METHOD OF PROMOTING WOUND HEALING

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of priority of SG patent application No. 201205614-9, filed 27 July 2012, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[002] The present invention generally relates to the field of biochemistry. In particular, the present invention relates to mi-RNAs, anti-miRNAs and polypeptides, which can be used for the treatment of wounds.

BACKGROUND OF THE INVENTION

[003] Diabetes is a group of diseases characterized by a high glucose level in blood that is caused by the subject's impaired ability to produce and/or use insulin. When diabetes is not well managed, diabetes can deteriorate steadily and cause complications such as blindness, nerve damage, kidney failure, heart disease and limb amputation. Many of these complications are due to damage to vasculature and the inability to heal wounds. Chronic wounds caused by diabetic patient's inability to heal wounds are a major global health burden and the most common cause of lower extremity amputations. In extreme cases, ineffective wound healing can lead to death.

[004] Wound healing requires an orchestrated integration of complex biological events including cell migration, proliferation and extracellular remodeling with matrix deposition, globally stimulated by TGF-β and other growth factors. In diabetes, these complex and interactive protective processes are disturbed. Accordingly, there is a need to provide a method or composition that can restore or improve normal wound healing.

SUMMARY OF THE INVENTION

[005] In a first aspect, the present invention refers to the use of ~~a miR-198 inhibitor and/or~~ a follistatin-like-1 (FSTL1) polypeptide in the manufacture of a medicament. In a second aspect, the present invention refers to the use of ~~a miR-198 inhibitor and/or~~ a follistatin-like-1 (FSTL1) polypeptide in the manufacture of a medicament for treating chronic cutaneous wounds. In a third aspect, the present invention refers to an in vitro method of identifying a chronic cutaneous would in an isolated sample, wherein the method comprises: ~~analyzing~~

~~expression level of miR-198 in the sample, wherein expression or an increased expression of miR-198 indicates that the wound is a chronic cutaneous wound; and/or~~ analyzing expression of FSTL1 gene and/or FSTL1 polypeptide level, wherein reduced or non-expression of FSTL1 gene and/or reduced level or absence of FSTL1 polypeptide indicates that the wound is a chronic cutaneous wound, wherein a reduced expression of FSTL1 or reduced presence of FSTL1 polypeptide is determined relative to a control expression or polypeptide level obtained from one or more wounds which are not chronic cutaneous wound. In one example, there is provided a method of promoting wound healing or wound closure. The method as described herein comprises administration of a miR-198 inhibitor and/or follistatin-like-1 (FSTL1) polypeptide.

[006] In another example, there is provided a method of treating chronic cutaneous wounds. The method of treating chronic cutaneous wounds may comprise the administration of a miR-198 inhibitor and/or follistatin-like-1 (FSTL1) polypeptide.

[007] In another example, there is provided a method of identifying a non-healing wound (chronic cutaneous wound). The method of identifying the chronic cutaneous wound may comprise analyzing expression level of miR-198, wherein expression or an increased expression of miR-198 indicates that the wound is a chronic cutaneous wound. The method of identifying a non-healing wound as described herein may optionally comprise analyzing expression of *FSTL1* gene and/or FSTL1 polypeptide level, wherein reduced or non-expression of *FSTL1* gene and/or reduced level or absence of FSTL1 polypeptide indicates that the wound is a chronic cutaneous wound.

[008] In another aspect, there is provided an *in vitro* use of ~~miR-198~~ FSTL1 polypeptide as a biomarker for identifying a non-healing wound (chronic cutaneous wound).

[009] In another aspect, there is provided a pharmaceutical composition comprising the compositions or compounds as described herein.

