Directing stem cells and progenitors towards neuronal differentiation – implications for experimental therapies for Parkinson's disease

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ABSTRACT

The insight that stem- and progenitor-cells contribute to replacement of nerve cells in the adult central nervous system is the basis of modern therapies for structural brain repair. Their goal is to protect, support and stimulate endogenous stem cells in areas affected by disease and to replace lost cells by transplanting in vitro generated, tailored nerve cells.

In the present thesis growth factors and signaling molecules were investigated for their potential to direct stem- and neural progenitor- cells towards neuronal cell fate. Involved signaling pathways were characterized and candidate molecules identified that might be beneficial for cell-based therapies in Parkinson's disease.

Results show that Bone Morphogenetic Proteins (BMPs) and Growth Differentiation Factors increase astroglial differentiation while inhibiting oligodendrocyte maturation in rat embryonic mesencephalic culture. None of the factors protect dopaminergic neurons against oxidative radicals in vitro. BMP5, 6 and 7, however, promote dopaminergic differentiation by directly targeting the neuronal cell population.

In cultures of adult rat hippocampus-derived progenitors (AHPs), endogenous BMPs were found to increase undesired astroglial differentiation via the BMP type I receptors ALK6 and ALK2. By viral transduction of dominant negative ALK2 or ALK6, respectively, BMP signaling was blocked in order to inhibit astroglial cell differentiation. Indeed, the expression of glial fibrillary acidic protein (GFAP), a marker for astrocytes, decreased. The number of oligodendrocytes increased and neurons were not affected. However, the strategy proved impractical since it induced cell death. RT-PCR results indicate that only the ALK6, but not the ALK2 receptor, is dynamically regulated in these cultures, suggesting that ALK6 is mainly responsible for glial differentiation and survival of AHPs.

Apoptosis signal-regulating kinase-1 is a ubiquitously expressed enzyme involved in apoptosis. Overexpression of its constitutively active form induced neuronal differentiation in AHP culture. At the same time GFAP expression was inhibited. The effect is mediated via p38 mitogen-activated protein kinase and via inhibition of GFAP promoter activity.

In order to generate transplantable dopaminergic neurons, human embryonic stem cells (hESCs) were cocultured with the stromal cell line PA6, known to instruct mouse and primate ESCs towards dopaminergic cell fate. About 11% of hESCs developed into tyrosine hydroxylase-positive (TH-pos) neurons with CNS identity. The hESC-derived neurons displayed action potential in vitro. However, they did not induce behavioral recovery after transplantation to the 6-hydroxydopamine -lesioned rat striatum. Extended differentiation time on PA6 in vitro decreased the risk for teratoma formation after transplantation, but did not elevate the low number of TH-pos neurons in the graft.

In conclusion, certain BMPs as well as ASK1 have been identified as molecules that increase neuronal differentiation. Their putative role in experimental CNS cell therapies is discussed. At the moment, however, the gap between experimental systems and biological reality is difficult to overcome. Further investigations that are necessary to reduce safety concerns in cell-based treatment strategies are outlined.

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I. **Brederlau A**, Faigle R, Kaplan P, Odin P, Funa K. Bone morphogenetic proteins but not growth differentiation factors induce dopaminergic differentiation in mesencephalic precursors. *Mol Cell Neurosci* 2002, 21(3):367-378.

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