS.

3. The isolated peptide of any preceding claim, wherein *m* is 1-4 and $^{5}X_{m}$ is any amino acid; or *m* = 1, ^{5}X = C or A; or *m* = 4 and $^{5}X_{m}$ = APMD or CPMD.

4. The isolated peptide of any preceding claim which consists of the amino acid sequence set forth in any one of SEQ ID NOS: 2-5 and 11.

5. The isolated peptide of any preceding claim which comprises two or three intrachain disulphide bonds.

6. An isolated nucleic acid encoding the isolated peptide of any one of Claims 1-5.

7. A genetic construct comprising the isolated nucleic acid of Claim 6.

8. A host cell comprising the genetic construct of Claim 7.

9. A method of producing an agent that promotes cell proliferation, migration and/or heals wounds, said method including the step of identifying, or screening an analogue, mimetic or agonist of the isolated peptide of any one of Claims 1-5 that promotes that heals wounds.

10. A pharmaceutical composition comprising the isolated peptide of any one of Claims 1-5, or the isolated nucleic acid of Claim 6, together with a pharmaceutically acceptable carrier, diluent or excipient.

11. Use of a composition in the manufacture of a medicament for wound healing, said composition comprising an isolated peptide of any one of Claims 1-5, the isolated nucleic acid of Claim 6, or the pharmaceutical composition of Claim 10, to thereby initiate, stimulate or facilitate proliferation and/or migration of the one or more cells.

12. Use of a composition in the manufacture of a medicament for healing a wound, said composition comprising an isolated peptide of any one of Claims 1-5, the isolated nucleic acid of Claim 6, or the pharmaceutical composition of Claim 10 to thereby at least partly heal the wound.