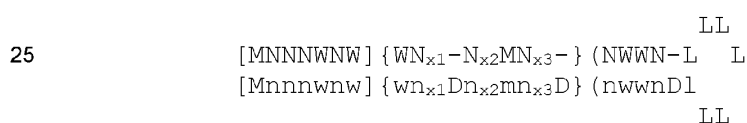


CLAIMS:

1. A pre-miRNA for targeting an exogenous nucleotide sequence to an exosome, wherein the pre-miRNA comprises an exogenous nucleotide sequence and a stem-loop structure, wherein the stem comprises at least one wobble pair.
2. A pre-miRNA according to claim 1, wherein the pre-miRNA comprises:
 - a. a 5' end comprising at least one wobble pair; and/or
 - b. an exogenous nucleotide sequence comprising one or more deletions and/or mismatches; and/or
 - c. a 3' end comprising a loop and at least one wobble pair.
3. A pre-miRNA according to any preceding claim, comprising the structure A-B-C, wherein:
 - a. A is a pre-miRNA 5' end, wherein the pre-miRNA 5' end comprises at least 50%, 60%, 70%, 80%, 90% or greater sequence identity to the 5' end of pre-miR-21;
 - b. B is an exogenous nucleotide sequence not naturally found in pre-miR-21; and
 - c. C is a pre-miRNA 3' end, wherein the pre-miRNA 3' end comprises at least 50%, 60%, 70%, 80%, 90% or greater sequence identity to the 3' end of pre-miR-21,
 optionally wherein the pre-miRNA comprises at least 20%, 30%, 40% or greater total sequence identity to the sequence of pre-miR-21.
4. A pre-miRNA according to any preceding claim, wherein the pre-mRNA comprises the structure:



wherein:

- N is any nucleotide and hybridises with n, optionally by Watson-Crick pairing;
- N_x is any length of nucleotide sequence that hybridises with n, optionally wherein N_{x1}, N_{x2}, and N_{x3} have different lengths;
- M is a nucleotide and is mismatched or does not hybridise with m;
- D is a nucleotide and is present on one side of the stem loop structure only;
- W is a nucleotide and forms a wobble pair with w;

L is a nucleotide that forms a loop structure and may hybridise with l; and

wherein the pre-miRNA comprises the structure A-B-C, wherein:

[...] is A, a miR-21 5' end structural mimic;

{...} is B, an exogenous nucleotide sequence; and

5 (...) is C, a miR-21 3' end structural mimic.

5. A pre-miRNA according to claim 4, wherein N_{x1} comprises from 4 to 12 nucleotides, N_{x2} comprises from 2 to 8 nucleotides, and N_{x3} comprises from 2 to 8 nucleotides.

6. A pre-miRNA according to any preceding claim, wherein:

10 the stem loop structure comprises an overall length of 60 to 80 nucleotides; optionally wherein the exogenous nucleotide sequence is 19-25 nucleotides in length; and/or

15 wherein the pre-miRNA comprises a transcription factor-regulated promoter upstream of the exogenous nucleotide sequence, optionally wherein the promoter is regulated by one or more of a TATA-binding protein, Activator protein 1 (AP-1), a CCAAT-enhancer-binding protein (C/EBP), Serum response factor (SRF), Signal transducer and activator of transcription 3 (STAT3), a GC-box binding protein, an E26 transformation-specific (ETS) family transcription factor optionally PU.1, a Nuclear Factor I (NFI) transcription factor, or p53.

20 7. A pre-miRNA according to any preceding claim, wherein the pre-mRNA comprises the structure:

$$\begin{array}{ccccccc} & & & & & LL & \\ [MNNNWNW] & \{ & WNNNNNNNNN-NNNNNNMNNNNN- & \} & (NWWN-L & L & \\ [Mnnnnwnw] & \{ & wnnnnnnnnnDnnnnnnmnnnnnD & \} & (nwwnDl & & \\ & & & & & LL & \end{array}$$

wherein:

N is any nucleotide and hybridises with n, optionally by Watson-Crick pairing;

M is a nucleotide and is mismatched or does not hybridise with m;

D is a nucleotide and is present on one side of the stem loop structure only;

30 W is a nucleotide and forms a wobble pair with w;

L is a nucleotide that forms a loop structure and may hybridise with l; and

wherein the pre-miRNA comprises the structure A-B-C, wherein:

[...] is A, a pre-miRNA 5' end;
 {...} is B, an exogenous nucleotide sequence; and
 (...) is C, a pre-miRNA 3' end.

