METHODS OF CELLULAR REPROGRAMMING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore application no. 10201401734X, filed 23 April 2014, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to cellular reprogramming. Specifically, the present invention relates to integrin modulated cellular reprogramming.

BACKGROUND OF THE INVENTION

[0003] Phenotypic stability is a hallmark of differentiated cells. This phenotypic stability is maintained by reciprocal interactions between the cell and its environment in a process known as dynamic reciprocity, where signals between the cellular microenvironment, such as the extracellular matrix (ECM), and the cell interact to influence and stabilize the cell phenotype within its niche environment.

[0004] The linkage between the cell and its microenvironment involves transmembrane cell adhesion proteins, of which the integrins form a superfamily. Integrins therefore play a critical role in cell adhesion to the ECM, which in turn, regulate cellular processes of adhesion, proliferation, migration and differentiation.

[0005] However, to date, there are few reports on reprogramming differentiated cells to stem-cell like cells, or of reprogramming differentiated cells to other differentiated cell types by manipulating the interaction of the cell with the ECM. There are also few reports on the role that integrins play in reprogramming the fate of differentiated cells.

[0006] Accordingly, there is a need to understand the interactions of the cell with the ECM in reprogramming cell fate and the role integrins play in this process.

SUMMARY OF THE INVENTION

In one aspect, there is provided a method for reprogramming a first cell type to an intermediate cell of a second cell type, comprising the step of: contacting said first cell type with a first

agent to modulate an integrin profile in the first cell type to provide said intermediate cell of said second cell type, wherein said first cell type is a fibroblast cell, and said intermediate cell is a stem-cell like cell, and wherein said first agent comprises integrin ligands of any one or more of integrin subunits selected from the group consisting of αv, α5, β3, β5 and β6, or nucleic acid molecules that increase the expression of any one or more of integrin subunits selected from the group consisting of α2, α3, α6, β1 and β4.

[0007] In one example, there is provided a reprogrammed cell obtained according to the method as disclosed herein.

[0008] In another aspect, there is provided a kit for reprogramming a first cell type to a second cell type, comprising one or more of the following components: (i) a composition comprising at least one first agent to effect reprogramming of a first cell type to an intermediate cell type; (ii) a composition comprising at least one second agent to effect reprogramming of an intermediate cell type to a second cell type; optionally comprising instructions for use, wherein said first cell type is a fibroblast cell, and said intermediate cell is a stem-cell like cell, and wherein said first agent comprises integrin ligands of any one or more integrin subunits selected from the group consisting of αv, α5, β3, β5 and β6, or nucleic acid molecules that increase the expression of any one or more integrin subunits α2, α3, α6, β1 and β4.

[0009] In one example, there is provided a method for treating a patient in need of cell-based therapy or tissue replacement, comprising administering to said patient a reprogrammed cell obtained according to the method as disclosed herein.

[0010] In another example, there is provided a reprogrammed cell obtained according to the method as disclosed herein for use in treating a patient in need of cell-based therapy or tissue replacement, wherein said reprogrammed cell is to be administered to said patient.

[0011] In another example, there is provided a use of a reprogrammed cell obtained according to the method as disclosed herein in the manufacture of a medicament for treating a patient in need of cell-based therapy or tissue replacement, wherein said medicament is to be administered to said patient.

[0012] In another example, there is provided a method for treating a patient in need of cancer therapy, comprising delivering a bioactive to said patient using as a vehicle, a reprogrammed cell obtained according to the method as disclosed herein.

[0013] In another example, there is provided a reprogrammed cell obtained according to the method as disclosed herein as a vehicle for delivering a bioactive for use in treating a patient in need of cancer therapy.

[0014] In another example, there is provided a use of a reprogrammed cell obtained according to the method as disclosed herein as a vehicle for delivering a bioactive in the manufacture of a medicament for treating a patient in need of cancer therapy.

[0015] In another example, there is provided a method of determining the suitability of a cancer therapy for a cancer patient, comprising preparing a reprogrammed cell according to

the method as disclosed herein, and administering to said reprogrammed cell one or more cancer therapy to assess efficacy of said cancer therapy on said reprogrammed cell.

[0016] In another example, there is provided a reprogrammed cell prepared according to the method as disclosed herein for use in determining the suitability of a cancer therapy for a cancer patient, wherein one or more cancer therapy is to be administered to said reprogrammed cell to assess efficacy of said cancer therapy on said reprogrammed cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] 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:

[0018] **Fig. 1**. Timeline for induction by selective integrin inhibition and ligation

[0019] **Fig. 2**. Selective integrin inhibition leading to the intermediate phenotype.(A) The intermediate phenotype in the form of spherical cell clusters (SCC) at 3 days induction; Changes in (B) MET markers and (C) Stem cell markers, with respect to isotype control; (D): Western blot showing upregulation of expression for the integrins α2 and α6, and to a lesser extent, integrin α3 and CXCR4

[0020] **Fig. 3**. Confocal micrographs of DAPI-stained (A) induced IMR90 fibroblasts (the intermediate phenotype) in comparison to (B) control fibroblasts, showing differences in chromatin and nuclear structure. Blue: DAPI staining; Green: phalloidin staining; corresponding panel below shows 3D reconstruction of confocal sections through nuclei.