[0010] The pharmaceutical composition may comprise a miR-198 inhibitor and TGF-β1; or the miR-198 inhibitor and a FSTL1 polypeptide; or the FSTL polypeptide and TGF-β1; or the miR-198 inhibitor and TGF-β1 and the FSTL1 polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0012] **Fig. 1** shows the expression of an exonic miRNA or linked ORF in context-specific physiological states. a) Schematic of FSTL1 gene in chromosome 3 (NCBI Reference Sequence: NM_007085), showing the exon-intron boundaries, the 3'-UTR sequence encoding the miR-198 precursor (nucleotides which are underlined indicate the mature miR-198) b) Bar graph of showing miR-198 expression as assayed by qRT-PCR in organ culture explants at indicated time points after injury. Significant down-regulation of miR-198 observed as early as 3 hours post-injury **[$P<0.001$]. c) Images obtained from *in situ* hybridization with LNA probes specific for mature miR-198 on normal skin at (left) 0 and (right) 24 hours after injury. Note, a marked down-regulation of miR-198 expression at 24 hours after wounding. d) Representative semi-quantitative-RT-PCR analysis of FSTL1 mRNA in explant samples before and after injury at indicated time points. e)

Claims

1. Use of a follistatin-like-1 (FSTL1) polypeptide in the manufacture of a medicament for promoting wound healing or wound closure.

2. Use of a follistatin-like-1 (FSTL1) polypeptide in the manufacture of a medicament for treating chronic cutaneous wounds.

3. The use of claim 1 or 2, further comprising a miR-198 inhibitor.

4. The use of claim 3, wherein miR-198 inhibitor and follistatin-like-1 (FSTL1) polypeptide are to be co-administered simultaneously or separately from each other.

5. The use of any one of claims 2 to 4, wherein the chronic cutaneous wounds are the wounds of a patient suffering or suspected to be suffering from diabetes mellitus.

6. The use of any one claims 2 to 4, wherein the chronic cutaneous wound is diabetic ulcer.

7. The use of any one of the preceding claims, wherein the medicament is to be administrated via systemic or topical administration.

8. The use of claim 7, wherein administration is via topical administration.

9. The use of any one of claims 3 to 8, wherein the miR-198 inhibitor and FSTL1 polypeptide are to be administered independently of each other in an amount of between about 10 μg to 300 mg/kg body weight.

10. The use of any one claims 3 to 9, wherein the miR-198 inhibitor is selected from the group of an anti-miR-198, peptide nucleic acid (PNA) derivatives of miR-198 inhibitor sequence and Tiny LNA anti-miRs for seed-sequence of the inhibitor.

11. The use of any one claims 3 to 10, wherein the miR-198 inhibitor has the sequence: 5'-GAACCUAUCUCCCCUCUGGACC-3' (SEQ ID NO: 1).

12. The use of claim 11, wherein the inhibitor is unmodified.

13. The use of claim 11, wherein the inhibitor is with at least one modification/s selected from the group consisting of 1) full or partial 2'-O-methoxy ethyl modification, 2) full or partial phosphorothioate modification, 3) cholesterol modification of 3' end of the miR-198 inhibitor and 4) full or partial locked nucleic acid modification (LNA) of nucleotides.

14. An *in vitro* method of identifying a chronic cutaneous wound in an isolated sample, wherein the method comprises:

analyzing expression of *FSTL1* gene and/or FSTL1 polypeptide level, wherein reduced or non-expression of *FSTL1* gene and/or reduced level or absence of FSTL1 polypeptide indicates that the wound is a chronic cutaneous wound,

wherein a reduced expression of *FSTL1* or reduced presence of FSTL1 polypeptide is determined relative to a control expression or polypeptide level obtained from one or more wounds which are not chronic cutaneous wound.

15. The method of claim 14, wherein the method further comprises:

analyzing expression level of miR-198 in the sample, wherein expression or an increased expression of miR-198 indicates that the wound is a chronic cutaneous wound,

wherein an increased expression of miR-198 is determined relative to a control expression level obtained from a sample from one or more wounds which are not chronic cutaneous wound.

16. The method of claim 15, wherein expression of miR-198, *FSTL1* gene and FSTL1 polypeptide are measured in keratinocytes in the epidermis at the wound edge.

