METHODS AND USES FOR DETERMINING OSTEOGENIC POTENTIAL OF *IN VITRO* DIFFERENTIATED CELLS

FIELD OF THE INVENTION

The invention relates to methods and uses for determining osteogenic potential of *in vitro* differentiated cells. More particularly, the invention relates to methods and uses for determining osteogenic potential of *in vitro* differentiated cells including measuring one or more cellular markers.

BACKGROUND OF THE INVENTION

Transplantation of stem cells capable of undergoing osteogenic differentiation, of cells that are committed towards osteogenic differentiation or of cells with bone-forming ability is a promising avenue for the treatment of bone-related diseases, in particular when the treatment requires production of new bone tissues.

Mesenchymal stem cells (MSC) have been used previously to treat bone disorders (Gangji et al., 2005 Expert Opin Biol Ther 5: 437-42). However, although such relatively undifferentiated stem cells can be transplanted, they are not committed to an osteoblastic lineage and contribution to the formation of bone tissue may be primarily mediated by paracrine effects. Moreover, the quantity of MSC obtainable from subjects for the therapeutic use is frequently unsatisfactory.

Several methods for *in vitro* expanding MSCs and obtaining osteoprogenitors, osteoblasts or osteoblast phenotype cells from MSCs have been developed. Cells obtained by such methods may have varying degrees of osteogenic potential *in vitro* and *in vivo*. As a result, the amount of new bone tissue produced *in vivo* upon transplantation of such cells may not always be predictable and in some cases not optimal for clinical purposes.

There exists a need to determine prior to transplantation of *in vitro* cultured cells, such as *in vitro* differentiated MSC-derived cells, whether the cells have an osteogenic potential which is clinically useful.

SUMMARY

In an aspect of the present disclosure, there is provided a method for determining osteogenic potential of *in vitro* differentiated cells, the method comprising measuring the quantity of CD10 on the cell surface of the *in vitro* differentiated cells, wherein the method comprises determining that the *in vitro* differentiated cells have osteogenic potential if the *in vitro* differentiated cells have a normalized Median of Fluorescence Intensity (nMFI) for CD10 of at least 40, preferably the $nMFI_{CD10}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE.

In another aspect of the present disclosure, there is provided a use of CD10 for determining osteogenic potential of *in vitro* differentiated cells.

In a further aspect of the present disclosure, there is provided a method for selecting a subject for preparing *in vitro* differentiated cells of chrondro-osteoblastic lineage, the method comprising:

- recovering MSC from a biological sample of a subject;
- obtaining *in vitro* differentiated cells from the MSC;
- determining the osteogenic potential of the *in vitro* differentiated cells by a method disclosed herein; and
- selecting the subject for preparing *in vitro* differentiated cells of chondro-osteoblastic lineage if the *in vitro* differentiated cells have clinically useful osteogenic potential.

As corroborated by the experimental section, which illustrates certain representative examples of the present disclosure, the inventors realized that the osteogenic potential of *in vitro* differentiated cells, such as mesenchymal stem cells (MSC)-derived cells, can be evaluated by determining a specific cell surface marker expression profile of said cells. More particularly, the inventors found that by measuring the quantity of the *in vitro* differentiated cells expressing any one or more of CD73, CD105, CD10 or CD44, preferably all of CD73, CD105, CD10 and CD44, and measuring the quantity of any one or more of CD73, CD105 or CD44, preferably all of CD73, CD105 and CD44, expressed

by the cells, one can determine whether the cells have osteogenic potential, more particularly osteogenic potential that renders the cells useful in clinical settings. Furthermore, the present inventors also found that using the method for determining the osteogenic potential of MSC-derived cells as disclosed herein a subject could be selected which is particularly suitable as a donor of MSCs for preparing MSC-derived cells of chondro-osteoblastic lineage.

Hence, in an example, the present disclosure provides the use of any one or more of CD73, CD105 or CD44 for determining osteogenic potential of *in vitro* differentiated cells.

Preferably, the present disclosure provides the use of CD73, CD105, CD44 and CD10 for determining osteogenic potential of *in vitro* differentiated cells.

In a further example the present disclosure provides a method for determining osteogenic potential of *in vitro* differentiated cells comprising measuring the quantity of the *in vitro* differentiated cells expressing any one or more of CD73, CD105, CD10 or CD44, and measuring the quantity of any one or more of CD73, CD105 or CD44 expressed by the *in vitro* differentiated cells.

Preferably, the present disclosure provides a method for determining osteogenic potential of *in vitro* differentiated cells comprising measuring the quantity of the *in vitro* differentiated cells expressing CD73, CD105, CD10 and CD44, and/or measuring the quantity of any one or more of CD73, CD105 or CD44 expressed by the *in vitro* differentiated cells.

In a further example the present disclosure provides a method for selecting a subject for preparing *in vitro* differentiated cells of chrondro-osteoblastic lineage, the method comprising:

- recovering MSC from a biological sample of a subject;
- obtaining *in vitro* differentiated cells from the MSC;
- determining the osteogenic potential of the *in vitro* differentiated cells by a method as taught herein; and
- selecting the subject for preparing *in vitro* differentiated cells of chondro-osteoblastic lineage if the *in vitro* differentiated cells have clinically useful osteogenic potential.

