Neurosci Bull April 1, 2013, 29(2): 144–154. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1317-z

·Review·

Promoting remyelination for the treatment of multiple sclerosis: opportunities and challenges

Yueting Zhang, Taylor B. Guo, Hongtao Lu*

Neuroimmunology Discovery Performance Unit, GlaxoSmithKline Research and Development Center, Shanghai, China

*Current address: Altimab Therapeutics, Inc., 3347 Helen Lane, Lafayette, California, 94549, USA

Corresponding author: Hongtao Lu. E-mail: htlu@altimab.com

© Shanghai Institutes for Biological Sciences, CAS and Springer-Verlag Berlin Heidelberg 2013

Multiple sclerosis (MS) is a chronic and devastating autoimmune demyelinating disease of the central nervous system. With the increased understanding of the pathophysiology of this disease in the past two decades, many disease-modifying therapies that primarily target adaptive immunity have been shown to prevent exacerbations and new lesions in patients with relapsing-remitting MS. However, these therapies only have limited efficacy on the progression of disability. Increasing evidence has pointed to innate immunity, axonal damage and neuronal loss as important contributors to disease progression. Remyelination of denuded axons is considered an effective way to protect neurons from damage and to restore neuronal function. The identification of several key molecules and pathways controlling the differentiation of oligodendrocyte progenitor cells and myelination has yielded clues for the development of drug candidates that directly target remyelination and neuroprotection. The long-term efficacy of this strategy remains to be evaluated in clinical trials. Here, we provide an overview of current and emerging therapeutic concepts, with a focus on the opportunities and challenges for the remyelination approach to the treatment of MS.

Keywords: multiple sclerosis; myelination; neurodegeneration; oligodendrocytes; disease progression; disease modifying therapy; drug target; animal models

Introduction

Multiple sclerosis (MS) mainly affects young adults, most being diagnosed between the ages of 20 and 50 years. Based on the clinical symptoms and course of the disease, MS is usually classified into four subtypes: relapsing-remitting (RR), primary progressive (PP), secondary progressive (SP), and progressive-relapsing (PR) MS. Approximately 85% of MS patients initially have the RR subtype, with acute attacks (relapse) followed by partial or full recovery (remission). About two-thirds of RRMS patients progress into the SP phase, in which neurologic disability accumulates without attacks. About 15% of MS patients experience progressive clinical worsening from the onset with no clinical attacks and thus are defined as PPMS. The last subtype, PRMS is a very rare form which is characterized by progression of disability from onset but with clear acute relapses, with or without full recovery.

Although the pathogenesis of MS is not fully understood, it is generally believed that relapses are driven by the adaptive immune system. This involves waves of CD4 T helper cell 1 (Th1), T helper cell 17 (Th17), and CD8 cells infiltrating the central nervous system (CNS), provoking attacks that result in demyelination and axonal damage. Remyelination usually occurs during the early phase of the disease but eventually fails as it progresses, leaving chronic lesions largely demyelinated. Pathological studies have shown that abundant oligodendrocyte progenitor cells (OPCs) exist in the adult, and OPCs, either endogenous or transplanted, migrate to and re-populate demyelinated lesions. However, they cannot effectively remyelinate the denuded axons[1-3]. A non-permissive environment for OPC

differentiation and myelination has been suggested to be the main cause for remyelination failure. This sustained demyelination, along with axonal damage and neurodegeneration, leads to the accumulation of neurological deficits, which are the main feature of the chronic progressive phase of the disease. While adaptive immunity accounts for the acute phase, increasing evidence indicates that innate immunity may be a driving force for disease progression[4].

Marketed Drugs and Emerging Therapies

The advancement of our understanding of the pathophysiology of MS has led to the development of many disease-modifying therapies in the past two decades. These treatments mainly target RRMS patients and have demonstrated reasonable efficacy in reducing the relapse rate and the formation of new lesions, but they are relatively ineffective in preventing the progression of disability. Pathologically, MS is a very heterogeneous disease. Lassman *et al.* have categorized it into four major types based on autopsies[5]; it is therefore not surprising that therapy against one type may not be effective against another.

So far, eight drugs have been approved for treating RRMS: three beta interferons (Betaferon, Rebif, and Avonex), glatiramer acetate (Copaxone), mitoxantrone (Novantrone), natalizumab (Tysabri), the first oral agent, fingolimod (Gilenya), and the newly-approved oral drug teriflunomide (Aubagio). An overview is presented in Table 1. These drugs can be classified into three categories based on their mechanisms of action: (1) immunomodulation, such as beta interferons and Copaxone, which are currently commonly used as first-line therapies, as they modestly reduce relapses and are generally well-tolerated; (2) general immunosuppression, such as by mitoxantrone and teriflunomide; and (3) blockade of the infiltration of immune cells into the CNS, such as natalizumab (anti-VLA4) and fingolimod (S1P receptor agonist). The evolution of current therapies reveals a trend from general immunomodulation and immunosuppression to more specific targeting with a better risk/benefit profile. Many new agents are still under

Table 1. US FDA-approved drugs for multiple sclerosis (MS)

MS treatment	Dosing route	Mechanism of action	Efficacy		Safety
			ARR reduction	Progression of disability	
Avonex (IFN-β1a)	Inj, sc	Immunomodulation	32%		Flu-like symptoms, injection-site reactions
Betaseron (IFN-β1b)	Inj, sc	Immunomodulation	30–50%		
Rebif (IFN-β1a)	Inj, sc	Immunomodulation	27–33%		
Copaxone (glatiramer acetate)	Inj, sc	Immunomodulation	34.4%		Headache, nasopharyngitis, injection-site reactions
Tysabri (natalizumab)	Inj (sc, iv, im)	Leukocyte trafficking	68%	42% (2 years)	Progressive multifocal leukodystrophy risk
Novantrone (mitoxantrone)	Inj, iv	Immunosuppression	61%		Nausea, vomiting, hair loss, potential risk for cardiotoxicity and leukemia
Gilenya (fingolimod)	Oral	Leukocyte trafficking	54–60%	30–32% (3 months) 37–40% (6 months)	Nasopharyngitis, low lymphocyte counts, upper respiratory tract infections, headache, diarrhea, back pain and cough, liver transaminase elevations
Aubagio (teriflunomide)	Oral	Immunosuppression	36.3%	31.5% (3 months)	Headache, alanine transaminase elevations, hair thinning, diarrhea, nausea and neutropenia

Inj, injectable; sc, subcutaneous; iv, intravenous; im, intramuscular; ARR, annual relapse rate. All efficacies refer to comparison with placebo.

development, but they all target the immune system. As shown in Table 2, most of the developing assets are monoclonal antibodies with only a few oral drugs in the pipeline.

