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CHAPTER 2

APPLICATION OF HUMAN OLIGODENDROCYTES IN (RE)MYELINATION RESEARCH

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ABSTRACT

Studies on myelination and oligodendrocyte development are inevitably linked with demyelinating conditions such as multiple sclerosis (MS), leukodystrophies or spinal cord injury (SCI). Chronic loss of myelin, subsequently leading to neurodegeneration, is the ultimate cause of severe and permanent disability. Thus, fast restoration of myelin (remyelination) is essential for circumventing demyelination-caused pathologies. Implantation of exogenous remyelinating cells has been considered as a potential remyelination strategy. Researchers have examined a variety of cell types endowed with myelin-forming capacity (oligodendrocytes, Schwann cells, olfactory ensheathing cells etc.) *in-vitro* and *in-vivo* for their potential application as myelin restoring cell grafts. This review gives a summary of studies on the generation and testing of pure suspensions of **human** oligodendrocytes as a clinically relevant, efficient cellular tool for treating myelin pathology. We start with a brief overview of the current knowledge on the development of human oligodendrocytes from the late stages of embryogenesis up to the early postnatal stage. Insight in the specific extrinsic and intrinsic factors regulating normal oligodendrogenesis is crucial in order to achieve and maintain a sufficient population of engraftable functional oligodendrocytes *in-vitro*. We discuss potential sources of human oligodendrocytes, including novel oligodendrocyte generation strategies employing induced pluripotent stem cells (iPSCs) and direct conversion technology. Finally, we provide a systematic overview of (the outcome of) experimental studies, in which human oligodendrocytes were tested for their (re)myelination capacity and efficiency.

INTRODUCTION

Myelination of central nervous system (CNS) tracts has long been recognized only as the prerequisite for rapid, saltatory transduction of action potentials along axons. In recent years, however, additional properties of myelin have been discovered, most of them associated with neuronal support. Myelin sheaths surrounding axons were identified to protect neurons with trophic, metabolic and structural support [1]. In myelinated axons, energy-consuming transmembrane ion pumps are confined to the small nodal areas; it is hypothesized that they have a much lower energy demand than unmyelinated ones and are less sensitive to oxidative stress [2], [3]. Loss or dysfunction of myelin will eventually lead to axonal and (retrogradely) neuronal degeneration as can be observed in chronically demyelinated MS lesions [4]. In this respect, myelin dysfunction has been recently indicated as a potential contributor to motor neuron degeneration in amyotrophic lateral sclerosis (ALS) [5].

Fast remyelination is the best option to stop degeneration of injured axons, to restore the lost saltatory conduction and to provide long-term neuronal survival. Spontaneous endogenous remyelination has been shown to be very efficient in animal models and in early stages of demyelinating disorders [6]. In chronic demyelinating diseases such as MS, endogenous remyelination by oligodendrocyte precursor cells (OPCs) eventually fails. Grafting of exogenous remyelinating cells might be a suitable treatment strategy in that case. Apart from MS, this may also apply for various white matter disorders (WMD), pediatric (e.g. periventricular leukomalacia in premature infants or hereditary leukodystrophies) as well as adult types. Most WMDs are primarily characterized by dysfunction of glia (oligodendrocytes or astrocytes or both), failure to form or restore myelin, formation of abnormal myelin, or myelin loss (demyelination) with axonal damage typically occurring secondarily. These WMDs primarily require replacement of glia (oligodendrocytes or astrocytes or both), preferably early in the disease course, before axonal loss is extensive and irreversible.

The aim of this review is to provide a comprehensive overview of *in-vitro* and *in-vivo* studies employing human exogenous oligodendrocyte precursor cells (OPCs) for myelin (re)generation.

OLIGODENDROCYTE DIFFERENTIATION *IN-VIVO*

During development of the CNS in mammals, oligodendrocytes originate from neural progenitor/stem cells in specific, well-defined domains and the time course of their appearance is tightly controlled. Most of our knowledge of this process comes from observations and research conducted in rodents. In the developing mouse nervous system, the first oligodendrocyte progenitor

cells arise around embryonic day 12.5 (E12.5) from the ventral region of the neural tube, the so-called pMN domain [7], [8]. This domain is also responsible for giving rise to motor neurons earlier in embryonic development. The switch from generation of motor neurons to OPCs occurs mainly due to the temporal establishment of morphogen gradients across the developing spinal cord, of which Sonic hedgehog (Shh) and bone morphogenetic protein (BMP) play a central role (Shh as a positive and BMP as a negative regulator of OPC emergence) [9], [10]. Exposure to a specific combination of these factors induces the expression of oligodendrogenic transcription factors (see below). Around embryonic day 15, OPCs originating from the pMN domain manage to populate the vast majority of the neural tube [11], [12]. A second, less abundant wave of OPCs arises from the dorsal compartment of the neural tube in a Shh-independent manner [12], [13]. The first signs of OPC maturation into oligodendrocytes and myelination can be observed around E17 and these processes continue after birth [14], [15]. In the developing brain, on the other hand, we can observe three waves of oligodendrocyte development. The first one emerges from the ventral telencephalic region called the medial ganglionic eminence (MGE) at E12.5, the second one arises from the lateral ganglionic eminence (LGE) around E15 and the third one develops from cortical brain regions only after birth [16]. This third OPC wave is the most abundant and eventually gives rise to most of the oligodendrocytes present in the adult brain [17].

Oligodendrocyte lineage specification in human shares many features with that in rodents. Similarly, there are two waves of oligodendrocyte development occurring in the neural tube [18], [19] and three in the telencephalon [20]. Also the process of human oligodendrocyte differentiation from an early progenitor cell to a fully mature myelin-forming oligodendrocyte is similar to that in rodents. The major difference between the two species is the time course of this process. While it takes only around 3 weeks for mouse OPCs to develop, maturate and start myelination, in human this process can take up to 22 weeks. Human OPC specification starts around 7.5 weeks of gestation (Buchet et al., 2011). These cells become functional not earlier than around 11 weeks of gestation in the spinal cord [22] and 30 weeks of gestation in the forebrain [23] when the first myelinated axons can be detected.

As mentioned before, in both species the course of oligodendrocyte differentiation follows the same pattern and can be divided into several, well-defined stages each characterized by the response to specific external factors, by the expression of a specific set of transcription factors and protein markers and by a specific cell morphology (Figure 1). In the spinal cord, the vast majority of early OPCs originate from neural precursor cells (NPCs) which express the transcription factors Olig2 and Ngn2 and can develop into both

oligodendrocytes and motor neurons [24], [25]. In response to Shh signaling, Ngn2 is downregulated and another transcription factor, Nkx2.2, starts to be expressed within the pMN domain [26], [27]. Co-expression of Olig2 and Nkx2.2 determines the oligodendrocytic fate of the differentiating cell [28]. The central role in this "neuron-OPC switch" has been recently ascribed to changes in the phosphorylation status of Olig2 [29]. OPCs are small, proliferating, bipolar or multipolar cells endowed with a high migratory potential. Besides the transcription factors mentioned earlier, they express among others Olig1 and Sox10, as well as a panel of membrane-bound markers: A2B5, platelet-derived growth factor receptor alpha (PDGFRα) and, at later developmental stages, proteoglycan NG2 [30]–[32]. As differentiation progresses, they lose their migratory and proliferative capacity and their morphology becomes more complex with numerous, long extensions. These so-called pre-myelinating oligodendrocytes can be identified with an array of markers such as O4, 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) and galactocerebroside (GalC) [33], [34]. Fully mature oligodendrocytes enwrap axons with compact, multilayer membrane sheaths, the myelin, in a process called myelination. At this stage, cells express specific myelin proteins i.e. myelin basic protein, myelin associated glycoprotein, myelin oligodendrocyte glycoprotein and myelin proteolipid protein (MBP, MAG, MOG and PLP), involved in the structural integrity of myelin [35], [36]. Interestingly, Olig1 transcription factor is redistributed from the nucleus to the cytoplasm at this final stage of oligodendrocyte development [37], [38].

