CLAIMS

1. A method of monitoring the state of a cell, the method comprising establishing, for a selected microRNA (miRNA) species secreted by the cell, a ratio of: (a) a precursor form of the miRNA species (pre-miRNA); to (b) a mature form of the miRNA species (mature miRNA); in which the pre- to mature miRNA ratio so established is indicative of the state of the cell.

2. A method according to Claim 1, in which: (a) the cell is comprised in a cell mass, tissue, organ or organism and the pre- to mature miRNA ratio is indicative of the state of the respective cell mass, tissue, organ or organism, or (b) the miRNA species is comprised in exosomes secreted by the cell and the method comprises obtaining such exosomes and obtaining precursor and mature forms of the miRNA species therefrom, in which for example the exosomes are obtained from a sample of an organism, which organism comprises the cell, the sample for example comprising a blood sample, a serum sample, a saliva sample or a urine sample.

3. A method according to Claim 1 or 2, in which: (a) the pre- to mature miRNA ratio is established by hybridisation to an array comprising nucleic acid sequences capable of binding to and distinguishing between precursor and mature forms of the miRNA species, or (b) the pre- to mature miRNA ratio is established by real time polymerase chain reaction (RT-PCR).

4. A method according to Claim 1, 2 or 3, which comprises establishing a profile comprising a plurality of pre- to mature miRNA ratios for a plurality of selected miRNA species, each indicative of the state of the cell, such as by hybridisation to an array comprising a plurality of probes capable of binding to and distinguishing between precursor and mature forms of the plurality of the selected miRNA species.

5. A method according to any preceding claim, in which the state of the cell comprises a physiological state, such as a cell cycle state, a differentiation state, a development state or a metabolic state or a pathological state, such as a disease state, a human disease state, a diabetic state, an immune disorder state, a neurodegenerative disorder state, an oncogenic state, a cancerous state or a tumour state.

6. A method:

(a) for detecting a change in state of a cell, the method comprising detecting a change in a pre- to mature miRNA ratio of the cell (or a profile comprising such a ratio), in which such a change indicates a change in state of the cell;

(b) for establishing that a cell is in a particular state, the method comprising comparing a pre- to mature miRNA ratio of the cell (or a profile comprising such a ratio) with a pre- to mature miRNA ratio (or a profile comprising such a ratio) of a cell known to be in that particular state;

(c) of monitoring the state of an organism, the method comprising obtaining a sample from or of that organism, performing a method according to any preceding claim on the sample, and establishing the state of the organism from the pre- to mature miRNA ratio thereby obtained;

(d) of detecting a disease in an organism, the method comprising obtaining a sample from or of that organism, performing a method according to any preceding claim on the sample, and comparing the pre- to mature miRNA ratio thereby obtained with a pre- to mature miRNA ratio of a sample known to be of or from a diseased organism; or

(e) of treatment or prevention of a disease in an organism, the method comprising detecting a disease in an organism according to (d), and administering a treatment for that disease to the organism.

7. A method according to any preceding claim, in which: (a) the cell comprises a mesenchymal stem cell (MSC); (b) the states of the cell comprise an untransformed state and a transformed state, such as a c-myc transformed state; (c) the microRNA comprises hsa-let-7b microRNA (miRBase Accession Number: MI0000063); or (d) the ratio is 2.8 higher in a transformed state compared to a normal state; or any combination thereof.

8. A array comprising a plurality of nucleic acid sequences capable of binding to and distinguishing between precursor and mature forms of a plurality of miRNA species.

9. A method comprising the steps of: (a) providing a mesenchymal stem cell (MSC); and (b) introducing an oncogene into the mesenchymal stem cell to thereby transform it; in which the transformed mesenchymal stem cell does not secrete a gene product of the oncogene into a medium in which it is grown.

10. A method according to Claim 9, in which: (a) the oncogene comprises c-myc; (b) the mesenchymal stem cell (MSC) comprises an established cell line such as huES9.E1; or (c) the mesenchymal stem cell (MSC) is derived from umbilical cord.

11. A method according to Claim 9 or 10, in which transformed mesenchymal stem cell: (a) is capable of bypassing senescence; (b) has an increased proliferation rate; (c) has a decreased population doubling time such as 24 hours; or (d) has an increased telomerase activity; as compared to a mesenchymal stem cell that has not been transformed.

12. A method of providing a conditioned medium, the method comprising the steps set out in Claim 9, 10 or 11, followed by culturing the transformed mesenchymal stem cell, or a descendent thereof, in a cell culture medium to condition it, and separating the conditioned from the transformed mesenchymal stem cell.

13. A method of providing an exosome, the method comprising: (a) obtaining a mesenchymal stem cell conditioned medium (MSC-CM) by a method according to Claim 12; (b) concentrating the mesenchymal stem cell conditioned medium, for example by ultrafiltration over a >1000 kDa membrane; (c) subjecting the concentrated mesenchymal stem cell conditioned medium to size exclusion chromatography, such as using a a TSK Guard column SWXL, 6 x 40 mm or a TSK gel G4000 SWXL, 7.8 x 300 mm column; and (d) selecting UV absorbant fractions, for example, at 220 nm, that exhibit dynamic light scattering, such as detected by a quasi-elastic light scattering (QELS); in which step (d) for example comprises collecting fractions which elute with a retention time of 11-13 minutes, such as 12 minutes.

14. A method of preparing a pharmaceutical composition, the method comprising obtaining a mesenchymal stem cell conditioned medium (MSC-CM) by a method according to Claim 12 or an exosome by a method according to Claim 13 and admixing the MSC-CM or exosome, as the case may be, with a pharmaceutically acceptable carrier or diluent.