Given the fact that current therapies have achieved good efficacy in reducing the relapse rate and new lesion formation, the RRMS market is reasonably served. These drugs, however, are only partially effective in preventing the progression of disability. Hence, there is a huge unmet need for effective therapies that halt neurodegenerative changes or even reverse the neurological deficits that occur as a consequence of axonal damage and gradual neuronal loss. Although direct insults (such as inflammatory responses and excitotoxicity) causing axonal damage have been investigated, sustained demyelination rendering the axons more susceptible to damage is the leading mechanism. To achieve neuroprotection, the best approach is to stimulate remyelination in MS lesions by promoting OPC differentiation and myelination which may protect neurons from further breakdown and ideally promote functional recovery. Indeed, at least in preclinical research, it has been demonstrated that agents capable of promoting OPC differentiation promote remyelination in animal models of MS[6-8].

In vitro and *in vivo* Models of OPC Differentiation and Myelination

Our understanding of the process of OPC differentiation and myelination has been greatly advanced using a number of increasingly sophisticated *in vitro* and *in vivo* tools. The most widely used *in vitro* model is primary culture of OPCs, as isolated oligodendrocytes in culture follow a developmental program that is very similar to that *in vivo*[9], suggesting that intrinsic regulation exists to instruct OPCs to differentiate into mature oligodendrocytes and produce myelin membrane even in the absence of axons. Although these cultures are invaluable in studies of the migration, proliferation and differentiation of OPCs, they do not allow the study of axonal-glial interactions and the myelination process, which requires some kind of a co-culture system. Broadly, there are three kinds of myelinating culture systems: mixed culture, co-culture of purified cells, and cerebellar slice culture. Mixed culture usually involves cells from the CNS; they take >2 weeks to myelinate, and the system does not allow the manipulation of individual cell types. Co-culture of purified OPCs with neurons, usually with dorsal root ganglion (DRG) neurons, offers the advantage of flexibility. The substitution of DRG neurons with CNS-derived neurons, retinal ganglion cells or hippocampal neurons, brings the system closer to the *in vivo* myelination process[10]. Slice cultures can, to some extent, maintain the 3D architecture of the cerebellar white and grey matter and provide a valuable *ex vivo* model to study demyelination/remyelination. Although it is hard to target a single cell type in the slice, a potential way to overcome this is the addition

Table 2. Emerging therapies for multiple sclerosis

Compound	Dosing route	Mechanism of action	Efficacy		Safety
			ARR reduction	Progression of disability	
Alemtuzumab (anti-CD52)	Inj	Immunomodulation	31–55%*	27%* (2 years)	Mild to moderate infusion-associated reactions
Daclizumab (anti-CD25)	Inj	Immunomodulation	50.5–51.4%	46%# (3 months)	Urinary and upper respiratory tract infections, rash, fatigue, lymphopenia and transient thrombocytopenia
Laquinimod	Oral	Immunomodulation	23%	30% (2 years)	Headache, nasopharyngitis and back pain
Dimethyl fumarate	Oral	Anti-inflammation, cytoprotective	48–53%	34–38% (2 years)	Flushing, gastrointestinal events, lymphopenia

All efficacies refer to comparison with placebo unless specified otherwise. Inj, injectable; ARR, annual relapse rate.

*, compared to beta interferon 1a (Rebif); #, in RRMS patients without highly-active disease.

of exogenous cells into the slice culture and then study the consequences.

Among the mechanism-related or disease-relevant animal models in the field of MS, the most widely-used is experimental autoimmune encephalomyelitis (EAE), which is induced by immunization with one or more myelin autoantigens, leading to inflammation of the CNS. There are different variants of EAE depending on the strain of animals used, but the common features are severe CNS lymphocytic inflammation and demyelination. This model has greatly furthered our understanding of immunology in general and neuroinflammation in particular, and remains a tool for evaluating potential MS therapeutics. However, it has always been difficult to separate the inflammatory component from the CNS component in EAE. Therefore, to evaluate the direct effect of an agent on demyelination or remyelination, demyelination models with minimal inflammation are needed. These models typically involve the local injection of toxins that either strip the myelin away or kill the oligodendrocytes. Lysolecithin (LPC) and ethidium bromide are the typical toxins used[11,12]. The cuprizone model is another widely used toxin-based method that offers the ease of handling, as cuprizone is usually mixed with animal chow and ingested. All of these models rely on pathological examination of CNS myelin content to show demyelination, which can only be assessed after animal sacrifice, and all these models suffer from low throughput. An animal model with a far higher throughput is desirable for the purpose of drug screening. For this, ethidium bromide-induced demyelination of the developing zebrafish embryo fits the bill[13,14]. This model provides a pair of powerful *in vivo* readouts in the transgenic fish, i.e. mobility and myelin imaging. On the other hand, since the cuprizone model results in profound demyelination of the corpus callosum in mammals, *in vivo* functional readouts such as rotarod behavior and translatable imaging markers such as diffuse tensor imaging have been attempted[15]. In addition, recording the electric conductance along the white matter in acutely-isolated slices may be another functional readout. It is important to realize that these toxin-based demyelination models do not mimic MS pathology, despite some recent information showing the activation of microglia, reactive astrocytes and the presence of Th17 cells. They are however still useful in testing the mechanisms of de- and re-myelination with minimal immunological involvement. It is noteworthy that increased use of transgenic mice with overexpression or conditional ablations of oligodendrocyte-specific genes has also significantly increased our knowledge of myelination.