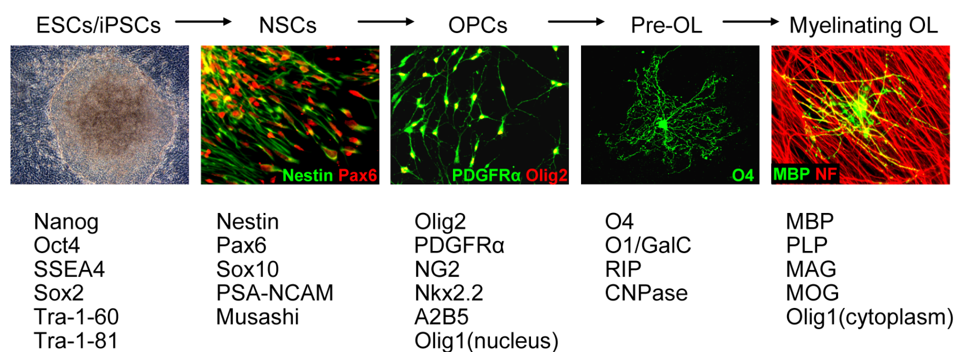

Figure 1. Stages of oligodendrocyte differentiation from pluripotent stem cells (iPSCs/ESCs) and markers commonly used for their characterization. Note, some markers might be expressed also in adjacent differentiation stages. ESCs/iPSCs: typical morphology of human embryonic stem cell/induced pluripotent stem cell colony; NSCs, OPCs, Pre-OL, Myelinating OL: Immunofluorescent stainings of neural stem cells, oligodendrocyte precursor cells, pre-oligodendrocyte and myelinating oligodendrocyte respectively.

POTENTIAL SOURCES OF HUMAN OPCs FOR MYELINATION STUDIES

For cell-based remyelination research, several sources of human OPCs have been proposed: OPCs can be isolated directly from embryonic human tissue or derived from neural stem cells (NSCs) isolated from embryonic human brain; they can be generated from human embryonic stem cells (hESCs) (commonly differentiated via an NSC stage) and more recently from human induced pluripotent stem cells (hiPSCs). We will present current strategies to induce the differentiation of stem cells into OPCs. An interesting, novel alternative might be the use of direct cell conversion technology for the generation of OPCs directly from somatic cells. Each of these possible cell sources has its specific advantages and disadvantages that will be shortly discussed within this paragraph.

OPCs ISOLATED FROM HUMAN TISSUE

OPCs isolated directly from human tissue have hardly been used in myelination experiments. Nonetheless, the rationality of such an approach has been first described by Gumpel et al. in 1987. In this study, however, the authors did not intend to isolate a pure population of OPCs but instead used small fragments of human embryonic brain for transplantation. Still, human OPCs migrating out from these fragments managed to differentiate and myelinate axons in a hypomyelinated mouse model [39], [40]. More recently, a number of papers were published describing in detail the procedure of OPC isolation from human tissue [41]–[44]. The major restriction of such an approach is certainly the limited availability of embryonic human tissue for OPC derivation. Another problem is the great heterogeneity of samples obtained from different human donors and different fetal ages making comparison of results very difficult. Moreover, knowing the limited proliferation potential of isolated OPCs *in-vitro*, the acquisition of a sufficient number of cells for experimental studies may be troublesome. Another, more frequently used source of human oligodendrocytes for myelination research are tissue-derived NSCs. NSCs with oligodendroglial lineage differentiation potential have been isolated from multiple fetal CNS regions including forebrain [45]–[47], subcortical white matter [48], cortex [49], [50], ganglionic eminences [51] and spinal cord [52]–[55], as well as adult postmortem brain samples [56]–[58]. These cells can be relatively easily ex-panded *in-vitro* as monolayer or in the form of floating cultures (so-called neuro-spheres) in the presence of specific growth factors such as FGF2 and EGF [46], [52], [59]–[61]; in some cases LIF [46], [52], [61]–[63] and PDGF [57], [59], [64] are also added to the medium. Importantly, human NSCs amplified *in-vitro* retain their multipotency, i.e. the ability to give rise to neurons, astrocytes and

oligodendrocytes, even after long-term culturing [46], [50], [65], [66]. Oligodendrocyte differentiation of the human NSCs is commonly achieved by growth factor withdrawal [33], [46], [47], [51], [67]–[70] or/and supplementation with a panel of induction and growth factors including Shh, PDGF, T3, NT-3, BDNF, CNTF and IGF-1 [47], [50], [68], [70]–[72]. An alternative approach for oligodendrocyte fate induction from NSCs has been recently presented by Wang et al. After comparing the gene expression profile of FACS-sorted human NSCs and OPCs, they came up with a panel of transcription factors that are significantly upregulated during OPC fate acquisition. Overexpression of one of these factors, Sox10, in human NSCs resulted in their oligodendrocyte commitment [73].

In most transplantation experiments, human NSCs are indeed differentiated into OPCs, or at least into glia restricted precursors. Yet, in some experimental set-ups multipotent NSCs have been used [21], [52], [54], [71], [74]–[80]. In these cases, experimental results largely rely on the intrinsic (default) NSC differentiation program. An interesting example of such a study is provided by Brustle et al., in which human NSCs were implanted into the ventricle of embryonic rat brains. The human cells appeared to incorporate into all major compartments of the host cerebrum and differentiated into neurons, astrocytes and oligodendrocytes, generating animals with CNS chimerism, a model for studying human neural development in a functional nervous system [81]. Unfortunately, derivation of NSCs from human tissue suffers from the same drawbacks as previously described for derivation of OPCs, i.e. limited accessibility to human samples and a vast heterogeneity. Yet, the relatively uncomplicated large-scale expansion of NSCs *in-vitro* should be considered a great advantage.

OPCs DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Since their first successful isolation in 1998 [82], human embryonic stem cells (hESCs) held great promise for fundamental research as well as for potential clinical applications in cell-based therapies. As an almost unlimited source for all kinds of differentiated cell types, they were considered the solution for the restricted access to human tissue-derived material/cells. With time, various strategies and protocols for the generation and differentiation of specific cell types from hESCs emerged, including cells of oligodendrocytic lineage. Up to date, several protocols have been established for the generation of oligodendrocytes from hESCs [68], [69], [83]–[86]. Strikingly, despite the common endpoint, different approaches were used among these protocols, each of them recapitulating the *in-vivo* conditions during embryonic and postnatal oligodendrogenesis (Figure 1). They reflect the fact that there are at least three

different waves of oligodendrocyte development *in-vivo* each characterized by distinct inductive stimuli from the environment of the neural stem cells. For instance, protocols proposed by Nistor et al. [83] and Izrael et al. [69] do not include Shh at any stage during differentiation pointing towards a more "dorsal origin" (Shh-independent) type of oligodendrocytes produced with their approach. Moreover, combinations of growth factors and morphogens used at specific differentiation checkpoints differed remarkably. In some protocols, certain differentiation stages with their own specific requirements of growth- and induction factors were not distinguished or recognized. Additionally, there appeared to be considerable differences in the time course of OPC differentiation of hESCs, varying from 25 days in the shortest [84] to 98 days in the longest scenario [85].

Indeed, hESCs may serve as a very adequate source of oligodendrocytes for basic research and transplantations. However, the actual use of these cells comes with considerable drawbacks. First of all, in many cases the oligodendrocyte differentiation procedures are very long and complicated; often they appear to be difficult to reproduce in other labs, although this difficulty might, at least in part, originate from intrinsic differences between the used hESC lines. Apart from the ethical concerns associated with hESCs, significant problems with their clinical use in future transplantations may arise from the fact that grafts of OPCs derived from hESCs will be allogenic, introducing a significant risk of graft rejection and hence the need for life-long immunosuppressive treatment.

The solution for the latter has come from a groundbreaking, Nobel prize winning development in stem cell research, the generation of human induced pluripotent stem cells (hiPSCs) [87]–[91]. iPSCs are equivalents of ESCs and are derived from somatic cells by forced expression of specific pluripotency-related transcription factors. Although the original use of integrative viral vectors for reprogramming and the need for (xenogenic) feeder layers for cell culturing were thought to hamper the potential clinical use of patient-derived iPSCs, the latest advancements in reprogramming now allow generation of xeno-free, zero-footprint hiPSCs without any genomic integrations of foreign DNA (hiPSCs generation strategies reviewed in [92]). hiPSCs may serve as an excellent autologous source for human oligodendrocytes, especially for disease modeling and transplantation studies (Figure 2). Several laboratories have reported the differentiation of oligodendrocytes from hiPSCs. In some cases oligodendrocytes were just a minor "byproduct" of differentiation into other CNS cell types (mostly NSCs or various types of neurons) [93]–[95]. A few research groups, however, particularly focused on establishing specific and effective protocols for the differentiation of hiPSCs into functional oligodendrocytes [70], [96]–[99] though with varying degrees of success. In the first documented attempt to

