[0006] Applicants previously determined that activation of the IGF1, Insulin, ERBB2 and ERBB3 receptors was a hallmark of proliferating hES cells. IGF1R/IR activation could be attributed to IGF1 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 PI3 kinase/AKT signal, and equivalent activation could be achieved with lower concentrations of LR3-IGF1, an IGF1 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 IGF1R 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-1β (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 disclosure~~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 a defined cell culture medium comprising a compound, wherein the compound is norepinephrine or a structural analog, derivative, or physiologically acceptable salt thereof with the proviso that the defined cell culture medium supports the low density survival and expansion of human pluripotent stem cells cultured therein in the absence of differentiation.

[0008b] In another aspect, there is provided a composition comprising: a) a defined cell culture medium comprising a compound, wherein the compound is norepinepherine or a structural analog, derivative, or physiologically acceptable salt thereof; and b) a human pluripotent stem cell; with the proviso that the defined cell culture medium supports the low density survival and expansion of the human pluripotent stem cell cultured therein in the absence of differentiation.

[0008c] In another aspect, there is provided a method for expanding a human pluripotent stem cell, comprising incubating the human pluripotent stem cell in a defined cell culture medium comprising a compound, wherein the compound is norepinepherine or a structural analog, derivative, or physiologically acceptable salt thereof wherein the cell is plated at low density and divides during the incubation but does not differentiate.

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