Signals that Control OPC Differentiation and Myelination

Signals controlling OPC differentiation and myelination can be classified into two categories: intrinsic signals and extrinsic signals/environmental cues. The interplay of these determinants regulates the differentiation of OPCs into mature oligodendrocytes. Below is a general discussion of the important players in the regulation of OPC differentiation and myelination. This does not mean, however, that these players are automatically good drug targets; understanding the molecules and the downstream pathways driven or regulated by them certainly offers good clues for potential drug intervention, if a good entry point can be identified.

Intrinsic Signals

It has long been appreciated that much of the regulation of oligodendrocyte differentiation is intrinsic in nature, with mechanisms like an internal “clock” limiting the number of cell divisions or cell cycle time in OPC cultures in the absence of neurons[16]. One important transcription factor is Olig2. The initial specification of the oligodendrocyte lineage is dependent on Olig2 and this lineage is absent from Olig2-null mice[17]. Olig2 induces transcription factors that are required for the generation of mature, postmitotic oligodendrocytes, notably Olig1, Sox-10, Nkx2.2, Nkx6.2, Ying Yang 1, and ZFP488[18-23]. In counterbalance, transcription factors that maintain OPCs in their undifferentiated state and repress myelin gene expression have also been identified, which include Id2, Id4, Hes5, and Sox6. Despite the discovery of transcription factors that regulate the differentiation of OPCs, little is known about the factors that control the conversion of premyelinating into myelinating oligodendrocytes. Transgenic animals and gene expression analysis such as DNA microarrays have allowed the identification of several oligodendrocyte-specific genes that regulate differentiation and myelination, including positive regulators such as myelin gene regulatory factor (MRF) zinc finger protein 191 (Zfp191), retinoid X receptor (RXR), and negative regulators such as Notch, Wnt and GPR17[24-26].

Myelin Gene Regulatory Factor

MRF (also known as Gene Model 98) is a critical transcrip-

tional regulator of oligodendrocyte maturation and CNS myelination. MRF is specifically expressed by postmitotic oligodendrocytes and promotes rapid OPC differentiation into myelin basic protein-positive and myelin oligodendrocyte glycoprotein-positive oligodendrocytes[24]. In mice lacking MRF in the oligodendrocyte lineage, the initial differentiation of OPCs into pre-myelinating oligodendrocytes is unaffected; however, these pre-myelinating oligodendrocytes fail to express the majority of myelin genes and the mice display severe neurological abnormalities[24]. Furthermore, MRF is reported to be required for the maintenance of myelin in mature oligodendrocytes. Ablation of MRF using an inducible conditional knockout strategy in myelinating cells causes delayed but severe CNS demyelination. These findings demonstrate an ongoing requirement for MRF in the maintenance of both mature oligodendrocyte identity and the myelin sheath[27].

Zinc Finger Protein 191

Zfp191 is another transcription factor identified as being required for CNS myelination[25]. In the absence of Zfp191, the oligodendrocyte lineage appears to stall at a much later stage than occurs in the absence of other factors such as Olig1, Sox10 and MRF; relatively normal numbers of CC1-positive oligodendrocytes are generated and capable of contacting axons, but fail to fully myelinate them.

Retinoid X Receptor-gamma

RXRγ was recently identified as a factor regulating myelination in a microarray analysis of separate stages of spontaneous remyelination after focal demyelination in the rat CNS[28]. RXRγ is highly expressed in remyelinating lesions, suggesting that it may be important in lesion repair. This was further investigated by gain/loss-of-function studies in purified OPC and cerebellar slice cultures. Knockdown of RXRγ by RNA interference or RXR-specific antagonists severely inhibits oligodendrocyte differentiation in culture, while the RXR agonist 9-*cis*-retinoic acid promotes remyelination in demyelinated cerebellar slice cultures and in demyelinated aged rats[28]. However, given the pleiotropic roles of RXRγ and its diverse partners, a general RXRγ agonist may have significant side effects. Hence, the identification of those RXR partners that are responsible for the remyelination effect may provide a good intervention point for drug targeting.

G Protein-Coupled Receptor 17

GPR17 is a Gi-coupled receptor with expression restricted to the early differentiation stages of oligodendrocyte-lineage cells, and it is downregulated during the peak period of myelination and in adulthood[29]. Gpr17-knockout mice show early-onset oligodendrocyte myelination, while Gpr17 overexpression inhibits oligodendrocyte differentiation and maturation both *in vivo* and *in vitro*[26]. However, another study showed that treatment with the endogenous GPR17 ligand UDP-glucose promotes the differentiation of OPCs into mature myelin basic protein-positive oligodendrocytes[30]. Follow-up studies further showed that GPR17 is restricted to NG2- and O4-positive OPCs and immature oligodendrocytes. Activation with natural ligands inhibits cAMP production and this inhibition is reversed by siRNA knockdown of GPR17. It was hypothesized that GPR17 keeps OPC in immature stages by inhibiting cAMP formation early in differentiation. When a critical stage of OPC differentiation is reached, GPR17 is downregulated to allow the reversal of cAMP to appropriate levels necessary for terminal maturation[31,32]. These studies together suggest that GPR17 is an intrinsic timer that controls OPC maturation.

Notch

The involvement of Notch signaling in OPC differentiation in both development and pathological conditions has been extensively studied. The canonical pathway through Jagged–Notch binding promotes the specification of oligodendrocyte-lineage cells from neural precursor cells while inhibiting their further differentiation into mature oligodendrocytes[33,34]. In MS, Jagged is re-expressed in astrocytes at the borders of active lesions[35]. Conditional ablation of Notch from Olig1-positive oligodendroglial cells results in accelerated remyelination after LPC-induced focal demyelination in the corpus callosum[36]. An earlier study using 2',3'-cyclic nucleotide phosphodiesterase promoter (CNP)-cre-driven deletion failed to show any effect on the rate of remyelination[37]. This discrepancy of outcomes may be due to the promoter used to drive the deletion of Notch, since CNP is only expressed from premature oligodendrocytes onwards. Furthermore, the non-canonical ligand contactin enhances oligodendrocyte differentiation and initiates the wrapping of contactin-expressing cells[33]. Therefore, the role of Notch signaling in the regulation of myelination is more complicated than first anticipated.