17. An *in vitro* use of FSTL1 polypeptide as a biomarker for identifying a chronic cutaneous wound.

18. The use of claim 17, further comprising miR-198 as a biomarker for identifying a chronic cutaneous wound.

19. A pharmaceutical composition comprising an miR-198 inhibitor and a FSTL1 polypeptide.

Claims

1. Use of a miR-198 inhibitor and/or a follistatin-like-1 (FSTL1) polypeptide in the manufacture of a medicament for promoting wound healing or wound closure.

2. Use of a miR-198 inhibitor and/or a follistatin-like-1 (FSTL1) polypeptide in the manufacture of a medicament for treating chronic cutaneous wounds.

3. The use of claim 1 or 2, further comprising administration of TGF-β1 either simultaneously or before or after administration of the miR-198 inhibitor and/or the FSTL1 polypeptide.

4. The use of any one of the preceding claims, wherein in case miR-198 inhibitor and follistatin-like-1 (FSTL1) polypeptide are used together they are to be administered simultaneously or separately from each other.

5. The use of any one of the preceding claims, wherein the chronic cutaneous wounds are the wounds of a patient suffering or suspected to be suffering from diabetes mellitus.

6. The use of any one of the preceding claims, wherein the chronic cutaneous wound is diabetic ulcer.

7. The use of any one of the preceding claims, wherein administration is via systemic or topical administration.

8. The use of claim 7, wherein administration is via topical administration.

9. The use of any one of the preceding claims, wherein the miR-198 inhibitor and FSTL1 polypeptide are to be administered independently of each other in an amount of between about 10 μg to 300 mg/kg body weight.

10. The use of any one of the preceding claims, wherein TGF-β1 is to be administered in an amount of between about 10 μg to 300 mg/kg body weight.

11. The use of any one of the preceding claims, wherein the miR-198 inhibitor is selected from the group of an anti-miR-198, peptide nucleic acid (PNA) derivatives of miR-198 inhibitor sequence and Tiny LNA anti-miRs for seed-sequence of the inhibitor.

12. The use of any one of the preceding claims, wherein the miR-198 inhibitor has the sequence: 5'-GAACCUAUCUCCCCUCUGGACC-3' (SEQ ID NO: 1).

13. The use of claim 12, wherein the inhibitor is unmodified.

14. The use of claim 12, wherein the inhibitor is with at least one modification/s selected from the group consisting of 1) full or partial 2'-O-methoxy ethyl modification, 2) full or partial phosphorothioate modification, 3) cholesterol modification of 3' end of the miR-198 inhibitor and 4) full or partial locked nucleic acid modification (LNA) of nucleotides.

15. An *in vitro* method of identifying a chronic cutaneous wound in an isolated sample, wherein the method comprises:

analyzing expression level of miR-198 in the sample, wherein expression or an increased expression of miR-198 indicates that the wound is a chronic cutaneous wound; and/or

analyzing expression of *FSTL1* gene and/or FSTL1 polypeptide level, wherein reduced or non-expression of *FSTL1* gene and/or reduced level or absence of FSTL1 polypeptide indicates that the wound is a chronic cutaneous wound.

16. The method of claim 15, wherein an increased expression of miR-198 is determined relative to a control expression level obtained from a sample from one or more wounds which are not chronic cutaneous wound.

17. The method of claim 15, wherein a reduced expression of *FSTL1* or reduced presence of FSTL1 polypeptide is determined relative to a control expression or polypeptide level obtained from one or more wounds which are not chronic cutaneous wound.

18. The method of any one of claims 15 to 17, wherein expression of miR-198, *FSTL1* gene and FSTL1 polypeptide are measured in keratinocytes in the epidermis at the wound edge.

19. Use of miR-198 and/or FSTL1 polypeptide as a biomarker for identifying a chronic cutaneous wound.

20. A pharmaceutical composition comprising

a) a miR-198 inhibitor and TGF-β1; or

b) the miR-198 inhibitor and a FSTL1 polypeptide; or

c) the FSTL polypeptide and TGF-β1; or

d) the miR-198 inhibitor and TGF-β1 and the FSTL1 polypeptide.