USE OF MI R-198 IN TREATING AND DIAGNOSING CUTANEOUS SQUAMOUS CELL CARCINOMA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore provisional application no. 10201604845W, filed 14 June 2016, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] 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 carcinomas.

BACKGROUND OF THE INVENTION

[0003] Carcinomas, for example squamous cell carcinomas (SCC), result from the uncontrolled growth of squamous cells, a subtype of epithelial cell. Another example, head and neck squamous cell carcinoma (HNSCC) develops from mucosal linings of the upper aero-digestive tract, mainly in the nasal cavity, paranasal sinuses, nasopharynx, larynx, trachea, oropharynx and oral cavity. Head and neck squamous cell carcinoma (HNSCC) is the sixth highest cancer by incidence worldwide, with 40% to 50% of patients with head and neck squamous cell carcinoma (HNSCC) having a median survival rate of only 5 years. Aggressive disease progression with high rates of metastasis contributes to poor patient survival rates. The identification of therapeutics targeting metastasis in carcinomas is therefore of clinical interest in the management of carcinomas, such as head and neck squamous cell carcinoma (HNSCC). There is thus a need to find a method of targeting and treating cancers, for example epithelial cancers.

SUMMARY

[0004] In one aspect, the present disclosure refers to a use of i) one or more of an oligonucleotide comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof; and/or ii) at least one oligonucleotide which reduces expression of any one or more of the following target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1),

Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU) in the manufacture of a medicament for treating a carcinoma. In another aspect, the present disclosure refers to a method of determining the presence of a carcinoma in a subject, wherein the method comprises: detecting the presence of miR-198 in a sample obtained from a subject; and/or detecting the presence of at least one or more target proteins in the sample; and comparing the detected level of miR-198 with the level of miR-198 detected in a control sample; and/or comparing the detected level of the at least one or more target proteins with the level of the at least one or more target proteins detected in the control sample, wherein the at least one or more target proteins is/are selected from a group consisting of follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), laminin subunit gamma-2 (LAMC2), urokinase-type plasminogen activator (PLAU) and combinations thereof. In yet another aspect, the present disclosure refers to a method of determining the presence of a carcinoma in a subject, wherein the method comprises: a. detecting the presence of miR-198 in a sample; b. comparing the detected level of miR-198 with the level of miR-198 detected in a control sample; and/or c. detecting the presence of at least one or more biomarkers in the sample; and d. comparing the detected level of the at least one or more biomarkers with the level of the at least one or more biomarkers detected in the control sample; wherein the at least one or more biomarkers is/are selected from a group consisting of miR-181a, epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) or a combination thereof.

[0004a] In one ~~aspect~~example, the present ~~invention~~disclosure refers to a method of treating a carcinoma, wherein the method comprises administration of i) one or more of an oligonucleotide comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof; and/or ii) at least one oligonucleotide which reduces expression of any one or more of the following target proteins: follistatin-related protein 1 (FSTL1), Protein

diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU).

[0005] In another ~~aspect~~example, the present ~~invention~~disclosure refers to a method of determining the presence or absence of a carcinoma in a subject, wherein the method comprises detecting the absence or presence of miR-198 in a sample obtained from a subject; and/or detecting the absence or presence of at least one or more target proteins in the sample; and comparing the detected level of miR-198 with the level of miR-198 detected in a control sample; and/or comparing the detected level of the at least one or more target proteins with the level of the at least one or more target proteins detected in the control sample, wherein the at least one or more target proteins is/are selected from a group consisting of follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), laminin subunit gamma-2 (LAMC2), urokinase-type plasminogen activator (PLAU) and combinations thereof.

[0006] In yet another example, the present ~~invention~~disclosure refers to a method of determining the presence or absence of a carcinoma in a subject, wherein the method comprises a. detecting the absence or presence of miR-198 in a sample; and b. comparing the detected level of miR-198 with the level of miR-198 detected in a control sample; and/or c. detecting the absence or presence of at least one or more biomarkers in the sample; and d. comparing the detected level of the at least one or more biomarkers with the level of the at least one or more biomarkers detected in the control sample; wherein the at least one or more biomarkers is/are selected from a group consisting of miR-181a, epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) or a combination thereof.

[0007] In a further ~~example~~aspect, the present ~~invention~~disclosure refers to a pharmaceutical composition comprising at least one or more oligonucleotides as disclosed herein.

[0008] In one example, the present ~~invention~~disclosure refers to a method of treating a carcinoma, wherein the method comprises administration of one or more oligonucleotides comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof, in combination with an expression inhibitor of any one of more of the target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU); wherein the expression inhibitor is selected from the group consisting of siRNA, shRNA and RNAi.

[0009] In another example, the present ~~invention~~disclosure refers to a method of treating carcinoma using siRNA and/or shRNA targeting the expression of one or more of the following proteins: follistatin-related protein 1 (FSTL1; SEQ ID NO: 3), Protein diaphanous homolog 1 (DIAPH1;

SEQ ID NO: 4), laminin subunit gamma-2 (LAMC2; SEQ ID NO: 5), and urokinase-type plasminogen activator (PLAU; SEQ ID NO: 6):

[0010] In yet another ~~example~~aspect, the present ~~invention~~disclosure refers to use of at least one or more oligonucleotides, as defined herein, in the manufacture of a medicament for treating a carcinoma.