Wnt Signaling

The importance of Wnt signaling in postnatal myelination

was discovered in a genome-wide screen of oligodendrocyte-derived transcription factors, which identified Tcf4, an effector of the Wnt–β-catenin pathway, as one such factor[38]. Tcf4 is expressed specifically in OPCs and is down-regulated in maturing oligodendrocytes. Enforced expression of constitutive β-catenin leads to delayed differentiation of OPCs in development. Dissociation of Tcf4/β-catenin through developmental down-regulation of Tcf4 itself, upregulation of the β-catenin antagonist adenomatous polyposis coli or competition for β-catenin binding by histone deacetylases or Groucho/Tle1, drives oligodendrocytes to differentiate and myelinate[39]. All these data suggest that promoting the degradation of β-catenin and thus blocking the Wnt pathway in OPCs may be an effective approach to facilitating OPC differentiation and myelination. This hypothesis has been tested in recent studies using pharmacological inhibitors of the Wnt pathway. Axin2 is a target of Wnt transcriptional activation that negatively feeds back on the pathway, promoting β-catenin degradation. The small-molecule inhibitor XAV939, which inhibits the enzymatic activity of tankyrase, stabilizes Axin2 levels in OPCs from the CNS and accelerates their differentiation and myelination after LPC-induced demyelination in cerebellar slice culture and *in vivo*[40]. Together, these findings indicate that Axin2 is an important regulator of remyelination and that tankyrase could be a good drug target for myelination.

Extrinsic Signals for OPC Differentiation and Myelination

Several signals in the environment also regulate OPC differentiation and myelination; many are inhibitory, such as the secreted factors BMP4 and LINGO expressed in neurons. It has been suggested that in MS, a prevailing non-permissive environment due to high levels of several inhibitory environmental cues, hampers OPC differentiation and remyelination.

Bone Morphogenetic Protein 4

BMP4 belongs to the TGFβ family. It promotes astroglial lineage determination at the expense of oligodendrogliogenesis and inhibits the differentiation of OPCs[41,42]. More recent studies showed that BMP4 is increased during demyelination. Besides, infusion of noggin, its natural antagonist, promotes oligodendrogenesis in the subventricular zone and increases the density of mature oligodendrocytes and remyelinated axons in the remyelinating corpus callosum[43,44]. Thus, inhibition of endogenous BMP signaling during demyelination promotes mature oligodendrocyte regeneration and remyelination. The interactions among the BMP, Notch, and Wnt signaling pathways have been reported. BMP4 increases the levels of Wnt (*Tbx3*) and Notch target genes (*Jag1*, *Hes1*, *Hes5*, *Hey1*, and *Hey2*)[45]. BMP and Wnt–β-catenin also upregulate ID2[42,46], an inhibitory factor for OPC differentiation, thereby enhancing the cross-talk among signaling pathways that are known to inhibit myelination and repair.

LINGO-1

LINGO-1 (leucine-rich repeat and Ig domain containing NOGO receptor interacting protein-1) is a CNS-specific protein expressed on both neurons and oligodendroglial cells. It forms a complex with the Nogo receptor and inhibits neurite outgrowth[47]. Blocking LINGO-1 leads to axonal regeneration, increased neuronal survival and functional recovery in models of spinal cord injury both *in vitro* and *in vivo*[47]. LINGO-1 was recently also identified as a negative regulator of OPC differentiation. In complex with TrkA, it inhibits OPC differentiation during development. Attenuation of LINGO-1 function in OPCs by siRNA, dominant-negative LINGO-1, LINGO-1-Fc or LINGO knockout, invariably promotes OPC differentiation and myelination *in vitro*[48-51]. Blockade of LINGO-1 signaling promotes myelination in OPC-DRG co-cultures and LPC-treated cerebellar slice cultures[50,52]. Injection of a LINGO-1-blocking antibody leads to remyelination in the EAE and LPC focal injection models. In MS lesions, LINGO-1 is expressed by reactive astrocytes, macrophages/microglia and neurons[53]. All these data support the suggestion that antagonizing LINGO-1 provides a therapeutic approach that favors both neuroprotection and remyelination. Indeed, an anti-LINGO-1 antibody is undergoing phase I clinical trials for treating MS.

Cell Adhesion Molecules

Myelination requires precisely-controlled cell-cell interactions and several cell adhesion molecules have been reported to be involved in this process.

Neural cell adhesion molecule (NCAM) and L1 cell adhesion molecule (L1CAM) are both members of the immunoglobulin superfamily. They are involved in many aspects of nervous system development, including axonal outgrowth and fasciculation, neuronal migration and survival, and synapse formation[54]. Polysialic acid NCAM (PSA-

NCAM) is a post-translationally-modified version of NCAM existing on both oligodendrocytes and axons[55-58]. Removal of PSA from axons[59] and oligodendrocytes[60] is a prerequisite for the initiation of myelination during development. In MS, PSA-NCAM is re-expressed on denuded axons in the plaque, and this could act as an inhibitor of remyelination and participate in disease progression[61].

L1CAM has also been reported to be involved in the initiation of myelin formation[62,63]. It is downregulated on myelinated axons and re-expressed in regenerating axons after spinal cord injury[64,65]. Exogenous L1CAM is beneficial in promoting axon growth and functional recovery after spinal cord injury[66] and optic nerve lesion[67] and is involved in the regenerative growth of Purkinje cell axons *in vivo*[68]. These data suggest that L1CAM is capable of overcoming the anti-regeneration cues in adult CNS lesions.

