[0006] Applicants previously determined that activation of the IGFl, Insulin, ERBB2 and ERBB3 receptors was a hallmark of proliferating hES cells. IGF1R/IR activation could be attributed to IGFl in serum or microgram/ml concentrations of insulin, for example, in serum replacer or N2/B27 cell culture supplements in a range of growth conditions. These activities provide a strong PB kinase/AKT signal, and equivalent activation could be achieved with lower concentrations of LR3-IGF1, an IGFl analog. *See* McLean et al, 2007 Stem Cells 25, 29-38. Phosphorylation of ERBB2/3 was consistent with the activity of an EGF family member, heregulin/neuregulin, in MEF-CM. *See* Wang et al, 2007 Blood 110, 4111-9. Inhibition of IGFlR and ERBB2 impacted self-renewal of hES cell, as did small molecule inhibitors of Activin and FGF signaling. See Robins and Schulz, 2009, "Novel methods of Stem Cell Culture and Maintenance: Media and extra cellular matrix requirements for large scale ESC growth." In Emerging Technology Platforms for Stem Cells (eds. U. Lakshmipathy J. D. Chesnut and B. Thyagarajan, pp. 251-247) Hoboken. John Wiley & Sons Inc.; and Vallier *et al*, 2005 J Cell Sci 118, 4495-509). This defined media was developed and termed DC-HAIF, which consisted of DMEM/F12, non essential amino acids, trace elements, ascorbic acid, β-mecaptoethanol, Penicillin/Streptomycin (optional), with the only proteins being caprylic acid extracted fatty acid- free BSA, transferrin, and recombinant heregulin-lβ (H), activin A (A), LR3-IGF1 (I), and FGF2 (F). DC-HAIF supported long term maintenance of pluripotent hES cells, as well as single cell passaging and scaled expansion of hES cell using ACCUTASE™. See Robins and Schulz (2009) *supra*; Wang *et al, supra.* A batch-tested commercial formulation of DC-HAIF is available from under license to Life Technologies and sold under the trade name StemPro® hES cell SFM.

[0007] Still there remains a need to identify alternative and/or additional signaling pathways with critical functions in pluripotent stem cells, e.g. hES and iPS cells, and that are able to be used for therapeutic purposes, wherein the culture compositions are defined and/or produced to GMP standard.

SUMMARY OF THE INVENTION

[0008] The invention relates to compositions comprising a basal salt nutrient solution for pluripotent cell growth comprising at least norepinephrine and dopamine, the composition being essentially free of serum.

[0008a] In one aspect, there is provided an in vitro composition comprising single human pluripotent cells whereby said pluripotent cells are plated as single cells at a low cell density in a medium comprising an effective amount of a neurotransmitter or a structural analog, derivative or physiologically acceptable salt thereof sufficient to achieve low density survival and expansion of the pluripotent cells.

[0008b] In one aspect, there is provided an in vitro suspension culture comprising single human pluripotent cells whereby said pluripotent cells are in suspension as single cells and an effective amount of a neurotransmitter or a structural analog, derivative or physiologically acceptable salt thereof.

[0008c] In one aspect, there is provided a method of expanding human pluripotent cells in vitro comprising:

a. obtaining single human pluripotent cells; and

b. plating said single human pluripotent cells as single cells at low cell density in a medium comprising an effective amount of a neurotransmitter or a structural analog, derivative or physiologically acceptable salt thereof sufficient to achieve low density survival and expansion of the pluripotent cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figure 1. Representation of antagonists/modulators of neurotransmitter/hormone receptors identified by compound screening in hES cells. Alkaline phosphatase (AP) staining of the primary library screen demonstrated no effect (+++, confluent growth), moderate effect (++), or severe (+) impact on cells; only rare cells (+/-), or no cells (-) are also indicated. Receptor antagonists identified in the primary screen are shown (left column). The screen was performed using compounds at 10 μM. The secondary dose titration assay is shown (50-0.1 μM columns), and receptor class targeted by each compound and action is indicated.

[0001] Figure 2. Low density plating assay of hES cells {e.g., BGO2 cells). 10000 cells/well were plated in 6-well trays in StemPro® hESC SFM (Life Technologies) medium and stained with alkaline

What is claimed is:

1. An in vitro composition comprising single human pluripotent cells whereby said pluripotent cells are plated as single cells at a low cell density in a medium comprising an effective amount of a neurotransmitter or a structural analog, derivative or physiologically acceptable salt thereof sufficient to achieve low density survival and expansion of the pluripotent cells.

2. The composition of claim 1, wherein the neurotransmitter is a neurotransmitter receptor agonist.

3. The composition of claim 2, wherein the neurotransmitter is norepinephrine.

4. The composition of claim 1, wherein the human pluripotent cells can expand in the absence of differentiation.

5. The composition of claim 1, further comprising at least one growth factor.

6. The composition of claim 5, wherein the at least one growth factor is selected from the group consisting of: an ErbB3 ligand or a functional fragment thereof; a TGF-β family member or a functional fragment thereof; an activator of insulin-like growth factor receptor (IGF-1R) or a functional fragment thereof; and an activator of a fibroblast growth factor (FGF) receptor or a functional fragment thereof.

7. The composition of claim 6, wherein the ErbB3 ligand is heregulin.

8. The composition of claim 1, wherein the pluripotent cells are plated at about 1000 cells per ml of the medium.

9. The composition of claim 1, wherein the pluripotent cells can be passaged at least 2 times.

10. The composition of claim 9, wherein the pluripotent cells retain expression of pluripotency markers upon passaging.

11. The composition of claim 1, wherein the composition is serum free.

12. The composition of claim 1, wherein the composition is Activin A free.

13. The composition of claim 1, further comprising at least one growth factor.

14. An in vitro suspension culture comprising single human pluripotent cells whereby said pluripotent cells are in suspension as single cells and an effective amount of a neurotransmitter or a structural analog, derivative or physiologically acceptable salt thereof.

15. The composition of claim 14, wherein the composition is Activin A free.

16. A method of expanding human pluripotent cells in vitro comprising:

a. obtaining single human pluripotent cells; and

b. plating said single human pluripotent cells as single cells at low cell density in a medium comprising an effective amount of a neurotransmitter or a structural analog, derivative or physiologically acceptable salt thereof sufficient to achieve low density survival and expansion of the pluripotent cells.

17. The method of claim 16, wherein the neurotransmitter is a neurotransmitter receptor agonist.

18. The method of claim 17, wherein the neurotransmitter receptor agonist is norepinepherine.

19. The method of claim 16, wherein the medium does not comprise Activin A.

20. The composition of claim 1, said composition being essentially free of serum and serum replacement.

21. The composition of claim 1, wherein the composition is free of a feeder cell layer.

22. The composition of claim 1, wherein the effective amount of the neurotransmitter or the structural analog, derivative or physiologically acceptable salt thereof is sufficient to achieve improved survival and expansion of the pluripotent cells as compared to a control composition without the neurotransmitter or the structural analog, derivative or physiologically acceptable salt thereof.

23. The composition of claim 14, wherein the neurotransmitter is norepinephrine.