[0021] **Fig. 4**. (A) SCCs were cultured with ECM overnight; media was changed to B27 containing FGF and EGF. (i) Appearance of cells before media change; Appearance of cells following media change, after (ii) 1 day; (iii) 3 days; (iv) 4 days; (B) SCCs cultured with ECM overnight; media was changed to DMEM without growth factors. Appearance of cells following media change, after (ii) 1 day; (iii) 3 days; (iv) 4 days; (C) Gene expression after 4 days of culture. (C): Integrins profile after 4 days in ECM (Geltrex/ Laminin 332) culture, with and without growth factors (GFs). (D) Epithelial and mesenchymal markers after 4 days in ECM (Geltrex/ Laminin 332) culture, with and without GFs. (E) Pluripotent and stemness markers after 4 days in ECM (Geltrex/ Laminin 332) culture, with and without GFs.

Claims

1. A method for reprogramming a first cell type to an intermediate cell of a second cell type, comprising the step of:

contacting said first cell type with a first agent to modulate an integrin profile in the first cell type to provide said intermediate cell of said second cell type,

wherein said first cell type is a fibroblast cell, and said intermediate cell is a stem-cell like cell, and

wherein said first agent comprises integrin ligands of any one or more of integrin subunits selected from the group consisting of αv, α5, β3, β5 and β6, or nucleic acid molecules that increase the expression of any one or more of integrin subunits selected from the group consisting of α2, α3, α6, β1 and β4.

2. The method as claimed in claim 1, wherein the integrin ligands are extracellular matrix (ECM) proteins or anti-integrin antibodies.

3. The method according to claim 1 or 2, wherein the integrin ligands areanti-integrin antibodies or fragments thereof.

4. The method of claim 3, wherein the anti-integrin antibodies or fragments thereof are integrin inhibitors.

5. The method according to any one of the preceding claims, wherein said first cell type is contacted with the first agent for a period sufficient to alter gene expression.

6. The method according to claim 5, wherein the period is selected from the group consisting of at least about 1 minute, about 3 minutes, about 5 minutes, about 10 minutes, about 15 minutes, about 30 minutes, about 60 minutes, about 90 minutes, about 2 hours, about 24 hours, about 2 days or about 5 days.

7. The method of claim 1, wherein the nucleic acid molecules are expression vectors, wherein each of the expression vectors comprises at least one integrin gene operably linked to a promoter to overexpress said integrin gene in said first cell type, wherein said integrin gene encodes for integrin subunit selected from the group consisting of integrin subunits α2, α3, α6, β1 and β4.

8. The method of claim 7, wherein the expression vector is a viral vector.

9. The method of claim 8, wherein the viral vector is a lentiviral vector.

10. The method as claimed in any of claims 1 to 9, further comprising the step of detecting expression of integrins, pluripotent and/or stem cell markers to determine that said first cell type has transitioned to said intermediate cell type.

11. The method as claimed in claim 10, wherein an increased expression of integrin β4 and integrin β6 relative to the first cell type is indicative of said intermediate cell type.

12. The method of any of claims 1 to 11, further comprising contacting the intermediate cell type with a composition comprising a second agent to effect the reprogramming of said intermediate cell type to the second cell type.

13. The method according to claim 12, wherein said second agent ligates one or more integrin subunits selected from the group consisting of α1, α2, α3, α4, α5, α6, α7, α8, α9, α10, α11, αv, αD, αL, αM, αX, αE, αIIb, β1, β2, β3, β4, β5, β6, β7 and β8.

14. The method according to claim 13, wherein said second agent ligates an integrin subunit selected from the group consisting of α2, α3, α6, αv, α5, β1, β3, β4, β5, β6, and combinations thereof.

15. The method according to claim 14, wherein the combination of subunits is selected from αvβ3, αvβ5, αvβ6, α6β1, α6β4, α2β1, α3β1 and α5β1.

16. The method according to claim 12, wherein said second agent comprises one or more ligands that binds to said at least one integrin to cause ligation of said integrin.

17. The method according to claim 16, wherein said one or more ligands comprises one or more extracellular matrix components.

18. The method according to claim 17, wherein the one or more extracellular matrix components are derived from basement membrane.