In addition to the above pathways, other factors have also been reported to play a role in regulating OPC differentiation and myelination, such as the protein tyrosine phosphatases Dusp15/VHY and PTPRZ, transcription factor nuclear factor IA, teneurin 4, and sema 6A[69-72]. As there are multiple players involved in different layers regulating OPC differentiation and myelination, the whole process is quite complicated and is far from being clearly worked out. One therefore needs to consider several general criteria in selecting a good drug target to promote remyelination: first, the protein or the pathway is dysregulated in MS; second, the protein is a druggable target, e.g. transcription factors are usually not druggable; third, intervention on the target has minimal side-effects (e.g. the target is not expressed everywhere); and fourth, the drug, when successfully developed, must be able to enter the brain to promote remyelination. Targets that fit all these criteria are rather scarce, which illustrates the difficulty in developing new therapies for remyelination, let alone the other challenges discussed below.

Challenges

The forgoing discussion reviewed the exciting discoveries in the field of oligodendrocyte development from which novel targets related to myelination may emerge. However, selection of a target with a strong scientific rationale is only the beginning, albeit an essential step in the long and arduous journey of drug development. Building a bridge across the basic science of how oligodendrocytes are developed and how myelin is laid down and the clinical science of how myelination (structure) and synaptic transmission (function) are measured will be essential. The history of neuroscience drug research is littered with potential drugs that failed in clinical trials due to a lack of efficacy either because of the absence of translatability between preclinical animal models and human systems, or a flawed biomarker that turned out to be poorly reflective of efficacy in pivotal trials. The lesson from a slew of expensive failures, if any, would be that understanding the neurobiology and neuropathology of human disease and developing the right measuring tools are absolutely critical to successful drug development.

In this regard, a great deal has emerged from studying the post-mortem brain tissues from patients with MS. Pathological studies of MS lesions spearheaded by Luccinetti, Ransohoff and Trapp's groups among others have shed new light on the presence of cortical demyelinating lesions[73-76]. In addition, several groups have reported pervasive subpial or meningeal inflammatory cell aggregates akin to ectopic germinal centers in the vicinity of cortical lesions[76,77]. Based on the anatomical proximity, it is plausible that inflammatory cells trafficking into the CNS through the choroid plexus may set up sites of chronic inflammation atop the cortex within the blood-brain barrier and contribute to grey-matter demyelination. This hypothesis remains to be tested in experimental systems, but if supported, would provide a rationale for therapies that target innate lymphoid tissue cells which are the initial seeds for these structures[78]. In addition to the traditional staining of brain sections, modern molecular techniques are being used to study the contents of the MS lesions. Despite concerns about how tissues are preserved for this purpose and the caveat that post-mortem tissues may reflect only the end-game rather the ongoing disease process, some limited initial transcriptomic and 2D-mass spectrometry proteomic profiling data have been reported[79]. A cursory examination of the data reveals an enrichment of proteins important for material transport, structural integrity and adhesion. But data-mining of this treasure trove with bioinformatic tools will no doubt yield new clues about the myelination and neurodegeneration process that may one day contribute to new therapeutic targets.

The demonstration of successful remyelination in MS patients cannot be achieved without significant advances in

clinical radiology. Several pioneers have developed novel imaging techniques (such as magnetic transfer ratio and diffuse tensor imaging) that measure myelin content and tissue organization using conventional magnetic resonance imagers[80-83]. As these techniques become more standardized and gain wider use, they will greatly enhance our ability to read the progression of the myelination process in patients. In fact, some late-phase trials have included pilot studies on these readouts just to gain experience on the nuances. Moreover, the proposed anti-LINGO proof-of-concept trial will use some of these imaging parameters as early indications of myelination efficacy (Biogen Idec public disclosure). Together with measurements of regional/cortical atrophy and retinal nerve fiber layer thickness, it is conceivable that we will soon be able to have a full array of imaging readouts from which to choose to assess the efficacy of a potential therapy on myelination and neurorepair in patients with MS.

Conclusions and Future Directions

Despite great success in combating relapses over the past two decades, we are still on a long way from significantly halting and reversing the progression of disability in MS. Finding the therapeutic approaches that provide for CNS repair remains a great challenge. Our understanding of how myelination in the CNS is regulated has greatly advanced in the past decade. The combined use of genetics, transcriptome analysis, *in vitro* and *in vivo* models has revealed many ligands and transcription factors in the myelination process. These findings and tools provide the foundations for drug discovery. It is noteworthy that phenotypic screening remains an essential tool for drug discovery[84,85]. However, translating the knowledge of oligodendrocyte development and myelination into therapeutic approaches remains a major challenge. A lack of translation across species is one concern. In addition, whether the mechanism of remyelination in disease recapitulates that in development is also a big question. Encouragingly, however, many of the mechanisms thus far identified as controlling developmental myelination appear to operate for remyelination in animal models. Scientists from the bench to the bedside are now coming together to mount a concerted effort to translate research into effective CNS-protective and restorative therapies. The time is ripe for this critical mass to produce a breakthrough.

Received date: 2012-11-04; Accepted date: 2013-02-06

REFERENCES

[1] Kuhlmann T, Miron V, Cui Q, Wegner C, Antel J, Brück W. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 2008, 131:1749–1758.

[2] Wolswijk G. Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. J Neurosci 1998, 18:601–609.

[3] Chang A, Tourtellotte W, Rudick R, Trapp B. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 2002, 346:165–173.

[4] Weiner H. A shift from adaptive to innate immunity: a potential mechanism of disease progression in multiple sclerosis. J Neurol 2008, 255: 3–11.

[5] Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Annals Neurol 2000, 47: 707–717.

[6] Xiao L, Guo D, Hu C, Shen W, Shan L, Li C, *et al.* Diosgenin promotes oligodendrocyte progenitor cell differentiation through estrogen receptor-mediated ERK1/2 activation to accelerate remyelination. Glia 2012, 60: 1037–1052.