8. A pre-miRNA according to any preceding claim comprising the sequence of pre-miR-21 and an exogenous nucleotide sequence in place of the mature miR-21 sequence.
9. A pre-miRNA according to any preceding claim, wherein the wobble pairs (W-w) comprise guanine-uracil (G-U or U-G), hypoxanthine-uracil (I-U or U-I), hypoxanthine-adenine (I-A or A-I), and/or hypoxanthine-cytosine (I-C or C-I).
10. A pre-miRNA according to any preceding claim, wherein the pre-miRNA comprises the structure:

$$\begin{array}{c} \text{UC} \\ \text{[ACAGUCU] \{ GNNNNNNNN-NNNNNMNNNNN- \} (GGUA-C} \\ \text{[UGUCGGG] \{ UnnnnnnnnDnnnnnnnnnnnnnnD \} (CUGUUG} \\ \text{AA} \end{array}$$

wherein:

N is any nucleotide and hybridises with n, optionally by Watson-Crick pairing;

M is a nucleotide and is mismatched or does not hybridise with m;

D is a nucleotide and is present on one side of the stem loop structure only; and

wherein the pre-miRNA comprises the structure A-B-C, wherein:

[...] is A, a pre-miRNA 5' end;
 {...} is B, an exogenous nucleotide sequence; and
 (...) is C, a pre-miRNA 3' end.

11. A pre-miRNA according to any preceding claim, wherein the exogenous nucleotide sequence is an siRNA, miRNA, anti-miR, antisense oligonucleotide (ASO), or CRISPR guide strand sequence.
12. A pre-miRNA according to any preceding claim, wherein the exogenous nucleotide sequence is targeted to the exosome during exosome biogenesis.
13. A pre-miRNA according to any preceding claim, wherein the exogenous nucleotide sequence modulates gene activity in a target cell.
14. A cassette comprising a pre-miRNA, said cassette comprising, in the following order:
- a. 5' pri-miR-21 sequence, optionally comprising a regulatory element;

- b. a pre-miRNA mimic according to any preceding claim; and
 - c. a 3' pri-miR-21 sequence, optionally comprising a regulatory element.
- 15. A vector comprising the cassette according to claim 14 or the pre-miRNA according to any one of claims 1 to 13.
- 5 16. A cell comprising a vector, cassette or pre-miRNA according to any preceding claim.
- 17. A cell according to claim 16, wherein the cell is a stem cell, optionally a neural stem cell, optionally a CTX0E03 cell, optionally a partially differentiated stem cell.
- 18. A cell according to claim 16 or claim 17, wherein the pre-miRNA is present within exosomes in the cell.
- 10 19. A method of loading exosomes with an exogenous nucleotide sequence comprising producing exosomes from a cell according to claim 16, 17 or 18.
- 20. A method of preparing exosomes, optionally comprising a pre-miRNA according to any of claims 1 to 13, comprising:
 - a. culturing cells according to claims 16, 17 or 18;
 - 15 b. harvesting of conditioned media; and optionally
 - c. purification of the exosomes; and/ or
 - d. validation of the exosomes.
- 21. An exosome obtainable or obtained by the method of claim 19 or claim 20, optionally wherein the exosome comprises a pre-miRNA according to any of claims 1 to 13.
- 20 22. A method of delivering an exogenous nucleotide sequence to a target cell:
 - using an exosome of claim 21, wherein the method comprises contacting the target cell with the loaded exosomes; or
 - using a pre-miRNA according to any of claims 1 to 13, a cassette of claim 14 or a vector of claim 15, which when transfected into a cell delivers the exogenous
 - 25 nucleotide sequence, optionally wherein the exogenous nucleotide sequence is selectively expressed in the target cell and optionally wherein the target cell is a diseased cell having dysregulated gene expression such as a cancer cell or a cell of autoimmune disease.
- 30 23. A method of modulating gene activity in a target cell, comprising administering an exosome according to 21.

24. A pharmaceutical composition comprising the exosome of claim 21.
25. An exosome according to claim 21 or 24, for use in therapy.
26. Use of one or more of the regulatory elements located in the upstream or downstream
5 region of the mir21 exon to control miRNA loading into exosomes,
optionally wherein the one or more regulatory elements are regulated by one or more
of a TATA-binding protein, Activator protein 1 (AP-1), a CCAAT-enhancer-binding
proteins (C/EBP), Serum response factor (SRF), Signal transducer and activator of
transcription 3 (STAT3), a GC-box binding protein, an E26 transformation-specific
10 (ETS) family transcription factor optionally PU.1, a Nuclear Factor I (NFI) transcription
factor, or p53.