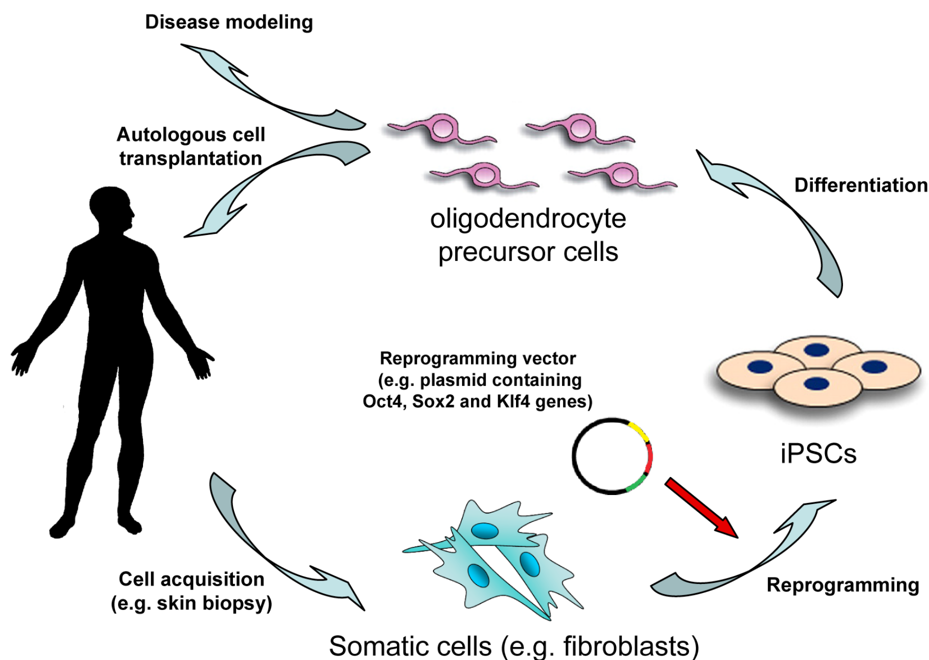

Figure 2. Potential use of patient-specific iPSCs for MS treatment and disease modeling. Somatic cells (e.g. fibroblasts) are acquired from MS patient and reprogrammed into iPSCs. Following differentiation, iPSCs-derived OPCs may be utilized as autologous grafts, or used for disease modeling *in-vitro*.

generate OPCs from hiPSCs, based on the protocol of Nistor et al. designed for hESCs [83], Ogawa et al. reported differentiation efficiencies as low as 0.01% [96]. Pouya et al. in contrast, while using a similar differentiation strategy, claimed an efficiency of ~80% for generating cells expressing OPC markers such as NG2 and PRGFRα [97], though their immunohistochemical evidence for that appeared to be inconclusive. The first truly successful generation of oligodendrocytes from hiPSCs can be ascribed to Wang et al. [98]. Utilizing a differentiation strategy adapted from another hESC-based protocols [69], [85], they not only managed to produce OPCs with a high efficiency (up to 79% based on Olig2/Nkx2.2 double-staining), but also demonstrated the functionality of these oligodendrocytes after transplantation into hypomyelinated animals [98]. Recently, a further optimized version of this protocol has been developed by Douvaras et al., who used it to generate oligodendrocytes from iPSCs derived from primary progressive MS (PPMS) patients [100].

Although hiPSC-oriented research carries a great promise for autologous cell replacement therapy, a number of issues still remain to be solved before introducing this new technology into clinical use. Firstly, the absolute safety of the current hiPSC production methods needs to be proven and established.

Additionally, ways to speed up the differentiation protocol for human OPCs need to be developed, since the present duration (in total ~150 days [98] or ~75 days [100] provides severe practical and logistical concerns. Eventually, the protocol needs to comply with the current good manufacturing practice (GMP) standards, entirely avoiding the use of xenogenic products throughout the procedure. Ultimately, contamination with undifferentiated hiPSCs should be excluded by stringent purification steps and verified before actual transplantation into a patient to eliminate the risk of teratoma formation.

Another method to obtain oligodendrocytes from human cell sources might come from the development of direct cell conversion technology. Two studies demonstrated the possibility to convert mouse somatic cells directly into OPCs using forced expression of specific transcription factors [101], [102]. Importantly, these directly converted OPCs behaved like normal primary OPCs, showing a similar gene expression profile and similar ability to myelinate axons *in-vitro* and *in-vivo* [101], [102]. Direct conversion technology is a relatively novel approach for generating clinically relevant cell types and the long-term functionality and stability of the converted cells need to be established. Safe, non-integrating, more efficient direct conversion methods need to be developed and most importantly, the applicability of this technique needs to be demonstrated for human OPCs.

APPLICATION OF HUMAN OLIGODENDROCYTES IN (RE)MYELINATION RESEARCH

The most prominent aspect of oligodendrocyte physiology during development is their ability to form compact myelin sheaths around axons. For regenerative medicine, however, the capacity of the cells to restore myelin within areas of demyelination, caused either by an acute insult (e.g. spinal cord injury), a chronic disorder (e.g. MS) or in WMDs, might be of even greater interest. In many pathological cases, rapid and efficient remyelination needs to be assured in order to rescue affected axons, prevent retrograde neuronal degeneration and with that irreversible disability. This goal can be achieved either by stimulating endogenous remyelination (reviewed elsewhere in details [103], [104]) or by grafting exogenous remyelinating cells (reviewed in [105], [106]). The latter strategy requires extensive characterization of the cell populations to be grafted with all available laboratory techniques and analysis methods, before one may proceed to a clinical trial with human subjects. Researchers have a number of model systems, both *in-vitro* and *in-vivo*, at their disposal to test the major functional aspects of oligodendrocyte physiology, i.e. migration, differentiation and (re)myelination. In the following paragraphs, examples of studies will be described that characterize human oligodendrocytes (tissue-isolated and stem cell-derived) in various model systems.

IN-VITRO MODEL SYSTEMS FOR MYELINATION STUDIES

Various model systems have been developed for studying (re)myelination *in-vitro*: co-cultures of oligodendrocytes with dorsal root ganglion (DRG) [107], [108] or CNS neurons [109], brain slice cultures from wild-type and mutant mice [110], [111], or CNS spheroids [112]. Oligodendrocytes were also shown to be able to enwrap artificial materials such as carbon [113], glass [114] and vicryl microfibers [115], polylactic acid nanofibers [116], or even paraformaldehyde-fixed axons [117] demonstrating that electrical activity of neurons, although beneficial, is not mandatory for myelination. While the vast majority of literature utilizing these models concerns rodent OPCs, also human oligodendrocytes have been tested for their myelination capacity *in-vitro* in several studies listed in table 1.

IN-VIVO EXPERIMENTS UTILIZING HUMAN OLIGODENDROCYTES

The functionality of human OPCs *in-vivo* has been thoroughly studied using numerous animal models. A list of *in-vivo* experiments with human OPCs derived from various sources is assembled in table 2.

A vast majority of the *in-vivo* experiments did confirm the applicability of human oligodendrocyte transplantation, demonstrating that human OPCs were able to survive, migrate and differentiate into mature functional oligodendrocytes after implantation. In many cases, formation of thick, compact myelin by transplanted cells was observed, accompanied by long-term beneficial effects on the “diseased” phenotype (table 2). Additionally, an interesting, myelination-independent effect of NSC/OPC transplantation was identified in EAE models, where significant alleviation of disease symptoms could be attributed to indirect, immunomodulatory/neurotrophic activity of grafted cells, presumably through secretion of specific bioactive factors [79], [134], [135].

Despite the promising outcome of most of the cited transplantation studies, the actual relevance of the used animal models for a specific human demyelination disorder and the extrapolation of findings from these studies to potential clinical application should be critically considered. The newborn shiverer mouse and other newborn transgenic mice may mimic various pediatric leukodystrophies (reviewed in [137] but apparently have less significance for studying MS-related demyelination and loss of myelin due to spinal cord injury in adults. More studies in novel, relevant, adult animal models are required for that, mimicking as closely as possible the specific characteristics, disease processes and myelin pathologies of the adult demyelination disorder under investigation.

Table 1. Examples of (re)myelination studies with human oligodendrocytes *in-vitro*.

Cell source	Cells used	Model system	Outcome (findings)	Year/Ref.
hESCs	hESCs-OPCs	Coculture with rat fetal hippocampal neurons	- Myelination: EM and ICC-confirmed	2007/[68]
hESCs	hESCs-OPCs	Coculture with shiverer organotypic cerebral slices	- Compact myelin formation: EM and ICC-confirmed - Noggin pretreatment markedly enhances myelination	2007/[69]
Human CNS	Fetal OPCs (endogenous)	Human forebrain organotypic slice cultures	- Axonal PSA-NCAM downregulation precedes myelination in human CNS	2007/[118]
hESCs	hESCs-(NG2+)OPCs	Coculture with hESC-derived neurons	- MBP-positive wraps formed around axons: Based on ICC and EM	2010/[86]
Human CNS	Fetal and Adult OPCs	Coculture with shiverer organotypic cerebral slices	- Myelin formation by human OPCs (limited): ICC-confirmed	2012/[119]
Human CNS	Fetal A2B5+OPCs	Coculture with rat DRG neurons	- Human Oligodendrocytes ensheath rat DRG axons: based on ICC - OPCs isolated from later gestation stages produce more myelin	2012/[120]
hiPSCs	hiPSC-OPCs	Coculture with human fetal cortical neurons	- Initiation of myelination based on ICC (myelination confirmed *in-vivo* – see table 2)	2013/[98]
hESCs	hESCs-OPCs	Coculture with hESC-derived neurons	- Efficient differentiation of OPCs from hESCs at low oxygen pressure - Oligodendrocyte differentiation enhanced by RXR agonist - Signs of axonal enwrapment by MBP-oligodendrocytes: ICC	2013/[121]

DRG – dorsal root ganglion; EM – electron microscopy; hESCs-OPCs – hESC-derived OPCs; ICC - immunocytochemistry; RXR – Retinoid X receptor; PSA-NCAM – polysialylated neural cell adhesion molecule

Table 2. (Re)myelination studies with human oligodendrocytes *in-vivo*.