[7] Silvestroff L, Bartucci S, Pasquini J, Franco P. Cuprizone-induced demyelination in the rat cerebral cortex and thyroid hormone effects on cortical remyelination. Exp Neurol 2012, 235: 357–367.

[8] Franco P, Silvestroff L, Soto E, Pasquini J. Thyroid hormones promote differentiation of oligodendrocyte progenitor cells and improve remyelination after cuprizone-induced demyelination. Exp Neurol 2008, 212: 458–467.

[9] Raff M, Lillien L, Richardson W, Burne J, Noble M. Platelet derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. Nature 1988, 333: 562–565.

[10] Watkins T, Emery B, Mulinyawe S, Barres B. Distinct stages of myelination regulated by gamma-secretase and astrocytes in a rapidly myelinating CNS coculture system. Neuron 2008, 60: 555–569.

[11] Jeffery N, Blakemore W. Remyelination of mouse spinal cord axons demyelinated by local injection of lysolecithin. J Neurocytol 1995, 24: 775–781.

[12] Matsushima G, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol 2001, 11: 107–116.

[13] Kirby BB, Takada N, Latimer AJ, Shin J, Carney TJ, Kelsh

RN, *et al. In vivo* time-lapse imaging shows dynamic oligodendrocyte progenitor behavior during zebrafish development. Nat Neurosci 2006, 9: 1506–1511.

[14] Lyons DA, Naylor SG, Scholze A, Talbot WS. Kif1b is essential for mRNA localization in oligodendrocytes and development of myelinated axons. Nat Genet 2009, 41: 854–858.

[15] Harsan L, Steibel J, Zaremba A, Agin A, Sapin R, Poulet P, *et al.* Recovery from chronic demyelination by thyroid hormone therapy: myelinogenesis induction and assessment by diffusion tensor magnetic resonance imaging. J Neurosci 2008, 28: 14189–14201.

[16] Kondo T, Raff M. Basic helix-loop-helix proteins and the timing of oligodendrocyte differentiation. Development 2000, 127: 2989–2998.

[17] Lu Q, Sun T, Zhu Z, Ma N, Garcia M, Stiles C, *et al.* Common developmental requirement for olig function indicates a motor neuron/oligodendrocyte connection. Cell 2002, 109: 75–86.

[18] Lu QR, Park JK, Noll E, Chan JA, Alberta J, Yuk D, *et al.* Oligodendrocyte lineage genes (*OLIG*) as molecular markers for human glial brain tumors. Proc Natl Acad Sci U S A 2001, 98: 10851–10856.

[19] Qi Y, Cai J, Wu Y, Wu R, Lee J, Fu H, *et al.* Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor. Development 2001, 128: 2723–2733.

[20] Zhou Q, Wang S, Anderson DJ. Identification of a novel family of oligodendrocyte lineage-specific basic helix–loop–helix transcription factors. Neuron 2000, 25: 331–343.

[21] He Y, Dupree J, Wang J, Sandoval J, Li J, Liu H, *et al.* The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation. Neuron 2007, 55: 217–230.

[22] Finzsch M, Stolt CC, Lommes P, Wegner M. Sox9 and Sox10 influence survival and migration of oligodendrocyte precursors in the spinal cord by regulating PDGF receptor alpha expression. Development 2008, 135: 637–646.

[23] Wang S, Dulin J, Wu H, Hurlock E, Lee S, Jansson K, *et al.* An oligodendrocyte-specific zinc-finger transcription regulator cooperates with Olig2 to promote oligodendrocyte differentiation. Development 2006, 133: 3389–3398.

[24] Emery B, Agalliu D, Cahoy JD, Watkins TA, Dugas JC, Mulinyawe SB, *et al.* Myelin gene regulatory factor is a critical transcriptional regulator required for CNS myelination. Cell 2009, 138: 172–185.

[25] Shen YH, Avila RL, Emery B, Traka M, Lin W, Watkins T, *et al.* Zfp191 is required by oligodendrocytes for CNS myelination. Genes Dev 2010, 24: 301–311.

[26] Chen Y, Wu H, Wang S, Koito H, Li J, Ye F, *et al.* The oligodendrocyte-specific G protein-coupled receptor Gpr17 is a cell-intrinsic timer of myelination. Nat Neurosci 2009, 12: 1398–1406.

[27] Koenning M, Jackson S, Hay CM, Faux C, Kilpatrick TJ, Willingham M, *et al.* Myelin gene regulatory factor is required for maintenance of myelin and mature oligodendrocyte identity in the adult CNS. J Neurosci 2012, 32: 12528–12542.

[28] Huang J, Jarjour A, Oumesmar B, Kerninon C, Williams A, Krezel W, *et al.* Retinoid X receptor gamma signaling accelerates CNS remyelination. Nat Neurosci 2011, 14: 45–55.

[29] Xin M, Yue T, Ma Z, Wu FF, Gow A, Lu QR. Myelinogenesis and axonal recognition by oligodendrocytes in brain are uncoupled in Olig1-null mice. J Neurosci 2005, 25: 1354–1365.

[30] Lecca D, Trincavelli ML, Gelosa P, Sironi L, Ciana P, Fumagalli M, *et al.* The recently identified P2Y-like receptor GPR17 is a sensor of brain damage and a new target for brain repair. PLoS One 2008, 3: 1–15.

[31] Fumagalli M, Daniele S, Lecca D, Lee P, Parravicini C, Fields R, *et al.* Phenotypic changes, signaling pathway, and functional correlates of GPR17-expressing neural precursor cells during oligodendrocyte differentiation. J Biol Chem 2011, 286: 10593–10604.

[32] Ceruti S, Viganò F, Boda E, Ferrario S, Magni G, Boccazzi M, *et al.* Expression of the new P2Y-like receptor GPR17 during oligodendrocyte precursor cell maturation regulates sensitivity to ATP-induced death. Glia 2011, 59: 363–378.

[33] Wang S, Sdrulla AD, diSibio G, Bush G, Nofziger D, Hicks C, *et al.* Notch receptor activation inhibits oligodendrocyte differentiation. Neuron 1998, 21: 63–75.