19. The method according to claim 17, wherein the extracellular matrix component is selected from a group consisting of laminin, collagen, entactin, and Geltrex® matrix (Life Technologies, USA).

20. The method according to claim 12, wherein contacting the intermediate cell type with a composition comprising said second agent is conducted for a duration sufficient to effect reprogramming to the second cell type.

21. The method according to claim 20, wherein the duration of contact is selected from at least about12 hours, about 18 hours, about 24 hours, about 30 hours, about 36 hours, about 48 hours, about 2.5 days, about 3 days, about 3.5 days, about 4 days, about 4.5 days, about 5 days, about 5.5 days, about 6 days, about 6.5 days or about 7 days.

22. The method according to any one of the preceding claims, wherein the method further comprises contacting said first cell type that has been contacted with said first agent to provide said intermediate cell of said second cell type and said second agent to effect the reprogramming of said intermediate cell type to the second cell type with an additional agent that is capable of effecting further reprogramming and/or modification of the growth and/or proliferative characteristics of the first cell type.

23. The method according to claim 22, wherein the additional agent is (a) a growth factor selected from the group consisting of fibroblast growth factor 2 (FGF2), epidermal growth factor (EGF), hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF); and/or

(b) a conditioned medium such as a conditioned medium from a rat insulinoma (Rin5f) cell line; and/or (c) a medium selected from B-27® supplement (comprising BSA, transferrin, insulin, progesterone, putrescine, sodium selenite, biotin, l-carnitine, corticosterone, ethanolamine, d(+)-galactose, glutathione (reduced), linolenic acid, linoleic acid, retinyl acetate, selenium, t3 (triodo-1-thyronine), dl-α-tocopherol (vitamine e), dl-α-tocopherol acetate, catalase, superoxide dismutase) and TeSR™2 (comprising DMEM/F12 (liquid), L-ascorbic Acid, selenium, transferrin, NaHCO3, glutathione, L-glutamine, defined lipids, thiamine, ß-mercaptoethanol, albumin, insulin, FGF2, TGFß1, pipecolic acid, LiCl, GABA).

24. The method according to claim 22 or 23, comprising providing the additional agent for a duration sufficient to effect the further reprogramming to the second cell type and/or modification of the growth and/or proliferative characteristics of the cell.

25. The method according to claim 24, wherein the duration is selected from the group consisting of at least about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 day and about 7 days.

26. The method according to any one of the preceding claims, further comprising providing a cellular support to support growth of the cells being reprogrammed.

27. The method according to claim 26, wherein said cellular support comprises a nanotopographical scaffold.

28. The method according to claim 26, wherein said nanotopographical scaffold comprises uniform arrays of silica nanoparticles.

29. The method according to any one of claims 1 to 28, wherein the second cell type is selected from the group consisting of epithelial cells, cells with epithelial characteristics, and neuronal cells.

30. The method of any of claims 1 to 29, wherein the method is conducted *in vitro*.

31. A kit for reprogramming a first cell type to a second cell type, comprising one or more of the following components:

(i) a composition comprising at least one first agent to effect reprogramming of a first cell type to an intermediate cell type;

(ii) a composition comprising at least one second agent to effect reprogramming of an intermediate cell type to a second cell type, optionally comprising instructions for use,

wherein said first cell type is a fibroblast cell, and said intermediate cell is a stem-cell like cell, and

wherein said first agent comprises integrin ligands of any one or more integrin subunits selected from the group consisting of αv, α5, β3, β5 and β6, or nucleic acid molecules that increase the expression of any one or more integrin subunits α2, α3, α6, β1 and β4.

32. The kit according to claim 31, further comprising (a) a growth factor selected from the group consisting of fibroblast growth factor 2 (FGF2), epidermal growth factor (EGF), hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF); and/or (b) a conditioned medium such as a conditioned medium from a rat insulinoma (Rin5f) cell line; and/or (c) a medium selected from B-27® supplement (comprising BSA, transferrin, insulin, progesterone, putrescine, sodium selenite, biotin, l-carnitine, corticosterone, ethanolamine, d(+)-galactose, glutathione (reduced), linolenic acid, linoleic acid, retinyl acetate, selenium, t3 (triodo-1-thyronine), dl-α-tocopherol (vitamine e), dl-α-tocopherol acetate, catalase, superoxide dismutase) and TeSR™2 (comprising DMEM/F12 (liquid), L-ascorbic Acid, selenium, transferrin, NaHCO3, glutathione, L-glutamine, defined lipids, thiamine, ß-mercaptoethanol, albumin, insulin, FGF2, TGFß1, pipecolic acid, LiCl, GABA); and

a scaffold for supporting growth of the cells being reprogrammed, wherein optionally, said scaffold comprises a nanotopographical scaffold, and wherein optionally said nanotopographical scaffold comprises uniform arrays of silica nanoparticles.