Cell source	Cells used	Model system	Outcome (findings)	Year/Ref.
Human CNS	Embryonic brain fragments (1-2mm)	Newborn shiverer mouse	- Good integration of the grafts - Myelination in multiple brain regions: (IHC and EM)	1987/[39]
Adult human CNS	White matter cells: 51% OL, 3% preOL, 1% OPCs	Ethidium bromide driven demyelination in rat spinal cords	- Survival of grafted cells and expression of myelin proteins - **No** migration and myelin sheaths formation	1996/[123]
Human CNS	Embryonic brain fragments (~1mm^3)	Newborn shiverer mouse	- Myelination, even after long term cryopreservation of the grafts (IHC)	1996/[40]
Human CNS	Fetal human brain cells (NSCs)	ICV implantation into embryonic rats	- Integration and migration of transplanted cells - Differentiation into mature OL; signs of myelination (CNP staining)	1998/[81]
Adult human CNS	NPCs (LacZ-transfected)	Ethidium bromide driven demyelination in rat spinal cords	- Differentiation into Schwann-like cells - Extensive remyelination (EM) - Restoration of normal conduction velocity in the remyelinated SC	2001/[124]
Adult human CNS	Glial progenitors (A2B5-sorted)	Lysolecithin-driven demyelination in rat brains	- Survival and migration of transplanted cells into demyelinated areas - Initiation of myelination (MBP staining)	2002/[42]
Adult human CNS	NPCs (A2B5-sorted)	ICV implantation into embryonic rats	- Integration of implanted cells - Differentiation into neurons, astrocytes and oligodendrocytes	2003/[48]
Human CNS	OPCs (A2B5-selected)	Newborn shiverer mouse	- Extensive myelination by xenografted cells (OPCs from adult human brain myelinate more extensively and rapidly than fetal ones)	2004/[125]
Human CNS	NPCs from fetal spinal cord	Ethidium bromide driven demyelination in rat spinal cords	- No evidence of oligodendrocyte differentiation and myelination - Remyelination by Schwann cells	2004/[53]
hESCs	OPCs	Shiverer mouse (3-4 week old)	- Integration, maturation and myelin formation by transplanted cells (IHC and EM)	2005/[83]
hESCs	OPCs	Spinal cord injury (rats)	- Survival, integration and ultimate differentiation of transplanted cells - Remyelination and improved locomotor ability (only in animals treated shortly after injury)	2005/[126]
Human CNS	NSCs	Spinal cord injury (NOD-*scid* and NOD-*scid*/shiverer mouse)	- Extensive survival, migration and differentiation of transplanted cells - Human cells-mediated remyelination (IHC and EM) - Induction of locomotor recovery	2005/[74]
hESCs	OPCs	ICV implantation into newborn shiverer mouse	- Transplanted cells enter the brain parenchyma and myelinate (MBP+ fibers: IHC) - Noggin pretreatment enhances myelination	2007/[69]

Cell source	Cells used	Model system	Outcome (findings)	Year/Ref.
Human CNS	Glial progenitors (A2B5-sorted)	Newborn shiverer-immunodeficient mouse	- Widespread and dense myelination in implanted animals (ICH and EM) - Significantly prolonged survival in treated group	2008/[127]
Human CNS	OPCs derived from NPCs (Olig2 overexpression)	Newborn shiverer mouse	- Extensive survival, migration and differentiation into MBP+ myelinating oligodendrocytes (IHC) - Olig2 overexpression enhances commitment of NSCs towards oligodendroglial fate	2009/[51]
Human CNS	HBI.F3 hNSCs cell line (Olig2 overexpression)	Spinal cord injury (rats)	- Enhanced myelination and increased volume of spared white matter in implanted animals - Improved locomotor recovery	2009/[128]
hESCs	OPCs	Newborn shiverer mouse	- Grafted cells survived and myelinated preferentially in corpus callosum (IHC and EM) - Study examines the role of Shh and FGF2 signaling during oligodendrocyte differentiation	2009/[72]
Human CNS	NSCs	Spinal cord injury (NOD-*scid* mouse)	- Successful cell engraftment, migration and limited proliferation - Transplantation does **not** induce changes in host microenvironment	2009/[129]
Human CNS	NSCs	EAE (common marmoset) iv & it transplantations	- Negligible invasion of brain tissue and differentiation of transplanted cells - Amelioration of disease symptoms in grafted animals (indirect, immunomodulatory effect)	2009/[79]
hESCs	OPCs	Mouse hepatitis virus-mediated demyelination	- Grafted cells do **not** survive past 2 weeks in the host - Remyelination by endogenous OPCs induced by human OPC transplantation	2009/[130]
hESCs	OPCs	Spinal cord injury (cervical)	- Significantly higher oligodendrocyte-remyelination efficiency compared to controls - Robust white and gray matter sparing - Preservation of motor neurons / locomotor recovery	2010/[131]
Human CNS	NSCs	Spinal cord injury (NOD-*scid* mouse)	- Engraftment, migration, limited proliferation and oligodendrocyte differentiation of transplanted NSCs - Remyelination of axons (CASPR staining co-localization with human cytoplasm marker)	2010/[77]
hESCs	OPCs	Spinal cord injury	- **No** MBP-positive cells shown *in-vitro* - **No** remyelination studied after transplantation - Increased neurological responses in implanted rats	2010/[132]
hESCs	OPCs	Spinal cord injury (complete transection)	- Survival, migration and oligodendrocyte differentiation of transplanted cells - Significantly enhanced locomotor functions compared to controls	2010/[133]
hiPSCs	OPCs	Lysolecithin-induced optic chiasm demyelination (rat)	- Functional improvement based on visual evoked potential (VEP) recordings	2011/[97]

Cell source	Cells used	Model system	Outcome (findings)	Year/Ref.
hESCs/hiPSCs	OPCs (O4-sorted)	Transplantation into striatum (rat)	- Extensive migration of implanted OPCs along white matter tracts	2011/[70]
Human CNS	NSCs	Lysolecithin-induced spinal cord demyelination (nude and shiverer mouse)	- Extensive survival and migration of transplanted NSCs - Differentiation towards oligodendrocytes *in-vivo* and formation of compact myelin (IHC and EM)	2011/[21]
Human CNS	NSCs	Newborn shiverer-immunodeficient mouse	- Differentiation towards oligodendrocytes *in-vivo* - Generation of compact myelin (IHC and EM) - Restoration of "wild-type" axon conduction velocities	2012/[76]
hESCs	OPCs	EAE (mouse)	- Grafted cells remain in the cerebroventricular system - Significant alleviation of EAE symptoms (score) - Beneficial effects associated with indirect, immunomodulatory activity of grafted cells	2012/[134]
Human CNS	Fetal OPCs	EAE (mouse)	- Significantly reduced EAE symptoms (score) - Beneficial effects associated with indirect, immunomodulatory activity of grafted cells	2012/[135]
hiPSCs	NSCs	Spinal cord injury (common marmoset)	- Survival and differentiation into all tree neural lineages *in-vivo* - beneficial effect on axonal sparing, angiogenesis and prevention of demyelination	2012/[80]
hiPSCs	NSCs	Spinal cord injury (mouse)	- Small fraction of transplanted cells (~1%) differentiated into MBP+ oligodendrocytes - Significant locomotor improvement in grafted animals	2012/[136]
Human CNS	NSCs (CD133+/CD24-)	Human Pelizaeus-Merzbacher disease	- Modest gain of neurological functions (3/4 subjects) - Myelination assessed based on MRI and MR spectroscopy - **No** adverse effects attributed to transplantations	2012/[63]
hiPSCs	OPCs	Newborn shiverer-immunodeficient mouse	- Widespread integration of transplanted cells and extensive myelination of host CNS (IHC and EM) - Compact myelin with evident nodes of Ranvier - Substantially increased survival of graft recipients	2013/[98]
Human CNS	NSCs (OPC-directed by Sox10 overexpression)	Newborn shiverer-immunodeficient mouse	- Robust oligodendrocyte differentiation of Sox10-NSCs (IHC) and enhanced myelination compared to controls (IHC) - Inhibition of astrocytic commitment in Sox10-NSCs	2014/[73]
hiPSCs (from PPMS patients)	OPCs	Newborn shiverer-immunodeficient mouse	- OPCs derived from PPMS patients' iPSCs (43-61% efficiency) - Robust OPC engraftment and myelination of the host Corpus Callosum (IHC and EM)	2014/[100]