[0010a] In another aspect, the present disclosure provides a use of one or more oligonucleotides comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof, and an expression inhibitor of any one of more of the target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU) in the manufacture of a medicament for combination therapy to treat a carcinoma; wherein the expression inhibitor is selected from the group consisting of siRNA, shRNA and RNAi. In another aspect, the present disclosure provides a use of one or more oligonucleotides comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof in the manufacture of a first medicament and use of an expression inhibitor of any one of more of the target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU) in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat a carcinoma; wherein the expression inhibitor is selected from the group consisting of siRNA, shRNA and RNAi. In yet another aspect, the present disclosure provides a use of one or more oligonucleotides comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof in the manufacture of a medicament for use in combinatorial therapy for treating a carcinoma, wherein said medicament is to be administered in combination with an expression inhibitor of any one of more of the target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU).

[0010b] In another aspect, the present disclosure provides a use of siRNA and/or shRNA targeting the expression of one or more of the following proteins: follistatin-related protein 1 (FSTL1; SEQ ID NO: 3), Protein diaphanous homolog 1 (DIAPH1; SEQ ID NO: 4), laminin subunit gamma-2 (LAMC2; SEQ ID NO: 5), and urokinase-type plasminogen activator (PLAU; SEQ ID NO: 6) in the manufacture of a medicament for treating carcinoma.

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 data showing a defective molecular switch in cutaneous SCC. a) shows the results of the expression of miR-198 and FSTL1 protein detected by *in situ* hybridization (left panel) and immunohistochemistry (right panel) respectively, in normal skin (n = 5). b) Expression of miR-198 detected by in situ hybridization (top panel) and immunohistochemical localization of FSTL1 protein (bottom panel) in SCC patient samples (n = 73); Scale bar: 100 μM.

[0013] **Fig. 2** shows data showing that expression of pro-invasive miR-198 target genes enhance invasion of carcinoma cells. a) Immunofluorescence staining of KRT14, PLAU, LAMC2 and DIAPH1 on normal skin and SCC patient samples. A significant increase in protein expression of target genes is clearly observed in all SCC patient samples compared to the normal skin (left column) (n = 73). b) Immunohistochemistry of c-MET in SCC patient samples and normal skin (n = 73). c) Boyden chamber invasion assay on SCC12 transfected with a non-targeting siRNA or siRNA against DIAPH1, LAMC2 and PLAU. Representative images of migrated cells detected with Giemsa staining. Cells were counted from six independent fields and histogram represents the relative number of invaded cells. **P<0.001 Error bars represent s.d d) Organotypic invasion assay with SCC12 cells expressing control shRNA or shRNA against LAMC2, DIAPH1 and PLAU. SCC12 cells were detected by immunohistochemistry against KRT14 (in red). Number of cells invading the dermal matrix were counted and plotted as relative percentage of invaded cells. **P<0.001 Error bars represent the standard deviation (s.d.)

[0014] **Fig. 3** presents data showing pro-invasive FSTL1 enhances carcinoma invasion. a) SCC12 cells transfected with control siRNA (left panel) or siRNA against FSTL1 (right panel) subjected to Boyden chamber assay. Representative images of migrated cells detected with Giemsa staining. b) Cells were counted from six independent fields and histogram represents relative number of invaded cells. c) Organotypic invasion assay performed using SCC12 cells transduced with control shRNA or shRNA against FSTL1. Sections were stained for KRT14 (in

CLAIMS

1. ~~A method of treating a carcinoma, wherein the method comprises administration~~ Use of i) one or more of an oligonucleotide comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof; and/or ii) at least one oligonucleotide which reduces expression of any one or more of the following target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU) in the manufacture of a medicament for treating a carcinoma.

2. The ~~method~~ use of claim 1, wherein the functional part of miR-198 comprises or consists of 5'GTCCAGAG 3' (SEQ ID NO: 2).

3. The use~~method~~ of any one of the preceding claims, wherein the target protein has an amino acid sequence selected from a group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6 and combinations thereof.

4. The use~~method~~ of any one of the preceding claims, wherein the one or more oligonucleotides under ii) are selected from the group consisting of siRNA, shRNA and RNAi.

5. The use~~method~~ of claim 4, wherein the one or more oligonucleotide is/are shRNA.

6. The use~~method~~ of claim 4, wherein the one or more siRNA comprises the sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10.

7. The use~~method~~ of any one of the preceding claims, wherein the oligonucleotide is unmodified, or is modified with one or more chemical groups selected from the group consisting of 2'O-methoxy, phosphorothioate, locked nucleic acid and cholesterol.

8. The use~~method~~ of any one of the preceding claims, wherein the carcinoma is selected from a group consisting of epithelial carcinoma, adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma, anaplastic carcinoma, large cell carcinoma, small cell carcinoma, basal cell neoplasm, basal cell carcinoma, hepatocellular carcinoma, adenoid cystic carcinoma, renal cell carcinoma, mucoepidermoid neoplasm, cystic neoplasm, mucinous neoplasm and cholangiocarcinoma.

