Embryonic stem cells for production of nerve myelinating cells and transplantation in dysmyelinated brain

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Development of Oligodendrocytes

In the embryonic neural tube, neural stem cells (NSC) give rise to neurons and to glial cells, among which oligodendrocytes that myelinate nerves in the central nervous system (CNS). Differentiation of oligodendrocytes proceeds through successive stages: 1) bipolar early progenitors, 2) multipolar oligodendrocyte precursors (OPC), 3) highly branched (arborized) pro-oligodendrocytes, and 4) mature cells forming myelin membrane sheaths. This complex developmental program can be reproduced by in vitro NSC cultures. NSC can be found in embryo or adult brain, but a more convenient and large-scale source would be pluripotent Embryonic Stem (ES) cell lines that have been established from blastocysts of mouse and human origin.

In vitro differentiation of oligodendrocytes from ES cells: Aims

Human ES cells could provide OPC populations for transplantation aimed at repairing the myelin in brain or spinal cord regions where myelin has been destroyed due to pathological processes or traumas. As important would be to find therapeutic agents

Fig. 1 Differentiation of oligodendrocytes from Embryonic Stem (ES) cells. A. Murine ES cells-derived mature oligodendrocytes stained for O4 sulfatide (green) are seen contacting neurons stained for βIII-tubulin (red). B. Human ES cells-derived oligodendrocytes (O4+), with elongated branches (Inset).
oligodendrocyte-specific O4 sulfatide shows multipolar cells, removed for differentiation. In 3-9 days, immunostaining for with FGF2, EGF and PDGF, and the growth factors are be stored frozen. NSC grow on adherent cationic substrates (neurospheres), from which we established NSC lines that can a two-week selection causing proliferation of NSC clusters (Fig. 1A). Murine ES cells are subjected to murine ES cells: role of IL6RIL6 MS.

or transplanting new myelinating cells could be of benefit in study if enhancing remyelination, protecting oligodendrocytes exhaustion of NSC) leading to neuronal loss. It is of interest to but this capacity is progressively exhausted (probably due to disability in MS. Remyelination occurs at the early stages of MS, for reducing relapse rate and progression of neurological of human Interferon-beta (Rebif®, Serono) widely used today and spinal cord and our previous work led to the development autoimmune process causes demyelinating lesions in the brain and spinal cord and our previous work led to the development of human Interferon-beta (Rebif®, Serono) widely used today for reducing relapse rate and progression of neurological disability in MS. Remyelination occurs at the early stages of MS, but this capacity is progressively exhausted (probably due to exhaustion of NSC) leading to neuronal loss. It is of interest to study if enhancing remyelination, protecting oligodendrocytes or transplanting new myelinating cells could be of benefit in MS.

**Fig. 2 Brain remyelinating capacity of oligodendrocytes precursor cells (OPC) derived from murine ES cells is stimulated by IL6RIL6. Neurosphere cells were cultured 3 days with FGF2 and EGF, then 1 day without growth factors (A) or the same with IL6RIL6 (B), and then transplanted on organotypic cultures of shiverer mouse brain slices. After 15 days, brain sections were stained for MBP (red). Inset: transplantation site in the hippocampus region (H) and area examined (rectangle). Bar graph: extent of MBP staining without (white) or with IL6RIL6 (black).**

that enhance differentiation, survival and myelinating capacity of oligodendrocytes, and could be administered to protect or regenerate the functions of the cells affected by disease.

Genetic diseases resulting in the loss of myelin (leukodystrophies, vanishing white matter) cause mental retardation or early death. An experimental model to test the benefits of OPC transplantation is provided by homozygous shiverer mice (shi-/-) having a deletion in the myelin basic protein (MBP) gene, and suffering from dysmyelination, tremors and a short life span. In multiple sclerosis (MS), an autoimmune process causes demyelinating lesions in the brain and spinal cord and our previous work led to the development of human Interferon-beta (Rebif®, Serono) widely used today for reducing relapse rate and progression of neurological disability in MS. Remyelination occurs at the early stages of MS, but this capacity is progressively exhausted (probably due to exhaustion of NSC) leading to neuronal loss. It is of interest to study if enhancing remyelination, protecting oligodendrocytes or transplanting new myelinating cells could be of benefit in MS.