EAE - experimental autoimmune encephalomyelitis; EM – electron microscopy; ICV – intracerebroventricular; IHC – immunohistochemistry; it – intrathecal; iv - intravenous; MRI – magnetic resonance imaging; NOD-*scid* mouse – nonobese diabetic-severe combined immunodeficient mouse; OL – oligodendrocytes; PPMS – primary progressive MS; SC – spinal cord

CONCLUSIONS & FUTURE PERSPECTIVES

Apart from re-establishing proper saltatory signal conduction, ensuring axonal rescue and neuronal survival are the key targets in the treatment of demyelinating disorders. Remyelination induced by grafting of exogenous myelinating cells has proven its efficacy in numerous animal models, justifying trials for clinical application.

A first 1-year open-label phase 1 study on the implantation of remyelinating cells has been conducted in 4 patients with the early severe form of Pelizaeus-Merzbacher disease (PMD) a rare leukodystrophy caused by a mutation of the PLP1 gene [63]. In this trial, human central nervous system stem cells (hCNS-SCs) were used, isolated from a single donated fetal brain and purified by fluorescence-activated cell sorting on CD133-positivity and CD24-negativity, characteristic for multipotent neural stem cells (NSCs). They were extensively characterized and expanded in vitro as neurospheres to obtain a sufficient number. The ability of these hCNS-SCs to produce functional myelin had been demonstrated before [76]. Production of the hCNS-SCs was under current GMP regulations. Each of the 4 patients received 3.0×10^8 hCNS-SCs, injected in 4 equal aliquots into each of the 4 frontal lobe sites. The findings from this phase 1 study indicated a favorable safety profile for hCNS-SCs in patients with PMD. The MRI results suggested durable cell engraftment and donor-derived myelin in the transplanted host white matter. Presently, clinical trials with the implantation of similar hCNS-SCs as remyelinating cells are being prepared for the treatment of spinal cord injuries.

The phase 1 study in the PMD patients illustrates the major drawbacks of this therapeutical implantation strategy. The PMD patients received injections with allogenic cells and so had to be exposed to a stringent immunosuppressive regimen. Obviously, if autologous NSCs would have been available, they would have required correction of the PLP1 mutation with the use of novel gene editing technologies (e.g. CRISPR/Cas9 editing). Moreover, long term culture of NSCs as neurospheres for multiplication can change the identity of the NSCs and may affect the ability to differentiate into a proper oligodendrocytic cell lineage.

It is clear that the iPSC technology provides an unprecedented source for an indefinite number of autologous OPCs that may be genetically modified/corrected if necessary. Specialized laboratories for the GMP production of well-characterized pure, xeno-free, zero-footprint hiPSCs are presently set up for the generation of patient-specific iPSCs. As yet, a major obstacle, as far as their application in remyelination-directed cell transplantation is concerned, is formed by the limited efficiency, the complexity and the long duration of current OPC differentiation protocols. Once these problems are solved, treatment of

specific pediatric leukodystrophies may be most suitable for a first trial with iPSC-derived OPCs. Clinical application of autologous iPSC-derived OPCs in chronic demyelination disorders such as MS will require careful planning and determination of the proper mode and time-point of administration. Moreover, since MS pathology comprises a complicated interaction between intrinsic neurodegenerative processes and an aberrantly reacting immune system, a remyelination strategy with implanted iPSC-derived autologous OPCs can only succeed in combination with current, efficient anti-inflammatory therapeutics.

REFERENCES:

[1] Nave, "Myelination and support of axonal integrity by glia.," *Nature*, vol. 468, no. 7321, pp. 244–52, Nov. 2010.

[2] Craner, Newcombe, Black, et al., "Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na+/Ca2+ exchanger.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 101, no. 21, pp. 8168–73, May 2004.

[3] Trapp and Stys, "Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis.," *Lancet Neurol.*, vol. 8, no. 3, pp. 280–91, Mar. 2009.

[4] Trapp and Nave, "Multiple Sclerosis: An Immune or Neurodegenerative Disorder?," *Annu. Rev. Neurosci.*, vol. 31, no. 1, pp. 247–269, Jul. 2008.

[5] Philips, Bento-Abreu, Nonneman, et al., "Oligodendrocyte dysfunction in the pathogenesis of amyotrophic lateral sclerosis.," *Brain*, vol. 136, no. Pt 2, pp. 471–82, Feb. 2013.

[6] Franklin and Ffrench-Constant, "Remyelination in the CNS: from biology to therapy.," *Nat. Rev. Neurosci.*, vol. 9, no. 11, pp. 839–55, Nov. 2008.

[7] Richardson, Pringle, Yu, et al., "Origins of spinal cord oligodendrocytes: possible developmental and evolutionary relationships with motor neurons.," *Dev. Neurosci.*, vol. 19, no. 1, pp. 58–68, Jan. 1997.

[8] Spassky, Goujet-Zalc, Parmantier, et al., "Multiple Restricted Origin of Oligodendrocytes," *J. Neurosci.*, vol. 18, no. 20, pp. 8331–8343, Oct. 1998.

[9] Kessaris, Pringle, and Richardson, "Ventral neurogenesis and the neuron-glial switch.," *Neuron*, vol. 31, no. 5, pp. 677–80, Sep. 2001.

[10] Tekki-Kessaris, Woodruff, Hall, et al., "Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon.," *Development*, vol. 128, pp. 2545–2554, 2001.

[11] Vallstedt, Klos, and Ericson, "Multiple dorsoventral origins of oligodendrocyte generation in the spinal cord and hindbrain.," *Neuron*, vol. 45, no. 1, pp. 55–67, Jan. 2005.

[12] Cai, Qi, Hu, et al., "Generation of oligodendrocyte precursor cells from mouse dorsal spinal cord independent of Nkx6 regulation and Shh signaling.," *Neuron*, vol. 45, no. 1, pp. 41–53, Jan. 2005.

[13] Fogarty, Richardson, and Kessaris, "A subset of oligodendrocytes generated from radial glia in the dorsal spinal cord.," *Development*, vol. 132, pp. 1951–1959, 2005.

[14] Qi, Tan, Hui, et al., "Gli2 is required for normal Shh signaling and oligodendrocyte development in the spinal cord.," *Mol. Cell. Neurosci.*, vol. 23, no. 3, pp. 440–50, Jul. 2003.

[15] Liu, Cai, Hu, et al., "Region-specific and stage-dependent regulation of Olig gene expression and oligodendrogenesis by Nkx6.1 homeodomain transcription factor.," *Development*, vol. 130, no. 25, pp. 6221–31, Dec. 2003.

[16] Richardson, Kessaris, and Pringle, "Oligodendrocyte wars.," *Nat. Rev. Neurosci.*, vol. 7, pp. 11–18, 2006.

[17] Kessaris, Fogarty, Iannarelli, et al., "Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage.," *Nat. Neurosci.*, vol. 9, no. 2, pp. 173–9, Feb. 2006.

[18] Hajihosseini, Tham, and Dubois-Dalcq, "Origin of Oligodendrocytes within the Human Spinal Cord," *J. Neurosci.*, vol. 16, no. 24, pp. 7981–7994, Dec. 1996.

[19] Jakovcevski and Zecevic, "Olig transcription factors are expressed in oligodendrocyte and neuronal cells in human fetal CNS.," *J. Neurosci.*, vol. 25, no. 44, pp. 10064–73, Nov. 2005.

[20] Jakovcevski and Zecevic, "Sequence of oligodendrocyte development in the human fetal telencephalon.," *Glia*, vol. 49, no. 4, pp. 480–91, Mar. 2005.

[21] Buchet, Garcia, Deboux, et al., "Human neural progenitors from different foetal forebrain regions remyelinate the adult mouse spinal cord.," *Brain*, vol. 134, no. Pt 4, pp. 1168–83, Apr. 2011.

[22] Weidenheim, Kress, Epshteyn, et al., "Early myelination in the human fetal lumbosacral spinal cord: characterization by light and electron microscopy.," *J. Neuropathol. Exp. Neurol.*, vol. 51, no. 2, pp. 142–9, Mar. 1992.

