DERIVATION, CHARACTERIZATION AND DIFFERENTIATION OF FEEDER-FREE HUMAN EMBRYONIC STEM CELLS

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs Universitet kommer att offentligen försvaras i Hjärtats aula, Vita stråket 12, Sahlgrenska Universitetssjukhuset, onsdagen den 5 maj 2010 kl 13.00

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Avhandlingen baseras på följande delarbeten:

I. **Narmin Bigdeli**, Maria Andersson, Raimund Strehl, Katarina Emanuelsson, Eva Kilmare, Johan Hyllner and Anders Lindahl. Adaptation of human embryonic stem cells to feeder-free and matrix-free culture conditions directly on plastic surfaces. **J Biotechnol** 2008 Jan 1; 133(1):146-53.

II. **Narmin Bigdeli**, Giuseppe Maria de Peppo, Anders Lindahl, Maria Lennerås, Raimund Strehl, Johan Hyllner, Camilla Karlsson. Extensive characterization of matrix-free growth adapted human embryonic stem cells; a comparison to feeder cultured human embryonic stem cells. **In manuscript.**

III. **Narmin Bigdeli**, Giuseppe Maria de Peppo, Maria Lennerås, Peter Sjövall, Anders Lindahl, Johan Hyllner,Camilla Karlsson. Superior osteogenic capacity of human embryonic stem cells adapted to matrix-free growth compared to human mesenchymal stem cells. **Submitted to Tissue Engineering Part A.**

IV. **Narmin Bigdeli**, Camilla Karlsson, Raimund Strehl, Sebastian Concaro, Johan Hyllner, Anders Lindahl. Co-culture of human embryonic stem cells and human articular chondrocytes results in significantly altered phenotype and improved chondrogenic differentiation. **Stem Cells** 2009;27:1812–1821.

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ABSTRACT

Human embryonic stem cells (hESCs) are pluripotent cells with self-renewal ability, derived from the inner cell mass of a human blastocyst. They have the remarkable potential to develop into different cell types and can thus be used to regenerate and restore damaged tissues and organs in the entire body. Hence, hESCs are of great importance when it comes to future cell-based therapies. In addition, hESCs are also suggested as the ultimate source of cells for drug screening, functional genomics applications and studying early human embryonic development. Despite the recent advances in culture techniques for undifferentiated hESCs, there is a great need for further improvements until hESCs can be applied to human medical conditions. Since hESCs are traditionally cultured on feeder-cells or a coating replacing feeder-cells, some of the issues to address are a less laborious system, cost-effectiveness, culture stability, well-defined components, xeno-free culture conditions and compatibility with good manufacturing practice. In order to use hESCs in clinical applications, it is further highly important to also compare their differentiation capacity towards different tissues to that of other cells sources. This thesis report an improved culture technique of undifferentiated hESCs in which the cells can be cultured directly on plastic surfaces without any supportive coating. This technique supports the undifferentiated state of the cells, which are denoted matrix-free growth-hESCs (MFG-hESCs). To our knowledge, this is the first study presenting a coating independent culture technique of undifferentiated hESCs. The MFG-hESCs highly resemble feeder-cultured hESCs, retaining the undifferentiated morphology characteristic of hESCs and further grow as colonies in monolayer. In addition, these cells display a high expression of markers for pluripotency like Oct-4, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81 and differentiate into tissues of all three germ layers while retaining a normal karyotype. Further characterization and genome-wide expression analysis in comparison to feeder-cultured hESCs revealed that MFG-hESCs have the advantage of increased expression of integrins and extracellular matrix (ECM) proteins, which might be the key factor(s) explaining their attachment and growth on the plastic. Studying the osteogenic ability of MFG-hESCs compared to human mesenchymal stem cells (hMSCs) revealed a superior ability of the MFG-hESCs to form mineralized matrix. Further results pointed out that these two cell types use different signalling pathways for differentiation into the osteogenic lineage. Microarray analysis revealed that several genes involved in ossification are differently expressed in undifferentiated cells from these two cell types. Quantitative PCR showed that MFG-hESCs had a significantly higher expression of *OPN* during osteogenic induction while the opposite was true for *ALP, TGFB2, RUNX2* and *FOXC1*. We also report an efficient differentiation method for the generation of chondroprogenitor cells from hESCs. This method is based on direct co-culture of hESCs and chondrocytes. In contrast to hESCs, the co-cultured hESCs can be expanded on plastic. Those cells are further able to produce significantly increased content of cartilage matrix, both in high density pellet mass cultures and hyaluronan-based scaffolds. They further form colonies in agarose suspension culture demonstrating differentiation towards chondroprogenitor cells.

Taken together, this thesis reveals improved culture technique of undifferentiated hESCs avoiding feeder-cells and coating matrix, which promotes stable culture condition of hESCs and facilitates large-scale production, making expansion of hESC less laborious and time-consuming. This thesis also demonstrates the potential of the culture environment to influence differentiation of hESCs towards the mesodermal lineage. In addition, this thesis demonstrates osteogenic and chondrogenic differentiation of hESCs which further can be used in experimental studies like toxicology testing and drug screening, and the differentiation potential demonstrated suggests a potential use of hESCs in future cell therapies.

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