| | | | |
|---|---|---|---|
| | | (100nM)<br>IBMX (250μM) | |

ITSE is a cell commercially available cell culture medium supplement containing transferrin and insulin; the remaining components are identical to the reagents used in Example 2.

Results are set forth in Figures 5 to 9, which are further described in Example 4.

**Example 4**

*Differentiation of BAT using second protocol*

Adipose derived stem cells are seeded 50,000 cells/cm$^2$ (day 0) in StemPro SFM (Invitrogen) 2 days later media are replaced by differentiation media according to the following formulations, again containing 1% penicillin and streptomycin. After day 6, media are refreshed every 2-3 days until day 14 or 15 of differentiation.

| Day | 0 | 2 | 3 | 6 | 6-15 |
|---|---|---|---|---|---|
| **Basal Media** | StemPro SFM | DMEM/F12<br>Insulin (5μg/ml)<br>Transferrin (10μg/ml) | Media and components as in day 2 refreshed | DMEM/F12<br>Insulin (100ng/ml)<br>Transferrin (10μg/ml) | Media and components refreshed every 2-3 days as in day 6 |
| **Components** | | BMP7 (50ng/ml)<br>T3 (2nM)<br>Dexamethasone (200nM)<br>Rosiglitazone (5μM)<br>IBMX (250μM) | | T3 (2nM)<br>Dexamethasone (30nM)<br>Rosiglitazone(100nM) | |

In order to monitor the generation of BAT, cells were analysed for the expression of various genes associated with the BAT cell type. Figure 5 illustrates a western blot of UCP-1 expression from tissues isolated according to the protocols of Examples 2, 3 and 4. The protocol of the present Example 4 is referred to as “MR”.

Figure 6 shows the changes in gene expression obtained with the three protocols, assessed by qPCR. In each case, UCP-1 expression is increased significantly as the

| | | | |
|---|---|---|---|
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Results are set forth in Figures 5 to 8 which are further described in Example 4.

**Example 4**

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| Day | 0 | 2 | 3 | 6 | 6-15 |
|---|---|---|---|---|---|
| **Basal Media** | StemPro SFM | DMEM/F12<br>Insulin (5μg/ml)<br>Transferrin (10μg/ml) | Media and components as in day 2 refreshed | DMEM/F12<br>Insulin (100ng/ml)<br>Transferrin (10μg/ml) | Media and components refreshed every 2-3 days as in day 6 |
| **Components** | | BMP7 (50ng/ml)<br>T3 (2nM)<br>Dexamethasone (200nM)<br>Rosiglitazone (5μM)<br>IBMX (250μM) | | T3 (2nM)<br>Dexamethasone (30nM)<br>Rosiglitazone(100nM) | |

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Figure 6 shows the changes in gene expression obtained with the three protocols, assessed by qPCR. In each case, UCP-1 expression is increased significantly as the

## Claims

1. A differentiated brown adipose tissue (BAT) cell which is derived from adult stem cells.

2. A differentiated BAT cell according to claim 1, wherein the adult stem cells are not transgenic.

3. A culture of differentiated BAT cells according to claim 1or claim 2, which culture is derived from adult stem cells.

4. A culture of differentiated BAT cells according to claim 3, wherein basal UCP1 expression is at a level at least 10 times that of white adipose tissue (WAT).

5. A culture of differentiated BAT cells according to claim 4, wherein basal UCP1 expression is at least 20 times the basal UCP1 expression in WAT.

6. A culture of differentiated BAT cells according to claim 3, which expresses PDRM16 at a basal level which is increased compared to WAT.

7. A culture of differentiated BAT cells according to claim 6, wherein PDRM16 is expressed at a level 2 times higher than the basal level in WAT.

8. A culture of differentiated BAT cells according to claim 3, which expresses PPRCG1a at a basal level which is increased compared to the basal expression level in WAT.

9. A culture of differentiated BAT cells according to claim 8, wherein PPRCG1a is expressed at a level at least 20 times higher than in WAT.

10. A culture of differentiated BAT cells according to claim 3, which does not express CIDEA at a level increased when compared with white adipose tissue (WAT).

11. A differentiated BAT cell or a culture of differentiated BAT cells according to any preceding claim, in which elevated basal expression of one or more of the following genes is observed compared to WAT: TBX15, FBXO31, EBF3, SHOX2, and TMEM26.

12. A differentiated BAT cell or a culture of differentiated BAT cells according to any preceding claim, which shows characteristics of a brite cell lineage.

13. A differentiated BAT cell or a culture of differentiated BAT cells according to any preceding claim, wherein the adult stem cell is an adipose-derived stem cell (ADSC).

14. A differentiated BAT cell or a culture of differentiated BAT cells according to claim 13, wherein the adult adipose-derived stem cell is an adMSC and/or a preadipocyte.

