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Summary of PhD-thesis themed: "Transplantation of neural pre-differentiated embryonic stem cells in rats after experimental stroke"

Summary

The reduced regeneration potential of the central nervous system after stroke remains one of the greatest challenges for experimental and clinical neuroscience (*Björklund und Lindvall, 2000*). In this study we investigated the survival and developmental potential of pre-differentiated embryonic stem cells (ES cells) after transplantation in rats with experimental stroke. ES cells derived from the β-actin-EGFP mouse were in vitro predifferentiated into Nestin+ cells and transplanted 1 week after stroke. Cells were applied directly into the necrotic area and near the lateral ventricle. Survival and differentiation of the EGFP+ cells were assessed 4 weeks and 3 months after transplantation.

Ki-67 immunohistochemistry revealed a strong proliferation of transplanted cells within the first week. Four weeks after transplantation proliferation was strongly reduced and transplanted cells had started to differentiate into neurons and astrocytes as well as oligodendrocytes. NeuN staining revealed that about one third of transplanted cells developed into mature neurons, whereas 6-9 % expressed the astrocytic marker GFAP. Neurons derived from transplanted ES cells expressed various markers such as glutamate decarboxylase, DARPP32, cholin acetyltransferase or parvalbumin demonstrating differentiation into neuronal subtypes normally present in striatum and cortex. In contrast to their great differentiation capacities transplanted ES cells displayed only very limited

migration potential. Thus, if injected to the contralateral side the EGFP+ cells were not able to migrate towards the lesion side.

An additional goal of this study was the functional characterisation of ES cell-derived neurons. *Patch Clamp* analysis recorded typcial neuronal properties like inward sodium currents, outward pottasium currents, action potentials as well as EPSPs and EPSCs from EGFP+ cells. Thus, our study shows for the first time that pre-differentiated ES cells developed into mature and functional neurons after transplantation in rats with experimental stroke.

In contrast to other transplantation studies with ES cells we observed no macroscopic signs of tumorigenesis and no animal died from tumor formation. This is probably caused by the xenogeneic transplantation paradigm, since we transplanted cells from mice into rats. However, a longlasting rejection process was detected over a time period of 12 weeks after transplantation. This resulted in a complete diminishment of transplanted cells or a strong volume reduction of the graft after 12 weeks. Thus, tumorigenesis and graft rejection remain the greatest challenges for a possible clinical application of ES cell based transplantation approaches.