[23] Back, Luo, Borenstein, et al., "Late Oligodendrocyte Progenitors Coincide with the Developmental Window of Vulnerability for Human Perinatal White Matter Injury," *J. Neurosci.*, vol. 21, no. 4, pp. 1302–1312, Feb. 2001.

[24] Lee, Lee, Ruiz, et al., "Olig2 and Ngn2 function in opposition to modulate gene expression in motor neuron progenitor cells.," *Genes Dev.*, vol. 19, no. 2, pp. 282–94, Jan. 2005.

[25] Novitch, Chen, and Jessell, "Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2.," *Neuron*, vol. 31, no. 5, pp. 773–89, Sep. 2001.

[26] Zhou, Choi, and Anderson, "The bHLH transcription factor Olig2 promotes oligodendrocyte differentiation in collaboration with Nkx2.2.," *Neuron*, vol. 31, no. 5, pp. 791–807, Sep. 2001.

[27] Briscoe, Sussel, Serup, et al., "Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling.," *Nature*, vol. 398, no. 6728, pp. 622–7, Apr. 1999.

[28] Fu, Qi, Tan, et al., "Dual origin of spinal oligodendrocyte progenitors and evidence for the cooperative role of Olig2 and Nkx2.2 in the control of oligodendrocyte differentiation.," *Development*, vol. 129, no. 3, pp. 681–93, Feb. 2002.

[29] Li, de Faria, Andrew, et al., "Phosphorylation regulates OLIG2 cofactor choice and the motor neuron-oligodendrocyte fate switch.," *Neuron*, vol. 69, no. 5, pp. 918–29, Mar. 2011.

[30] Sim, McClain, Schanz, et al., "CD140a identifies a population of highly myelinogenic, migration-competent and efficiently engrafting human oligodendrocyte progenitor cells.," *Nat. Biotechnol.*, vol. 29, no. 10, pp. 934–41, Oct. 2011.

[31] McKinnon, Waldron, and Kiel, "PDGF alpha-receptor signal strength controls an RTK rheostat that integrates phosphoinositol 3'-kinase and phospholipase Cgamma pathways during oligodendrocyte maturation.," *J. Neurosci.*, vol. 25, no. 14, pp. 3499–508, Apr. 2005.

[32] Polito and Reynolds, "NG2-expressing cells as oligodendrocyte progenitors in the normal and demyelinated adult central nervous system.," *J. Anat.*, vol. 207, no. 6, pp. 707–16, Dec. 2005.

[33] Zhang, Ge, and Duncan, "Tracing human oligodendroglial development in vitro.," *J. Neurosci. Res.*, vol. 59, no. 3, pp. 421–9, Feb. 2000.

[34] Zhang, "Defining glial cells during CNS development.," *Nat. Rev. Neurosci.*, vol. 2, no. 11, pp. 840–3, Nov. 2001.

[35] Shiota, Miura, and Mikoshiba, "Developmental profile and differential localization of mRNAs of myelin proteins (MBP and PLP) in oligodendrocytes in the brain and in culture.," *Brain Res. Dev. Brain Res.*, vol. 45, no. 1, pp. 83–94, Jan. 1989.

[36] Simons and Trotter, "Wrapping it up: the cell biology of myelination.," *Curr. Opin. Neurobiol.*, vol. 17, no. 5, pp. 533–40, Oct. 2007.

[37] Arnett, Fancy, Alberta, et al., "bHLH transcription factor Olig1 is required to repair demyelinated lesions in the CNS.," *Science*, vol. 306, no. 5704, pp. 2111–5, Dec. 2004.

[38] Othman, Frim, Polak, et al., "Olig1 is expressed in human oligodendrocytes during maturation and regeneration.," *Glia*, vol. 59, no. 6, pp. 914–26, Jun. 2011.

[39] Gumpel, Lachapelle, Gansmuller, et al., "Transplantation of human embryonic oligodendrocytes into shiverer brain.," *Ann. N. Y. Acad. Sci.*, vol. 495, pp. 71–85, Jan. 1987.

[40] Seilhean, Gansmüller, Baron-Van Evercooren, et al., "Myelination by transplanted human and mouse central nervous system tissue after long-term cryopreservation.," *Acta Neuropathol.*, vol. 91, no. 1, pp. 82–8, Jan. 1996.

[41] Roy, Wang, Harrison-Restelli, et al., "Identification, isolation, and promoter-defined separation of mitotic oligodendrocyte progenitor cells from the adult human subcortical white matter.," *J. Neurosci.*, vol. 19, no. 22, pp. 9986–95, Nov. 1999.

[42] Windrem, Roy, Wang, et al., "Progenitor cells derived from the adult human subcortical white matter disperse and differentiate as oligodendrocytes within demyelinated lesions of the rat brain.," *J. Neurosci. Res.*, vol. 69, no. 6, pp. 966–75, Sep. 2002.

[43] Ruffini, Arbour, Blain, et al., "Distinctive properties of human adult brain-derived myelin progenitor cells.," *Am. J. Pathol.*, vol. 165, no. 6, pp. 2167–75, Dec. 2004.

[44] Miron, Jung, Kim, et al., "FTY720 modulates human oligodendrocyte progenitor process extension and survival.," *Ann. Neurol.*, vol. 63, no. 1, pp. 61–71, Jan. 2008.

[45] Flax, Aurora, Yang, et al., "Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes.," *Nat. Biotechnol.*, vol. 16, no. 11, pp. 1033–9, Nov. 1998.

[46] Carpenter, Cui, Hu, et al., "In vitro expansion of a multipotent population of human neural progenitor cells.," *Exp. Neurol.*, vol. 158, no. 2, pp. 265–78, Aug. 1999.

[47] Neri, Maderna, Ferrari, et al., "Robust generation of oligodendrocyte progenitors from human neural stem cells and engraftment in experimental demyelination models in mice.," *PLoS One*, vol. 5, no. 4, p. e10145, Jan. 2010.

[48] Nunes, Roy, Keyoung, et al., "Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain.," *Nat. Med.*, vol. 9, no. 4, pp. 439–47, Apr. 2003.

[49] Arsenijevic, Villemure, Brunet, et al., "Isolation of multipotent neural precursors residing in the cortex of the adult human brain.," *Exp. Neurol.*, vol. 170, no. 1, pp. 48–62, Jul. 2001.

[50] Sun, Pollard, Conti, et al., "Long-term tripotent differentiation capacity of human neural stem (NS) cells in adherent culture.," *Mol. Cell. Neurosci.*, vol. 38, no. 2, pp. 245–58, Jun. 2008.

[51] Maire, Buchet, Kerninon, et al., "Directing human neural stem/precursor cells into oligodendrocytes by overexpression of Olig2 transcription factor.," *J. Neurosci. Res.*, vol. 87, no. 15, pp. 3438–46, Nov. 2009.

[52] Uchida, Buck, He, et al., "Direct isolation of human central nervous system stem cells.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 97, no. 26, pp. 14720–5, Dec. 2000.

[53] Chandran, Compston, Jauniaux, et al., "Differential generation of oligodendrocytes from human and rodent embryonic spinal cord neural precursors.," *Glia*, vol. 47, no. 4, pp. 314–24, Sep. 2004.

[54] Iwanami, Kaneko, Nakamura, et al., "Transplantation of human neural stem cells for spinal cord injury in primates.," *J. Neurosci. Res.*, vol. 80, no. 2, pp. 182–90, Apr. 2005.

[55] Yan, Xu, Welsh, et al., "Extensive neuronal differentiation of human neural stem cell grafts in adult rat spinal cord.," *PLoS Med.*, vol. 4, no. 2, p. e39, Mar. 2007.

[56] Palmer, Schwartz, Taupin, et al., "Cell culture. Progenitor cells from human brain after death.," *Nature*, vol. 411, no. 6833, pp. 42–3, May 2001.

[57] Schwartz, Bryant, Fuja, et al., "Isolation and characterization of neural progenitor cells from post-mortem human cortex.," *J. Neurosci. Res.*, vol. 74, no. 6, pp. 838–51, Dec. 2003.

[58] Van Strien, Sluijs, Reynolds, et al., "Isolation of Neural Progenitor Cells From the Human Adult Subventricular Zone Based on Expression of the Cell Surface Marker CD271.," *Stem Cells Transl. Med.*, vol. 3, no. 4, pp. 470–80, Apr. 2014.

[59] Sah, Ray, and Gage, "Bipotent progenitor cell lines from the human CNS.," *Nat. Biotechnol.*, vol. 15, no. 6, pp. 574–80, Jun. 1997.

[60] Svendsen, ter Borg, Armstrong, et al., "A new method for the rapid and long term growth of human neural precursor cells.," *J. Neurosci. Methods*, vol. 85, no. 2, pp. 141–52, Dec. 1998.