15. A method according to any preceding claim, in which transformed mesenchymal stem cell, the conditioned medium, exosome or pharmaceutical composition comprises at least one biological property of a mesenchymal stem cell, for example cardioprotection, or in which the conditioned medium, exosome or pharmaceutical composition is capable of reducing infarct size for example as assayed in a mouse or pig model of myocardial ischemia and reperfusion

injury, or which is capable of reducing oxidative stress for example as assayed in an *in vitro* assay of hydrogen peroxide (H_2O_2)-induced cell death.

16. An immortalised mesenchymal stem cell obtainable by a method according to Claim 9, 10 or 11, a conditioned medium obtainable by a method according to Claim 12, an exosome obtainable by a method according to Claim 13 or a pharmaceutical composition obtainable by a method according to Claim 14.

17. A method, array, immortalised mesenchymal stem cell, conditioned medium, exosome or pharmaceutical composition substantially as hereinbefore described with reference to and as shown in Figures 1 to 13 of the accompanying drawings.

CLAIMS

1. A method of monitoring the transformation state of a mesenchymal stem cell (MSC), the method comprising establishing, for hsa-let-7b microRNA (miRBase Accession Number: MI0000063) secreted by the mesenchymal stem cell, a ratio of: (a) a precursor form of hsa-let-7b microRNA (pre-miRNA); to (b) a mature form of hsa-let-7b microRNA (mature miRNA); in which the pre- to mature miRNA ratio so established is indicative of the transformation state of the mesenchymal stem cell.

2. A method according to Claim 1, in which the hsa-let-7b microRNA is comprised in exosomes secreted by the mesenchymal stem cell.

3. A method according to Claim 2, in which the method comprises obtaining such exosomes and obtaining precursor and mature forms of the hsa-let-7b microRNA therefrom.

4. A method according to Claim 1, 2 or 3, which comprises establishing a profile comprising a plurality of pre- to mature miRNA ratios for a plurality of selected miRNA species, each indicative of the state of the cell.

5. A method according to any preceding claim, in which the pre- to mature miRNA ratio is established by hybridisation to an array comprising nucleic acid sequences capable of binding to and distinguishing between precursor and mature forms of the miRNA species.

6. A method according to any of Claims 1 to 4, in which the pre- to mature miRNA ratio is established by real time polymerase chain reaction (RT-PCR).

7. A method according to any preceding claim, in which the pre- to mature miRNA ratio is 2.8 higher in a transformed state compared to a normal state.

8. A method according to any preceding claim, in which the mesenchymal stem cell is in an untransformed state or a c-myc transformed state.

9. An array comprising a plurality of probes capable of binding to and distinguishing between precursor and mature forms of a plurality of selected miRNA species, in which the plurality of selected miRNA species includes hsa-let-7b microRNA (miRBase Accession Number: MI0000063).

10. A method comprising the steps of:

(a) providing a mesenchymal stem cell (MSC);

(b) introducing an oncogene into the mesenchymal stem cell to thereby transform it; and

(c) establishing a pre- to mature miRNA ratio of hsa-let-7b microRNA secreted by the mesenchymal stem cell by a method according to any preceding claim so as to detect such transformation;

in which the transformed mesenchymal stem cell does not secrete a gene product of the oncogene into a medium in which it is grown.

11. A method according to Claim 10, in which: (a) the oncogene comprises c-myc; (b) the mesenchymal stem cell (MSC) comprises an established cell line; or (c) the mesenchymal stem cell (MSC) is derived from umbilical cord.

12. A method according to Claim 10 or 11, in which transformed mesenchymal stem cell: (a) is capable of bypassing senescence; (b) has an increased proliferation rate; (c) has a decreased population doubling time preferably 24 hours; or (d) has an increased telomerase activity; as compared to a mesenchymal stem cell that has not been transformed.

13. A method of providing a conditioned medium, the method comprising the steps set out in Claim 10, 11 or 12, followed by culturing the transformed mesenchymal stem cell, or a descendent thereof, in a cell culture medium to condition it, and separating the conditioned from the transformed mesenchymal stem cell.

14. A method of providing an exosome, the method comprising: (a) obtaining a mesenchymal stem cell conditioned medium (MSC-CM) by a method according to Claim 13; (b) concentrating the mesenchymal stem cell conditioned medium, preferably by ultrafiltration over a >1000 kDa membrane; (c) subjecting the concentrated mesenchymal stem cell conditioned medium to size exclusion chromatography, preferably using a a TSK Guard column SWXL, 6 x 40 mm or a TSK gel G4000 SWXL, 7.8 x 300 mm column; and (d) selecting UV absorbant fractions, preferably, at 220 nm, that exhibit dynamic light scattering, preferably detected by a quasi-elastic light scattering (QELS); in which step (d) preferably comprises collecting fractions which elute with a retention time of 11-13 minutes, preferably 12 minutes.

15. A method of preparing a pharmaceutical composition, the method comprising obtaining a mesenchymal stem cell conditioned medium (MSC-CM) by a method according to Claim 13

or an exosome by a method according to Claim 14 and admixing the MSC-CM or exosome, as the case may be, with a pharmaceutically acceptable carrier or diluent.

16. A method according to any preceding claim, in which transformed mesenchymal stem cell, the conditioned medium, exosome or pharmaceutical composition comprises at least one biological property of a mesenchymal stem cell, preferably cardioprotection, or in which the conditioned medium, exosome or pharmaceutical composition is capable of reducing infarct size preferably as assayed in a mouse or pig model of myocardial ischemia and reperfusion injury, or which is capable of reducing oxidative stress preferably as assayed in an *in vitro* assay of hydrogen peroxide (H_2O_2)-induced cell death.