15. A differentiated BAT cell or a culture of differentiated BAT cells according to claim 13 or claim 14, wherein the adult adipose-derived stem cell is derived from WAT.

16. A method for deriving a BAT cell from an adult stem cell, comprising culturing the stem cell in mesechymal stem cell growth medium, and transferring the cell to a medium comprising Dexamethasone, IBMX and BMP-7.

17. A method according to claim 16, in which the cells are not transfected with transgenes.

18. A method according to claim 16 or claim 17, wherein 20% or more of the adult stem cells are differentiated into BAT cells.

19. A method according to claim 18, wherein 50% or more of the adult stem cells are differentiated into BAT cells

20. A method according to any one of claims 16 to 19, comprising exposing the stem cell to the following media combinations:

(i) MSC SFM and BMP-7;

(ii) DMEM/F12, insulin, transferrin, BMP-7, T3, Dexamethasone, Rosiglitazone and IBMX; and

(iii) DMEM/F12, insulin, transferrin, BMP-7, T3, Dexamethasone and Rosiglitazone.

21. A method according to claim 20, wherein step (iii) is repeated by refreshing the medium.

22. A method according to any one of claims 16 to 21, wherein the cells are exposed to the media sequentially, at three-day intervals.

23. A method according to any one of claims 16 to 21, wherein the cells are exposed to the media contemporaneously.

24. A method according to any one of claims 16 to 19, comprising exposing the stem cell to the following media combinations:

(i) MSC SFM;

(ii) DMEM/F12, insulin, transferrin, BMP-7, T3, Dexamethasone, Rosiglitazone and IBMX; and

(iii) DMEM/F12, insulin, transferrin, T3, Dexamethasone and Rosiglitazone.

25. A method according to claim 24, wherein steps (ii) and (iii) are repeated by refreshing the medium.

26. A differentiated BAT cell or cell culture derived by a method according to any one of claims 16 to 25.

27. A differentiated BAT cell or cell culture according to any one of claims 1 to 15, derived by a method according to any one of claims 16 to 25.

28. A differentiated BAT cell or cell culture according to any one of claims 1 to 15 for use in treating a patient suffering from a disease characterised by excessive adipose tissue accumulation, wherein the cell is implanted into the patient.

29. A method for treating a patient suffering from a disease characterised by excessive adipose tissue accumulation, comprising implanting in a patient in need of said treatment one or more cells according to any one of claims 1 to 15.

30. A method according to claim 29, wherein the disease is diabetes.

31. A kit for deriving a BAT cell from an adult stem cell, comprising two or more of the agents set forth in claim 20 or claim 23.

**CLAIMS**

1. A method for deriving a BAT cell from an adult stem cell, comprising culturing the stem cell in mesenchymal stem cell growth medium, and transferring the cell to a medium comprising Rosiglitazone, Dexamethasone, IBMX and BMP-7.

2. A method according to Claim 1, in which the cells are not transfected with transgenes.

3. A method according to Claim 1 or 2, in which 20% or more of the adult stem cells are differentiated into BAT cells.

4. A method according to Claim 3, in which 50% or more of the adult stem cells are differentiated into BAT cells.

5. A method according to any preceding claim, comprising exposing the stem cell to the following media combinations:

(i) StemPro MSC SFM (Invitrogen);

(ii) DMEM/F12, insulin, transferrin, BMP-7, T3, Dexamethasone, Rosiglitazone and IBMX; and optionally

(iii) DMEM/F12, insulin, transferrin, BMP-7, T3, Dexamethasone and Rosiglitazone.

6. A method according to Claim 5, in which step (iii) is repeated by refreshing the medium.

7. A method according to any preceding claim, in which the cells are exposed to the media sequentially, at three-day intervals.

8. A method according to any of Claims 1 to 6, in which the cells are exposed to the media contemporaneously.

9. A method according to any of Claims 1 to 4, comprising exposing the stem cell to the following media combinations:

(i) MSC SFM;

(ii) DMEM/F12, insulin, transferrin, BMP-7, T3, Dexamethasone, Rosiglitazone and IBMX; and

(iii) DMEM/F12, insulin, transferrin, BMP-7, T3, Dexamethasone and Rosiglitazone.

10. A method according to Claim 9, in which steps (ii) and (iii) are repeated by refreshing the medium.

11. A kit for deriving a BAT cell from an adult stem cell, comprising Rosiglitazone, Dexamethasone, IBMX and BMP-7.

12. A kit for deriving a BAT cell from an adult stem cell according to Claim 11, further comprising one or more of the following agents: BMP; insulin; transferrin; and T3.

13. A kit for deriving a BAT cell from an adult stem cell according to Claim 11 or 12 further comprising a basal media and/or preadipocytes and/or adMSCs and/or instruments for the extraction of adipose tissue.