9. The use~~method~~ of claim 8, wherein the carcinoma is cutaneous squamous cell carcinoma or head and neck squamous cell carcinoma.

10. The use~~method~~ of any one of the preceding claims, wherein the one or more oligonucleotides are to be administered either simultaneously, or separately, or sequentially from each other.

11. A method of determining the presence of a carcinoma in a subject, wherein the method comprises

detecting the presence of miR-198 in a sample obtained from a subject; and/or

detecting the presence of at least one or more target proteins in the sample; and

comparing the detected level of miR-198 with the level of miR-198 detected in a control sample; and/or

comparing the detected level of the at least one or more target proteins with the level of the at least one or more target proteins detected in the control sample,

wherein the at least one or more target proteins is/are selected from a group consisting of follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), laminin subunit gamma-2 (LAMC2), urokinase-type plasminogen activator (PLAU) and combinations thereof.

12. The method of claim 11, wherein the at least one or more target protein is FSTL1.

13. The method of claim 11, wherein the presence of an increased level of follistatin-related protein 1 (FSTL1), protein diaphanous homolog 1 (DIAPH1), laminin subunit gamma-2 (LAMC2) and urokinase-type plasminogen activator (PLAU), compared to the control sample, is indicative of the presence of carcinoma cells in the sample.

14. The method of claim 11, wherein the presence of an increased level of FSTL1 is indicative of the presence of carcinoma cells in the sample.

15. A method of determining the presence of a carcinoma in a subject, wherein the method comprises

a. detecting the presence of miR-198 in a sample; and

b. comparing the detected level of miR-198 with the level of miR-198 detected in a control sample; and/or

c. detecting the presence of at least one or more biomarkers in the sample; and

d. comparing the detected level of the at least one or more biomarkers with the level of the at least one or more biomarkers detected in the control sample;

wherein the at least one or more biomarkers is/are selected from a group consisting of miR-181a, epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) or a combination thereof.

16. The method of any one of claims 11 to 15, wherein the presence of a decreased level of miR-198, compared to the control sample, is indicative of the presence of carcinoma cells.

17. The method of claim 15, wherein the presence of an increased level of miR-181a, EGF and EGFR is indicative of the presence of carcinoma cells in the sample.

18. The method of any one of claims 11 to 17, wherein the control sample is a sample obtained from a carcinoma-free subject.

19. Use of at least one or more oligonucleotides as defined in claim 1 in the manufacture of a medicament for treating a carcinoma.

20. The use of claim 19, wherein the one or more oligonucleotides is/are an shRNA and/or an siRNA directed against one or more of the following targets: a gene encoding for FSTL1, a gene encoding for DIAPH1, a gene encoding for LAMC2, and a gene encoding for PLAU.

21. The use of claim 19 or 20, wherein the siRNA sequence is selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10 and combinations thereof.

22. A pharmaceutical composition comprising at least one or more oligonucleotides as defined in claim 1.

23. The composition of claim 22, wherein the one or more oligonucleotides is/are miR-198.

24. The composition of claims 22 and 23, wherein the one or more oligonucleotides is/are an shRNA and/or an siRNA directed against one or more of the following targets: a gene encoding for FSTL1, a gene encoding for DIAPH1, a gene encoding for LAMC2, a gene encoding for PLAU.

25. The composition of claim 22 to 24, wherein the siRNA sequence is selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO:10 and combinations thereof.

26. ~~A method of treating a carcinoma, wherein the method comprises administration~~ Use of one or more oligonucleotides comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof, ~~in combination with~~and an expression inhibitor of any one of more of the target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU) in the manufacture of a medicament for combination therapy to treat a carcinoma; wherein the expression inhibitor is selected from the group consisting of siRNA, shRNA and RNAi.

27. Use of one or more oligonucleotides comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof in the manufacture of a first medicament and use of an expression inhibitor of any one of more of the target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU) in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat a carcinoma; wherein

the expression inhibitor is selected from the group consisting of siRNA, shRNA and RNAi.

28. Use of one or more oligonucleotides comprising or consisting of the sequence of miR-198 (SEQ ID NO: 1), a functional part thereof, or a combination thereof in the manufacture of a medicament for use in combinatorial therapy for treating a carcinoma, wherein said medicament is to be administered in combination with an expression inhibitor of any one of more of the target proteins: follistatin-related protein 1 (FSTL1), Protein diaphanous homolog 1 (DIAPH1), Laminin subunit gamma-2 (LAMC2) or Urokinase-type plasminogen activator (PLAU).

~~27~~29. ~~A method of treating carcinoma using~~ Use of siRNA and/or shRNA targeting the expression of one or more of the following proteins: follistatin-related protein 1 (FSTL1; SEQ ID NO: 3), Protein diaphanous homolog 1 (DIAPH1; SEQ ID NO: 4), laminin subunit gamma-2 (LAMC2; SEQ ID NO: 5), and urokinase-type plasminogen activator (PLAU; SEQ ID NO: 6) in the manufacture of a medicament for treating carcinoma.