

Derivation, propagation and differentiation of human stem and progenitor cells

Akademisk avhandling

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av

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Avhandlingen baseras på följande delarbeten:

- I. Eva Sjögren-Jansson, **Mathilda Zetterström**, Karina Moya, Jenny Lindqvist, Raimund Strehl, and Peter S. Eriksson. "Large-Scale Propagation of Four Undifferentiated Human Embryonic Stem Cell Lines in a Feeder-Free Culture System". *Developmental Dynamics*, 233:1304–1314, 2005.
- II. **Mathilda Zetterström Axell**, Suzana Zlateva, Maurice A. Curtis. "A method for rapid derivation and propagation of neural progenitors from human embryonic stem cells". *Submitted*.
- III. Björn Carlberg*, **Mathilda Zetterström Axell***, Ulf Nannmark, Johan Liu, H. Georg Kuhn. "Electrospun polyurethane scaffolds for proliferation and neuronal differentiation of human embryonic stem cells".* **equal contribution**. *Biomed. Mater.* 4 (2009) 045004.
- IV. Maurice A. Curtis, Monica Kam, Ulf Nannmark, Michelle F. Anderson, **Mathilda Zetterström Axell**, Carsten Wikkelso, Stig Holtås, Willeke M. C. van Rooon-Mom, Thomas Björk-Eriksson, Claes Nordborg, Jonas Frisé, Michael Dragunow, Richard L. M. Faull, Peter S. Eriksson. "Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension". *Science*. 2007 Mar 2;315(5816):1243-9. Epub 2007 Feb 15.



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Abstract

Neuronal loss is a common feature of many neurological disorders, including stroke, Parkinson's disease, Alzheimer's disease and traumatic brain injury. Human embryonic stem cells (hESCs) and hESC-derived neural progenitors (NPs) may provide a number of new ways for studying and treating diseases and injuries in the brain. Studying the proliferation and differentiation characteristics of hESCs and NPs is important for three main reasons: 1, they represent an almost unlimited source of cells for neuron replacement therapies after neurodegeneration in the brain; 2, they are a good source of normal human cells for studying functional genomics, proteomics or for drug screening; and 3, they allow us to study early human brain development.

The general aims of this thesis were four-fold: 1, to develop efficient and simple methods for the large scale propagation of hESCs and hESC-derived NPs; 2, to optimise NP differentiation into mature neurons and glia; 3, to find suitable materials to promote migration and differentiation of stem and progenitor cells, and; 4, to uncover critical differentiation factors expressed in common between neuroblasts in the rostral migratory stream (RMS; the only long distance cell migration system in the human brain) and that of hESC-derived NPs.

To address these aims, we used a range of techniques including cell culture, morphometric analysis, immunocytochemistry, immunohistochemistry and RT-PCR.

Here we report the development of an improved method for the transfer and culture of undifferentiated hESCs in the absence of a cell feeder-layer, which is more cost effective and reduces the contact with murine feeder cells that render the hESCs unusable for future transplantation into humans. We have also developed a simple method for producing NPs from hESCs, suitable for large scale expansion and long term propagation of NPs. The production of large quantities of NPs allows us to readily compare the properties of NPs in culture to those in the human brain. Studying the differentiation of hESCs on permissive substrates has also been a focus and is of importance because of the relevance to the developing and adult human brain, where a complex extracellular matrix exists as scaffolding for neuronal development. We found electrospun fibrous scaffolds suitable for propagation and differentiation of hESCs, deriving predominantly tyrosine hydroxylase positive neurons indicating a dopaminergic fate. Finally, we studied the adult human brain for the presence of progenitor cells with migratory characteristics. We used a combination of serial sectioning, immunostaining and RT-PCR of human post-mortem brain material. This was the first study to reveal the presence of a human RMS by which neuroblasts migrate long distances from the subventricular zone to the olfactory bulb where they differentiate into mature neurons. Further, we discovered a number of differentiation factors expressed (Pax6, NCAM, DCX, β III-tubulin) in common between the human RMS neuroblasts and hESC-derived NPs. Taken together, this thesis reveals improved ways to propagate and differentiate hESCs in culture, and has uncovered common differentiation factors present in both human neuroblasts and NPs. These studies further our understanding of human brain development, allow large scale production of NPs for further study, and may one day be useful for treating central nervous system disorders.

Key Words: Human embryonic stem cells, neural progenitor cells, stem cells, differentiation, propagation, migration, cell culturing, rostral migratory stream, electrospun scaffolds

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