[34] Hu QD, Ang BT, Karsak M, Hu WP, Cui XY, Duka T, *et al.* F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. Cell 2003, 115: 163–175.

[35] John G, Shanker S, Shafit-Zagardo B, Massimi A, Lee SC, Raine CS, *et al.* Multiple sclerosis: re-expression of a developmental pathway that restricts oligodendrocyte maturation. Nat Med 2002, 8: 1115–1121.

[36] Zhang Y, Argaw AT, Gurfein BT, Zameer A, Snyder BJ, Ge C, *et al.* Notch1 signaling plays a role in regulating precursor differentiation during CNS remyelination. Proc Natl Acad Sci U S A 2009, 106: 19162–19167.

[37] Stidworthy MF, Genoud S, Li WW, Leone DP, Mantei N, Suter U, *et al.* Notch1 and Jagged1 are expressed after CNS demyelination, but are not a major rate-determining factor during remyelination. Brain 2004, 127: 1928–1941.

[38] Fancy SP, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, *et al.* Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. Genes Dev 2009, 23: 1571–1585.

[39] Ye F, Chen Y, Hoang T, Montgomery R, Zhao X, Bu H, *et al.* HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting β-Catenin-TCF interaction. Nat Neurosci 2009, 12: 829–838.

[40] Fancy S, Harrington E, Yuen T, Silbereis J, Zhao C, Baranzini S, *et al.* Axin2 as regulatory and therapeutic target in new-

born brain injury and remyelination. Nat Neurosci 2011, 14: 1009–1016.

[41] Gross R, Mehler M, Mabie P, Zang Z, Santschi L, Kessler J. Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. Neuron 1996, 17: 595–606.

[42] Samanta J, Kessler J. Interactions between ID and OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. Development 2004, 131: 4131–4142.

[43] Sabo J, Aumann T, Merlo D, Kilpatrick T, Cate H. Remyelination is altered by bone morphogenic protein signaling in demyelinated lesions. J Neurosci 2011, 31: 4504–4510.

[44] Cate H, Sabo J, Merlo D, Kemper D, Aumann T, Robinson J, *et al.* Modulation of bone morphogenic protein signalling alters numbers of astrocytes and oligodendroglia in the subventricular zone during cuprizone-induced demyelination. J Neurochem 2010, 115: 11–22.

[45] Wu M, Hernandez M, Shen S, Sabo JK, Kelkar D, Wang J, *et al.* Differential modulation of the oligodendrocyte transcriptome by sonic hedgehog and bone morphogenetic protein 4 via opposing effects on histone acetylation. J Neurosci 2012, 32: 6651–6664.

[46] Cheng X, Wang Y, He Q, Qiu M, Whittemore SR, Cao Q. Bone morphogenetic protein signaling and olig1/2 interact to regulate the differentiation and maturation of adult oligodendrocyte precursor cells. Stem Cells 2007, 25: 3204–3214.

[47] Mi S, Lee X, Shao Z, Thill G, Ji B, Relton J, *et al.* LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat Neurosci 2004, 7: 221–228.

[48] Mi S, Miller RH, Lee X, Scott ML, Shulag-Morskaya S, Shao Z, *et al.* LINGO-1 negatively regulates myelination by oligodendrocytes. Nat Neurosci 2005, 8: 745–751.

[49] Mi S, Hu B, Hahm K, Luo Y, Kam Hui ES, Yuan Q, *et al.* LINGO-1 antagonist promotes spinal cord remyelination and axonal integrity in MOG-induced experimental autoimmune encephalomyelitis. Nat Med 2007, 10: 1228–1233.

[50] Lee X, Yang Z, Shao Z, Rosenberg SS, Levesque M, Pepinsky RB, *et al.* NGF regulates the expression of axonal LINGO-1 to inhibit oligodendrocyte differentiation and myelination. J Neurosci 2007, 27: 220–225.

[51] Zhao XH, Jin WL, Ju G. An *in vitro* study on the involvement of LINGO-1 and Rho GTPases in Nogo-A regulated differentiation of oligodendrocyte precursor cells. Mol Cell Neurosci 2007, 36: 260–269.

[52] Mi S, Miller R, Tang W, Lee X, Hu B, Wu W, *et al.* Promotion of central nervous system remyelination by induced differentiation of oligodendrocyte precursor cells. Annals Neurol 2009, 65: 304–315.

[53] Satoh J, Tabunoki H, Yamamura T, Arima K, Konno H. TROY and LINGO-1 expression in astrocytes and macrophages/microglia in multiple sclerosis lesions. Neuropathol Appl Neurobiol 2007, 33: 99–107.

[54] Maness P, Schachner M. Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. Nat Neurosci 2007, 10: 19–27.

[55] Trotter J, Bitter-Suermann D, Schachner M. Differentiation-regulated loss of the polysialylated embryonic form and expression of the different polypeptides of the neural cell adhesion molecule by cultured oligodendrocytes and myelin. J Neurosci Res 1989, 22: 369–383.

[56] Seki T, Arai Y. Expression of highly polysialylated NCAM in the neocortex and piriform cortex of the developing and the adult rat. Anat Embryol (Berl) 1991, 184: 395–401.

[57] Seki T, Arai Y. Distribution and possible roles of the highly polysialylated neural cell adhesion molecule (NCAM-H) in the developing and adult central nervous system. Neurosci Res 1993, 17: 265–290.

[58] Oumesmar BN, Vignais L, Duhamel-Clerin E, Avellana-Adalid V, Rougon G, Baron-Van Evercooren A. Expression of the highly polysialylated neural cell adhesion molecule during postnatal myelination and following chemically induced demyelination of the adult mouse spinal cord. Eur J Neurosci 1995, 7: 480–491.

[59] Charles P, Hernandez MP, Stankoff B, Aigrot MS, Colin C, Rougon G, *et al.* Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. Proc Natl Acad Sci U S A 2000, 97: 7585–7590.

