Maryam Hatami

Dr. sc.hum.

Title: Combination of Prox1/NeuroD1 Transcription Factor Overexpression Boosts Generation of Dentate Gyrus Granule Neurons from Pluripotent Stem Cells

Fach/ Einrichtung: Anatomie und Zellbiologie

Doktorvater: Prof. Dr. med. Thomas Skutella

ESCs have the capacity of unlimited self-renewal and pluripotency, which are promising tools ranging from basic research in developmental biology to future therapeutic applications. Following the establishment of basic techniques for ESC cultivation and neuronal differentiation, the main objective was to direct the differentiation towards specific types of neurons. For instance, our goal was to direct the differentiation of ESCs towards the DG granule neurons.

So far, two major reports have been published in regard to the hippocampal induction from ESC and IPS cells (Yu, Di Giorgio et al. 2014); (Sakaguchi, Kadoshima et al. 2015). Both strategies are based on the role of growth factors related to the hippocampus development. Initially, the online platforms GenePaint.org and Allen Brain Atlas as well as previous studies dealing with the cellular localization of both growth factors and transcription factors in the DG and molecular mechanisms contributing to DG differentiation were used as underlying scenario. Resulting from that the question arises which growth factor combination would be of stronger influence to the differentiation into DG granule neurons from mESCs.

The growth factor cocktail with DKK1 has proven to be more inductive in telencephalic neuronal progenitor cells and also more prone to the generation of highly enriched mouse DG progenitor-like colonies - which expressed DG markers such as Prox1, Neurod1 and Tbr2.

In the next part, the role of the transcription factors Emx2, Prox1 and NeuroD1 in the production and induction of DG granule neurons was investigated. A high expression of Emx2 suppresses the differentiation of telencephalic neuronal progenitor cells to DG progenitor cells and DG granule neurons, while NeuroD1 and Prox1 overexpression lead to a strong up-regulation of hippocampal progenitor markers.

The strongest granule cell differentiation expression profile was reached with the combined overexpression of PROX1 and NEUROD1. Morphological observations clearly demonstrate a considerable increase in neurogenesis. This phenomenon can be seen in other groups as well. A comparison of the growth factor effects with those of the transcription factors do show that the growth factors will lead to an increase in the induction of neuro-progenitor cells. By contrast, the transcription factors, and especially the combined prox1 and neurod1, pushed the cells to neuronal phenotype.

In summary, it can be concluded that the combination of central DG transcription factors mainly influences DG granule neuron differentiation in vitro. Furthermore, it became obvious that the combination of a DG specific growth factor cocktail and transcription factors will lead to a significant differentiation of DG granule neurons.