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TECHNOLOGY LICENSING OPPORTUNITIES

STEM CELL TECHNOLOGIES

Method for cell culture using Inter-alpha-Inhibitor (IαI)

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Invention Summary

A method for cell culture using one or more IαI (inter-alpha trypsin inhibitor or Inter-alpha inhibitor) protein(s) as a component in a cell culture media or a coating on a cell culture surface material.

Background

Pluripotent stem (PS) cells e.g. embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have the ability to maintain pluripotency during long-term culture and yet induce differentiation into multiple lineages and therefore potentially offer novel cell sources for e.g. basic research, toxicological screening, in vitro modeling of genetic disorders or therapeutic cell replacement. There are still many obstacles to overcome until these endpoints can be fully realized. For instance, it will be necessary to find culture conditions that support safe, simple and robust derivation, growth, maintenance and large-scale expansion, while maintaining self-renewal, of these difficult to culture cells. Especially important is the need for methods for maintenance of human PS cells in vitro. These methods need be good enough to maintain the population of cells without inducing mutagenesis, high levels of differentiation or loss of pluripotency.

Mouse ES cells are extensively used in basic research to e.g. study normal and pathological development and function and the knowledge obtained using these cells is often transferred to human systems. Most mouse ES (mES) cell lines used today are grown on pre-plated mitotically inactivated mouse embryonic fibroblast (MEF) feeder cells in media supplemented with selected batches of fetal bovine serum (FBS) and Leukemia inhibitory factor (LIF). The feeder cells provide a matrix that support mES cell attachment and secrete various growth factors that enhance the survival and propagation of mES cell growth whereas FBS provides hormones and essential nutrients, as well as altering the physiological/physiochemical properties of the medium. LIF drastically improves the derivation and maintenance of the pluripotency of mES cells. Some mES cell lines have been derived and adapted to grow feeder-free on 0.1% Gelatin coating (heterogeneous mixture of water-soluble proteins of high average molecular weight present in collagen and extracted from bovine skin) in serum and LIF containing media. Both these cell culture protocols have the shortcoming that many of their components (i.e. FBS, bovine serum albumin or BSA, gelatin) are not defined and are animal-derived. FBS, for instance, contains various growth factors and other undefined components that promote ES cell growth, but it has also been suggested to contain potential differentiation factors that can affect mES cell plating efficiency, growth and differentiation. Therefore FBS batches need to be pre-screened and ES-qualified to ensure that the net-effect of serum factors that sustain mES cell maintenance and growth outweighs the effects of differentiation-inducing factors. Feeders in their turn secrete a plethora of factors impossible to control and are a possible source of pathogenic contamination.

Human PS (hPS) cells and their differentiated cells are most commonly cultured in the presence of surfaces or media containing animal-derived components, such as feeder layers (both mouse-derived, typically MEFs, and human-derived, typically human foreskin fibroblasts or HFFs), Matrigel® (soluble basement membrane extract of the Engelbreth-Holm-

Swarm EHS tumor), knock out serum replacement (KOSR) and/or derivatives like BSA. These animal-derived reagents added to the culture environment expose the cells to potentially harmful viruses or other infectious agents, which could be transferred to patients or compromise general culture and maintenance of the hPS cells. In addition, such biological products are vulnerable to batch variations, immune responses and limited shelf-life, and the exposure of the cells to molecules from other species also creates changes that could create an immune response in the recipient, if the cells were to be used in cell therapy.

There is still a need to understand all the different components necessary for the growth and maintenance of undifferentiated, non-mutated, pluripotent stem cells. It is important to get the right combination of extracellular matrix (ECM) and media factors for an optimal maintenance, especially for the human PS cell lines, otherwise the cells show low attachment, survival and proliferation rates, as well as high levels of differentiation.

For the sake of cell survival and proliferation rate, current protocols for cell culture still use FBS or derivatives such as BSA in the cell culture media and, thus, there is still need of an improved serum free protocol that does not compromise the cells, the culture conditions or the pluripotency.

Technology

The technology covers the novel use of IαI family proteins(s) or part(s) thereof, in particular HC2 (heavy chain 2), as a surface coating and/or media additive for cell adhesion and long-term culture, maintenance and growth of pluripotent stem cells for at least twenty passages, in the presence of partially or completely chemically defined media, without inducing noticeable differentiation or karyotype abnormalities.

The invention provides a method for stem or progenitor cell culture, which involves the addition of one or more protein(s) from the IαI protein family to a serum-free culture of cells. In one example, the cells are stem cells. The addition promotes self-renewal, attachment, survival and, in the case of PS cells, also pluripotency. The IαI proteins(s) or part(s) thereof are isolated from serum, produced as a recombinant protein, or synthesized chemically.

Additional Information

Pijuan-Galitó S, Tamm C, and Annerén C. Serum Inter-α-inhibitor activates the Yes tyrosine kinase and YAP/TEAD transcriptional complex in mouse embryonic stem cells. J Biol Chem. 2014 Nov 28;289(48):33492-33502

Material for evaluation

GEHC may be able to provide small quantities of materials for evaluation at cost and could connect licensees with potential manufacturers of IαI.