[60] Fewou SN, Ramakrishnan H, Bussow H, Gieselmann V, Eckhardt M. Down-regulation of polysialic acid is required for efficient myelin formation. J Biol Chem 2007, 282: 16700–16711.

[61] Charles P, Reynolds R, Seilhean D, Rougon G, Aigrot MS, Niezgoda A, *et al.* Re-expression of PSA-NCAM by demyelinated axons: an inhibitor of remyelination in multiple sclerosis? Brain 2002, 125:1972–1979.

[62] Laursen L, Chan C, Ffrench-Constant C. An integrin–contactin complex regulates CNS myelination by differential Fyn phosphorylation. J Neurosci 2009, 29: 9174–9185.

[63] White R, Gonsior C, Krämer-Albers E, Stöhr N, Hüttelmaier S, Trotter J. Activation of oligodendroglial Fyn kinase enhances translation of mRNAs transported in hnRNP A2-dependent RNA granules. J Cell Biol 2009, 181: 579–586.

[64] Kubasak MD, Hedlund E, Roy RR, Carpenter EM, Edgerton VR, Phelps PE. L1 CAM expression is increased surrounding the lesion site in rats with complete spinal cord transection as neonates. Exp Neurol 2005, 194(2): 363–375.

[65] Martini R, Schachner M. Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and myelin-associated glycoprotein) in regenerating adult mouse

sciatic nerve. J Cell Biol 1988, 106: 1735–1746.

[66] Roonprapunt C, Huang W, Grill R, Friedlander D, Grumet M, Chen S, *et al.* Soluble cell adhesion molecule L1-Fc promotes locomotor recovery in rats after spinal cord injury. J Neurotrauma 2003, 20: 871–882.

[67] Xu G, Nie DY, Wang WZ, Zhang PH, Shen J, Ang BT, *et al.* Optic nerve regeneration in polyglycolic acid-chitosan conduits coated with recombinant L1-Fc. Neuroreport 2004, 15: 2167–2172.

[68] Zhang Y, Bo X, Schoepfer R, Holtmaat AJ, Verhaagen J, Emson PC, *et al.* Growth-associated protein GAP-43 and L1 act synergistically to promote regenerative growth of Purkinje cell axons *in vivo*. Proc Natl Acad Sci U S A 2005, 102: 14883–14888.

[69] Schmidt F, Eijnden M, Gobert R, Saborio G, Carboni1 S, Alliod C, *et al.* Identification of VHY/Dusp15 as a regulator of oligodendrocyte differentiation through a systematic genomics approach. PLoS One 2012, 7: e40457.

[70] Bernard F, Moreau-Fauvarque C, Heitz-Marchaland C, Zagar Y, Dumas L, Fouquet S, *et al.* Role of transmembrane semaphorin Sema6A in oligodendrocyte differentiation and myelination. Glia 2012, 60: 1590–604.

[71] Fancy S**,** Glasgow S**,** Finley M**,** Rowitch D**,** Deneen B**.** Evidence that nuclear factor IA inhibits repair after white matter injury. Annals Neurol 2012, 72: 224–233.

[72] Kuboyama K, Fujikawa A, Masumura M, Suzuki R, Matsumoto M, Noda M. Protein tyrosine phosphatase receptor type z negatively regulates oligodendrocyte differentiation and myelination. PLoS One 2012, 7: e48797.

[73] Bö L, Vedeler CA, Nyland HI, Trapp BD, Mök SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. J Neuropathol Exp Neurol 2003, 62: 723–732.

[74] Peterson JW, Bö L, Mök S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol 2001, 50: 389–400.

[75] Kutzelnigg A, Lucchinetti CF, Stadekmann C, Brück W, Rauschka H, Bergmann M, *et al.* Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 2005, 128: 2705–2712.

[76] Lucchinetti CF, Popescu BF, Bunyan RF, Moll NM, Roemer SF, Lassmann H, *et al.* Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med 2011, 365: 2188–2197.

[77] Howell O, Reeves C, Nicholas R, Carassiti D, Radotra B, Gentleman S, *et al.* Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain 2011, 134: 2755–2771.

[78] Victora GD, Nussenzweig MC. Germinal centers. Annu Rev Immunol 2012, 30: 429–457.

[79] Han M, Huwang S, Roy D, Lundgren D, Price J, Ousman S, *et al.* Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. Nature 2008, 451: 1076–1081.

[80] MacKay A, Whittall K, Adler J, Li D, Paty D, Graeb D. *In vivo* visualization of myelin water in brain by magnetic resonance. Magn Reson Med 1994, 31: 673–677.

[81] Narayanan S, Francis SJ, Sled JG, Santos AC, Antel S, Levesque I, *et al.* Axonal injury in the cerebral normal-appearing white matter of patients with multiple sclerosis is related to concurrent demyelination in lesions but not to concurrent demyelination in normal-appearing white matter. Neuroimage 2006, 29: 637–642.

[82] Stankoff B, Freeman L, Aigrot MS, Chardain A, Dolle F, Williams A, *et al.* Imaging central nervous system myelin by positron emission tomography in multiple sclerosis using [methyl-(1)(1)C]-2-(4′-methylaminophenyl)-6-hydroxybenzothiazole. Ann Neurol 2011, 69: 673–680.

[83] Stankoff B, Wang Y, Bottlaender M, Aigrot MS, Dolle F, Wu C, *et al.* Imaging of CNS myelin by positron-emission tomography. Proc Natl Acad Sci U S A 2006, 103: 9304-9309.

[84] Joubert L, Foucault I, Sagot Y, Bernasconi L, Duval F, Alliod C, *et al.* Chemical inducers and transcriptional markers of oligodendrocyte differentiation. J Neurosci Res 2010, 88: 2546–2557.

[85] Taupin P. Thirteen compounds promoting oligodendrocyte progenitor cell differentiation and remyelination for treating multiple sclerosis: WO2010054307. Expert Opin Ther Pat 2010, 20: 1767–1773.