**Differentiation of mature oligodendrocytes from murine ES cells: role of IL6RIL6**

We developed a procedure converting ES cells into mature oligodendrocytes (Fig. 1A). Murine ES cells are subjected to a two-week selection causing proliferation of NSC clusters (neurospheres), from which we established NSC lines that can be stored frozen. NSC grow on adherent cationic substrates with FGF2, EGF and PDGF, and the growth factors are removed for differentiation. In 3-9 days, immunostaining for oligodendrocyte-specific O4 sulfate shows multipolar cells, then arborized cells and then myelinating cells (MBP). We found a strong enhancement of the differentiation by adding a recombinant cytokine made by fusing IL-6 to its soluble IL-6 receptor (IL6RIL6) and acting as a potent ligand of gp130, the common receptor of the IL-6 family. IL6RIL6 produces a marked increase in the size of the O4+ cells, in the number of branches and in their length. Moreover, IL6RIL6 is essential to observe accumulation of MBP in peripheral branches and in myelin membrane sheets, indicating maturation of myelinating cells.

**IL6RIL6 activates the gene for Stathmin-2, a regulator of microtubule dynamics**

Gene expression profiling by DNA microarrays (Affymetrix) revealed a number of genes either increased or decreased by IL6RIL6 treatment of NSC. One of the genes induced to high-level of expression by IL6RIL6 was Stmn2 encoding the Stathmin-like-2 (SCG10) protein, which is an inhibitor of tubulin polymerization regulating microtubule extension. Silencing of Stmn2 by siRNA demonstrates that this IL6RIL6-induced gene is essential for oligodendrocyte development: when the Stmn2 gene is knocked down, the cells develop long and thick processes without sub-branches. Moreover, the MBP is not distributed in the branches, suggesting that Stmn2 may be necessary for the transport of the MBP mRNA along the microtubule. Our strategy is now to identify additional genes involved in oligodendrocyte differentiation by using microarray analysis combined with gene knock-down (or conversely overexpression) with the aim to find ways to further enhance myelination.

**Differentiation of oligodendrocytes from human ES cells**

Potential medical applications would require effective means to derive oligodendrocytes from human ES cells. With human ES cell lines from Prof. Joseph Itskovitz-Eldor (Technion, Haifa), we found a method producing numerous oligodendrocytes (Fig. 1B). This procedure effectively extinguishes expression of genes specific of undifferentiated ES cells (e.g. Oct-4) while switching on the lineage-specific genes.

**Increased myelinating capacity of ES cell-derived neurosphere cells in shiverer brain**

Since the brain of shi-/- mice lacks MBP, any MBP staining after transplantation is proof of remyelination. Transplanted cells must migrate into the brain tissue and mature oligodendrocytes migrate minimally. Hence we chose ES cell-derived NSC cultured only 4 days (to the OPC stage) and examined the effect of IL6RIL6. To allow accurate comparison of different cell preparations, we adapted as transplantation model organotypic cultures of shi-/- brain slices (400 μm thick). OPC were placed in the hippocampus region (H in Fig. 2 inset) and have to migrate into adjacent entorhinal cortex (E.C.) where nerves are normally highly myelinated. As seen in Fig. 2, strong remyelination was observed with IL6RIL6 treatment (panel B). IL6RIL6 significantly increased the length and number of remyelinated nerve fibers, and the MBP mRNA surface (Fig. 2, bars). Similarly, remyelination by human ES-NSc and stimulation by IL6RIL6 were observed in organotypic slices as well as after in vivo transplantation to newborn shi-/- mice by intraventricular injection.
Peripheral nerve myelination: effect of IL6RIL6 on Schwann cell differentiation

Myelination of peripheral nerves is impaired in neuropathies caused by diabetes or by chemotherapy, and administration of IL-6 has shown protective effects on nerve function and on myelination in rat models of neuropathies. We continue to study the role of IL-6 signaling on Schwann cells (SC). We previously showed that the gene encoding the major myelin protein of peripheral nerves, myelin protein zero (MPZ/Po), is induced by IL6RIL6 and we defined sequences in the Po gene promoter that respond to the IL6RIL6 stimulus. Transcription factor ZBP-99 was identified as binding to one of the sequences, which is flanked by Sox-10 binding sites. By chromatin immunoprecipitation (ChIP) in rat primary Schwann cells, we can show that IL6RIL6 treatment recruits both ZBP-99 and Sox-10 to the promoter. ZBP-99 gene knock-down by siRNA leads to loss of Po protein in the SC, demonstrating that ZBP-99 is an essential factor for myelination. Microarray analysis reveals that ZBP-99 is also involved in MBP gene expression and has multiple gene targets in SC. We investigate the functions of ZBP-99 in SC as well as in oligodendrocytes, where knock-down of ZBP-99 also affects differentiation.

Selected publications

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