These and further preferred examples of the present disclosure are described in the following sections and in the appended claims. The subject-matter of the appended claims is hereby specifically incorporated in this specification.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the bone neo-formation on a murine bone calvaria coronal section evidenced by murine and human calcium-binding fluorochromes, 2 weeks after administration of excipient alone (control condition), MSC-derived bone-forming cells A (generated with FGF-2 and TGFβ1) or MSC-derived bone-forming cells B (generated with FGF-2, TGFβ1 and heparin).

CLAIMS

1. A method for determining osteogenic potential of *in vitro* differentiated cells, the method comprising measuring the quantity of CD10 on the cell surface of the *in vitro* differentiated cells, wherein the method comprises determining that the *in vitro* differentiated cells have osteogenic potential if the *in vitro* differentiated cells have a normalized Median of Fluorescence Intensity (nMFI) for CD10 of at least 40, preferably the $nMFI_{CD10}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE.

2. The method according to claim 1, wherein the method comprises determining that the *in vitro* differentiated cells have osteogenic potential if the *in vitro* differentiated cells have a nMFI for CD10 of at least 50, preferably the $nMFI_{CD10}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE.

3. The method according to claim 1 or 2, the method comprising:

   - measuring the fraction of the *in vitro* differentiated cells expressing any one or more of CD73, CD105, CD10 or CD44 on the cell surface of the *in vitro* differentiated cells;
   - measuring the quantity of CD10 on the cell surface of the *in vitro* differentiated cells; and
   - determining that the *in vitro* differentiated cells have osteogenic potential if at least 90% of the *in vitro* differentiated cells express any one or more of CD73, CD105, CD10 or CD44, and if the *in vitro* differentiated cells have a nMFI for CD10 of at least 40, preferably the $nMFI_{CD10}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE.

4. The method according to any one of claims 1 to 3, the method comprising:

   - measuring the fraction of the *in vitro* differentiated cells expressing CD73, CD105, CD10 and CD44 on the cell surface of the *in vitro* differentiated cells;
   - measuring the quantity of CD10 on the cell surface of the *in vitro* differentiated cells; and
   - determining that the *in vitro* differentiated cells have osteogenic potential if at least 90% of the *in vitro* differentiated cells express CD73, CD105, CD10 and CD44, and if the *in vitro* differentiated cells have a nMFI for CD10 of at least 40, preferably the $nMFI_{CD10}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE.

5. The method according to any one of claims 1 to 4, wherein the method further comprises measuring the quantity of any one or more of CD73, CD105 or CD44 expressed by the *in vitro* differentiated cells.

6. The method according to claim 5, wherein the method comprises determining that the *in vitro* differentiated cells have osteogenic potential if the *in vitro* differentiated cells have a nMFI for

CD10 of at least 40, and if the *in vitro* differentiated cells have any one or more of a nMFI for CD73 of at least 500, a nMFI for CD44 of at least 100 or a nMFI for CD105 of at most 150, preferably wherein the $nMFI_{CD10}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE, the $nMFI_{CD73}$ is measured with an excitation wavelength of 633 nm and an emission wavelength of 660 nm for APC, the $nMFI_{CD44}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE, and/or the $nMFI_{105}$ is measured with an excitation wavelength of 633 nm and an emission wavelength of 660 nm for APC.

7. The method according to any one of claims 1 to 6, wherein the method further comprises measuring the quantity of CD73, CD105 and CD44 expressed by the *in vitro* differentiated cells.

8. The method according to claim 6, wherein the method comprises determining that the *in vitro* differentiated cells have osteogenic potential if the *in vitro* differentiated cells have a nMFI for CD10 of at least 40, and if the *in vitro* differentiated cells have a nMFI for CD73 of at least 500, a nMFI for CD44 of at least 100 and a nMFI for CD105 of at most 150, preferably wherein the $nMFI_{CD10}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE, the $nMFI_{CD73}$ is measured with an excitation wavelength of 633 nm and an emission wavelength of 660 nm for APC, the $nMFI_{CD44}$ is measured with an excitation wavelength of 488 nm and an emission wavelength of 580 nm for PE, and the $nMFI_{105}$ is measured with an excitation wavelength of 633 nm and an emission wavelength of 660 nm for APC.

9. Use of CD10 for determining osteogenic potential of *in vitro* differentiated cells.

10. The use according to claim 9, further comprising the use of any one or more of CD73, CD105 or CD44 for determining osteogenic potential of *in vitro* differentiated cells, or further comprising the use of CD73, CD105 and CD44 for determining osteogenic potential of *in vitro* differentiated cells.

11. The method according to any one of claims 1 to 8, or the use according to claim 9 or 10, wherein the *in vitro* differentiated cells are obtained from mesenchymal stem cells (MSC).

12. The method according to any one of claims 1 to 8, or 11, or the use according to any one of claims 9 to 11, wherein the *in vitro* differentiated cells are human cells.

13. A method for selecting a subject for preparing *in vitro* differentiated cells of chrondro-osteoblastic lineage, the method comprising:

    - recovering MSC from a biological sample of a subject;
    - obtaining *in vitro* differentiated cells from the MSC;

- determining the osteogenic potential of the *in vitro* differentiated cells by a method as defined in any one of claims 1 to 8, 11 or 12; and
- selecting the subject for preparing *in vitro* differentiated cells of chondro-osteoblastic lineage if the *in vitro* differentiated cells have clinically useful osteogenic potential.

14. The method according to claim 13, wherein the subject is a human subject.