[61] Li, Xu, Bai, et al., "Isolation and characterization of neural stem cells from human fetal striatum.," *Biochem. Biophys. Res. Commun.*, vol. 326, no. 2, pp. 425–34, Jan. 2005.

[62] Pagano, Impagnatiello, Girelli, et al., "Isolation and characterization of neural stem cells from the adult human olfactory bulb.," *Stem Cells*, vol. 18, no. 4, pp. 295–300, Jan. 2000.

[63] Gupta, Henry, Strober, et al., "Neural stem cell engraftment and myelination in the human brain.," *Sci. Transl. Med.*, vol. 4, no. 155, p. 155ra137, Oct. 2012.

[64] Chojnacki, Kelly, Hader, et al., "Distinctions between fetal and adult human platelet-derived growth factor-responsive neural precursors.," *Ann. Neurol.*, vol. 64, no. 2, pp. 127–42, Aug. 2008.

[65] Hong, Kang, Isacson, et al., "Neural precursors derived from human embryonic stem cells maintain long-term proliferation without losing the potential to differentiate into all three neural lineages, including dopaminergic neurons.," *J. Neurochem.*, vol. 104, no. 2, pp. 316–24, Jan. 2008.

[66] Akesson, Piao, Samuelsson, et al., "Long-term culture and neuronal survival after intraspinal transplantation of human spinal cord-derived neurospheres.," *Physiol. Behav.*, vol. 92, no. 1–2, pp. 60–6, Sep. 2007.

[67] Pistollato, Chen, Schwartz, et al., "Oxygen tension controls the expansion of human CNS precursors and the generation of astrocytes and oligodendrocytes.," *Mol. Cell. Neurosci.*, vol. 35, no. 3, pp. 424–35, Jul. 2007.

[68] Kang, Cho, Seo, et al., "Efficient induction of oligodendrocytes from human embryonic stem cells.," *Stem Cells*, vol. 25, no. 2, pp. 419–24, Feb. 2007.

[69] Izrael, Zhang, Kaufman, et al., "Human oligodendrocytes derived from embryonic stem cells: Effect of noggin on phenotypic differentiation in vitro and on myelination in vivo.," *Mol. Cell. Neurosci.*, vol. 34, no. 3, pp. 310–23, Mar. 2007.

[70] Major, Menon, Auyeung, et al., "Transgene Excision Has No Impact on In Vivo Integration of Human iPS Derived Neural Precursors," *PLoS One*, vol. 6, no. 9, p. e24687, Sep. 2011.

[71] Zhang, Wernig, Duncan, et al., "In vitro differentiation of transplantable neural precursors from human embryonic stem cells.," *Nat. Biotechnol.*, vol. 19, no. 12, pp. 1129–33, Dec. 2001.

[72] Hu, Du, Li, et al., "Human oligodendrocytes from embryonic stem cells: conserved SHH signaling networks and divergent FGF effects.," *Development*, vol. 136, no. 9, pp. 1443–52, May 2009.

[73] Wang, Pol, Haberman, et al., "Transcription factor induction of human oligodendrocyte progenitor fate and differentiation.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 2014, Jun. 2014.

[74] Cummings, Uchida, Tamaki, et al., "Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 102, no. 39, pp. 14069–74, Oct. 2005.

[75] Aharonowiz, Einstein, Fainstein, et al., "Neuroprotective effect of transplanted human embryonic stem cell-derived neural precursors in an animal model of multiple sclerosis.," *PLoS One*, vol. 3, no. 9, p. e3145, Jan. 2008.

[76] Uchida, Chen, Dohse, et al., "Human neural stem cells induce functional myelination in mice with severe dysmyelination.," *Sci. Transl. Med.*, vol. 4, no. 155, p. 155ra136, Oct. 2012.

[77] Salazar, Uchida, Hamers, et al., "Human neural stem cells differentiate and promote locomotor recovery in an early chronic spinal cord injury NOD-scid mouse model.," *PLoS One*, vol. 5, no. 8, p. e12272, Jan. 2010.

[78] Nori, Okada, Yasuda, et al., "Grafted human-induced pluripotent stem-cell-derived neurospheres promote motor functional recovery after spinal cord injury in mice.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 108, no. 40, pp. 16825–30, Oct. 2011.

[79] Pluchino, Gritti, Blezer, et al., "Human neural stem cells ameliorate autoimmune encephalomyelitis in non-human primates.," *Ann. Neurol.*, vol. 66, no. 3, pp. 343–54, Sep. 2009.

[80] Kobayashi, Okada, Itakura, et al., "Pre-Evaluated Safe Human iPSC-Derived Neural Stem Cells Promote Functional Recovery after Spinal Cord Injury in Common Marmoset without Tumorigenicity," *PLoS One*, vol. 7, no. 12, p. e52787, Dec. 2012.

[81] Brüstle, Choudhary, Karram, et al., "Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats.," *Nat. Biotechnol.*, vol. 16, no. 11, pp. 1040–4, Nov. 1998.

[82] Thomson, "Embryonic Stem Cell Lines Derived from Human Blastocysts," *Science (80).*, vol. 282, no. 5391, pp. 1145–1147, Nov. 1998.

[83] Nistor, Totoiu, Haque, et al., "Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation.," *Glia*, vol. 49, no. 3, pp. 385–96, Feb. 2005.

[84] Gil, Woo, Shim, et al., "Vitronectin promotes oligodendrocyte differentiation during neurogenesis of human embryonic stem cells.," *FEBS Lett.*, vol. 583, no. 3, pp. 561–7, Feb. 2009.

[85] Hu, Du, and Zhang, "Differentiation of human oligodendrocytes from pluripotent stem cells.," *Nat. Protoc.*, vol. 4, no. 11, pp. 1614–22, Jan. 2009.

[86] Sundberg, Skottman, Suuronen, et al., "Production and isolation of NG2+ oligodendrocyte precursors from human embryonic stem cells in defined serum-free medium.," *Stem Cell Res.*, vol. 5, no. 2, pp. 91–103, Sep. 2010.

[87] Takahashi, Tanabe, Ohnuki, et al., "Induction of pluripotent stem cells from adult human fibroblasts by defined factors.," *Cell*, vol. 131, no. 5, pp. 861–72, Nov. 2007.

[88] Nakagawa, Koyanagi, Tanabe, et al., "Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts.," *Nat. Biotechnol.*, vol. 26, no. 1, pp. 101–6, Jan. 2008.

[89] Yu, Hu, Smuga-Otto, et al., "Human induced pluripotent stem cells free of vector and transgene sequences.," *Science*, vol. 324, no. 5928, pp. 797–801, May 2009.

[90] Soldner, Hockemeyer, Beard, et al., "Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors.," *Cell*, vol. 136, no. 5, pp. 964–77, Mar. 2009.

[91] Aasen, Raya, Barrero, et al., "Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes.," *Nat. Biotechnol.*, vol. 26, no. 11, pp. 1276–84, Nov. 2008.

[92] Malik and Rao, "A review of the methods for human iPSC derivation.," *Methods Mol. Biol.*, vol. 997, pp. 23–33, Jan. 2013.

[93] Kim, Lee, Kim, et al., "Highly pure and expandable PSA-NCAM-positive neural precursors from human ESC and iPSC-derived neural rosettes.," *PLoS One*, vol. 7, no. 7, p. e39715, Jan. 2012.

[94] Hu, Weick, Yu, et al., "Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 107, no. 9, pp. 4335–40, Mar. 2010.

[95] Swistowski, Peng, Liu, et al., "Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions.," *Stem Cells*, vol. 28, no. 10, pp. 1893–904, Oct. 2010.

[96] Ogawa, Tokumoto, Miyake, et al., “Induction of oligodendrocyte differentiation from adult human fibroblast-derived induced pluripotent stem cells.,” *In Vitro Cell. Dev. Biol. Anim.*, vol. 47, no. 7, pp. 464–9, Aug. 2011.

[97] Pouya, Satarian, Kiani, et al., “Human induced pluripotent stem cells differentiation into oligodendrocyte progenitors and transplantation in a rat model of optic chiasm demyelination.,” *PLoS One*, vol. 6, no. 11, p. e27925, Jan. 2011.

[98] Wang, Bates, Li, et al., “Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination.,” *Cell Stem Cell*, vol. 12, no. 2, pp. 252–64, Feb. 2013.

[99] Jang, Kang, Kim, et al., “Induced pluripotent stem cell models from X-linked adrenoleukodystrophy patients.,” *Ann. Neurol.*, vol. 70, no. 3, pp. 402–9, Sep. 2011.

[100] Douvaras, Wang, Zimmer, et al., “Efficient Generation of Myelinating Oligodendrocytes from Primary Progressive Multiple Sclerosis Patients by Induced Pluripotent Stem Cells,” *Stem Cell Reports*, vol. 3, no. 2, pp. 250–259, Jul. 2014.

[101] Yang, Zuchero, Ahlenius, et al., “Generation of oligodendroglial cells by direct lineage conversion.,” *Nat. Biotechnol.*, vol. 31, no. 5, pp. 434–9, May 2013.

[102] Najm, Lager, Zaremba, et al., “Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells.,” *Nat. Biotechnol.*, vol. 31, no. 5, pp. 426–33, May 2013.

[103] Huang, Fancy, Zhao, et al., “Myelin regeneration in multiple sclerosis: targeting endogenous stem cells.,” *Neurotherapeutics*, vol. 8, no. 4, pp. 650–8, Oct. 2011.

[104] Franklin and Gallo, “The translational biology of remyelination: past, present, and future.,” *Glia*, vol. 62, no. 11, pp. 1905–15, Nov. 2014.

[105] Martino, Franklin, Baron Van Evercooren, et al., “Stem cell transplantation in multiple sclerosis: current status and future prospects.,” *Nat. Rev. Neurol.*, vol. 6, no. 5, pp. 247–55, May 2010.

[106] Goldman, Nedergaard, and Windrem, “Glial progenitor cell-based treatment and modeling of neurological disease.,” *Science*, vol. 338, no. 6106, pp. 491–5, Oct. 2012.

[107] Wood, Okada, and Bunge, “The use of networks of dissociated rat dorsal root ganglion neurons to induce myelination by oligodendrocytes in culture,” *Brain Res.*, vol. 196, no. 1, pp. 247–252, Aug. 1980.

[108] Wang, Colognato, and Ffrench-Constant, “Contrasting effects of mitogenic growth factors on myelination in neuron-oligodendrocyte co-cultures.,” *Glia*, vol. 55, no. 5, pp. 537–45, Apr. 2007.

[109] Watkins, Emery, Mulinyawe, et al., “Distinct stages of myelination regulated by gamma-secretase and astrocytes in a rapidly myelinating CNS coculture system.,” *Neuron*, vol. 60, no. 4, pp. 555–69, Dec. 2008.

[110] Zhang, Jarjour, Boyd, et al., “Central nervous system remyelination in culture - a tool for multiple sclerosis research.,” *Exp. Neurol.*, vol. 230, no. 1, pp. 138–48, Jul. 2011.

[111] Billings-Gagliardi, Hall, Stanhope, et al., “Cultures of shiverer mutant cerebellum injected with normal oligodendrocytes make both normal and shiverer myelin.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 81, no. 8, pp. 2558–61, Apr. 1984.

[112] Vereyken, Fluitsma, Bolijn, et al., “An in vitro model for de- and remyelination using lysophosphatidyl choline in rodent whole brain spheroid cultures.,” *Glia*, vol. 57, no. 12, pp. 1326–40, Sep. 2009.

[113] Althaus, Montz, Neuhoff, et al., “Isolation and cultivation of mature oligodendroglial cells.,” *Naturwissenschaften*, vol. 71, no. 6, pp. 309–15, Jun. 1984.

[114] Bullock and Rome, “Glass micro-fibers: a model system for study of early events in myelination.,” *J. Neurosci. Res.*, vol. 27, no. 3, pp. 383–93, Nov. 1990.

[115] Howe, "Coated glass and vicryl microfibers as artificial axons.," *Cells. Tissues. Organs*, vol. 183, no. 4, pp. 180–94, Jan. 2006.

[116] Lee, Leach, Redmond, et al., "A culture system to study oligodendrocyte myelination processes using engineered nanofibers.," *Nat. Methods*, vol. 9, no. 9, pp. 917–22, Sep. 2012.

[117] Rosenberg, Kelland, Tokar, et al., "The geometric and spatial constraints of the microenvironment induce oligodendrocyte differentiation.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 105, no. 38, pp. 14662–7, Oct. 2008.

[118] Jakovcevski, Mo, and Zecevic, "Down-regulation of the axonal polysialic acid-neural cell adhesion molecule expression coincides with the onset of myelination in the human fetal forebrain.," *Neuroscience*, vol. 149, no. 2, pp. 328–37, Oct. 2007.

[119] Bin, Leong, Bull, et al., "Oligodendrocyte precursor cell transplantation into organotypic cerebellar shiverer slices: a model to study myelination and myelin maintenance.," *PLoS One*, vol. 7, no. 7, p. e41237, Jan. 2012.

[120] Cui, D'Abate, Fang, et al., "Human fetal oligodendrocyte progenitor cells from different gestational stages exhibit substantially different potential to myelinate.," *Stem Cells Dev.*, vol. 21, no. 11, pp. 1831–7, Jul. 2012.

[121] Stacpoole, Spitzer, Bilican, et al., "High yields of oligodendrocyte lineage cells from human embryonic stem cells at physiological oxygen tensions for evaluation of translational biology.," *Stem cell reports*, vol. 1, no. 5, pp. 437–50, Jan. 2013.

[122] O'Guin, Gruber, Raine, et al., "Gas6 enhances axonal ensheathment by MBP+ membranous processes in human DRG/OL promyelinating co-cultures.," *ASN Neuro*, vol. 6, no. 1, pp. 29–42, Jan. 2014.

[123] Targett, Sussman, Scolding, et al., "Failure to achieve remyelination of demyelinated rat axons following transplantation of glial cells obtained from the adult human brain.," *Neuropathol. Appl. Neurobiol.*, vol. 22, no. 3, pp. 199–206, Jul. 1996.

[124] Akiyama, Honmou, Kato, et al., "Transplantation of clonal neural precursor cells derived from adult human brain establishes functional peripheral myelin in the rat spinal cord.," *Exp. Neurol.*, vol. 167, no. 1, pp. 27–39, Jan. 2001.

[125] Windrem, Nunes, Rashbaum, et al., "Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain.," *Nat. Med.*, vol. 10, no. 1, pp. 93–7, Jan. 2004.

[126] Keirstead, Nistor, Bernal, et al., "Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury.," *J. Neurosci.*, vol. 25, no. 19, pp. 4694–705, May 2005.

[127] Windrem, Schanz, Guo, et al., "Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse.," *Cell Stem Cell*, vol. 2, no. 6, pp. 553–65, Jun. 2008.

[128] Hwang, Kim, Kim, et al., "Transplantation of human neural stem cells transduced with Olig2 transcription factor improves locomotor recovery and enhances myelination in the white matter of rat spinal cord following contusive injury.," *BMC Neurosci.*, vol. 10, p. 117, Jan. 2009.

[129] Hooshmand, Sontag, Uchida, et al., "Analysis of host-mediated repair mechanisms after human CNS-stem cell transplantation for spinal cord injury: correlation of engraftment with recovery.," *PLoS One*, vol. 4, no. 6, p. e5871, Jan. 2009.

[130] Hatch, Schaumburg, Lane, et al., "Endogenous remyelination is induced by transplant rejection in a viral model of multiple sclerosis.," *J. Neuroimmunol.*, vol. 212, no. 1–2, pp. 74–81, Jul. 2009.

[131] Sharp, Frame, Siegenthaler, et al., "Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury.," *Stem Cells*, vol. 28, no. 1, pp. 152–63, Jan. 2010.

[132] Kerr, Letzen, Hill, et al., “Efficient differentiation of human embryonic stem cells into oligodendrocyte progenitors for application in a rat contusion model of spinal cord injury.,” *Int. J. Neurosci.*, vol. 120, no. 4, pp. 305–13, May 2010.

[133] Erceg, Ronaghi, Oria, et al., “Transplanted oligodendrocytes and motoneuron progenitors generated from human embryonic stem cells promote locomotor recovery after spinal cord transection.,” *Stem Cells*, vol. 28, no. 9, pp. 1541–9, Sep. 2010.

[134] Kim, Walczak, Kerr, et al., “Immunomodulation by transplanted human embryonic stem cell-derived oligodendroglial progenitors in experimental autoimmune encephalomyelitis.,” *Stem Cells*, vol. 30, no. 12, pp. 2820–9, Dec. 2012.

[135] Kim, Walczak, Muja, et al., “ICV-transplanted human glial precursor cells are short-lived yet exert immunomodulatory effects in mice with EAE.,” *Glia*, vol. 60, no. 7, pp. 1117–29, Jul. 2012.

[136] Fujimoto, Abematsu, Falk, et al., “Treatment of a mouse model of spinal cord injury by transplantation of human induced pluripotent stem cell-derived long-term self-renewing neuroepithelial-like stem cells.,” *Stem Cells*, vol. 30, no. 6, pp. 1163–73, Jun. 2012.

[137] Goldman, “Progenitor cell-based treatment of the pediatric myelin disorders.,” *Arch. Neurol.*, vol. 68, no. 7, pp. 848–56, Jul. 2011.