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Promoting peripheral myelin repair

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Abstract

Compared to the central nervous system (CNS), peripheral nerves have a remarkable ability to regenerate and remyelinate. This regenerative capacity to a large extent is dependent on and supported by Schwann cells, the myelin-forming glial cells of the peripheral nervous system (PNS). In a variety of paradigms, Schwann cells are critical in the removal of the degenerated tissue, which is followed by remyelination of newly-regenerated axons. This unique plasticity of Schwann cells has been the target of myelin repair strategies in acute injuries and chronic diseases, such as hereditary demyelinating neuropathies. In one approach, the endogenous regenerative capacity of Schwann cells is enhanced through interventions such as exercise, electrical stimulation or pharmacological means. Alternatively, Schwann cells derived from healthy nerves, or engineered from different tissue sources have been transplanted into the PNS to support remyelination. These transplant approaches can then be further enhanced by exercise and/or electrical stimulation, as well as by the inclusion of biomaterial engineered to support glial cell viability and neurite extension. Advances in our basic understanding of peripheral nerve biology, as well as biomaterial engineering, will further improve the functional repair of myelinated peripheral nerves.

Keywords

Schwann cell; remyelination; nerve injury; demyelinating neuropathy; nerve repair

Introduction

Peripheral nerves have a remarkable plasticity to regenerate and remyelinate allowing for functional recovery in affected body regions. As compared to target tissue re-innervation, remyelination of regenerated axons appears to be the simpler aspect of peripheral nerve repair, most of the studies in the nerve injury literature focus on promoting axon regeneration rather than remyelination. Nonetheless, in certain hereditary disorders and in

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Conflict of interest

LN holds a patent on liposome formulation cited in Lee et al., 2013 article.

selected toxin-induced nerve injury, myelin is the primary site of damage, sparing the axons at least during initial stages of the insult. If the Schwann cells are continuously stressed, such as in genetic disorders, axonal degeneration will ensue. Therefore, maintenance of healthy Schwann cells is of vital importance in peripheral nerve biology.

During peripheral nerve development, Schwann cells differentiate from neural crest cells to form non-myelinating and myelin-forming glia (Jessen and Mirsky, 2005). Non-myelinating Schwann cells can be further divided into Remak and terminal Schwann cells. Remak Schwann cells ensheath small diameter axons and typically associate with more than one fiber (Griffin and Thompson, 2008). Perisynaptic, terminal Schwann cells similarly do not form myelin and are believed to play a role in synaptic mechanisms. Distinct from oligodendrocytes, myelin-forming Schwann cells maintain a close, one-to-one relationship with individual axons, as each internodal myelin segment is formed by one glial cell. Each committed myelinating Schwann cell is postmitotic and adapts a polarized cellular architecture that is dependent on the correct expression of specific myelin proteins and membrane lipids (Scherer and Chance, 1995). Many aspects of peripheral nerve myelination are still under investigation, including the developmental mechanisms by which these various types of Schwann cells arise. Throughout lifespan, peripheral axons and glia are dependent on one-another for vitality and maintenance of the differentiated cellular phenotype. Therefore, attempts to repair peripheral nerve damage should include approaches that will benefit both axons and Schwann cells.

A wealth of knowledge about peripheral nerve biology and Schwann cell myelination has been obtained from studies in acute nerve injury paradigm. In response to crush or cut injuries, distal to the injury site peripheral nerves undergo the process of Wallerian degeneration, which is followed by neurite regeneration and remyelination (Scheib and Hoke, 2013). This inherent repair capacity of the PNS is evident in chronic conditions such as hereditary neuropathies which can involve hyperproliferation of Schwann cells and onion bulb formations, the results of repeated remyelination efforts. Unraveling the genetics of hereditary neuropathies and establishing animal models for the various PNS myelin proteins (Martini, 1997; Mathis et al., 2015) have provided key information for current efforts in myelin repair. Thorough understanding of the signaling mechanisms and the biological activities of peripheral nerve proteins, including those linked with peripheral neuropathies, are required for the design of effective and long-lasting myelin repair strategies that will support functional recovery.

In this review we will discuss approaches to repair peripheral nerve myelin damage in injury or genetic disease either through enhancing the endogenous capacity of Schwann cells to remyelinate, or through the application or transplantation of exogenous materials, such as pharmaceutics, growth factors, cells and/or scaffold material. A number of combinatorial approaches have also been explored with success, some of which will be cited here.

Remyelination after acute nerve injury

Remyelination is of great interest in post-injury regenerated peripheral nerves as it supports functional reinnervation of the target tissue. The early work of Aguayo and colleagues

elegantly demonstrated in multiple models that peripheral nerves are able to regenerate and regrow over long distances with fairly good accuracy (Aguayo et al., 1981), although the recovery of function is better after distal injuries. This unique regenerative property of PNS axons is dependent on environmental cues that are in large part derived from Schwann cells. After nerve transection injury, Schwann cells migrate out of the nerve stumps and through interactions with fibroblasts they provide a bridge for the regenerating axons (Parriinello et al., 2010). In addition to fibroblasts, a recent study identified newly-formed blood vessels that also support the directional migration of Schwann cells across the injury site, which in turn lays the framework for axon regrowth (Cattin et al., 2015). Therefore, the pro-regenerative response of Schwann cells to nerve injury is facilitated by intercellular interactions with fibroblasts as well as with newly-formed polarized vasculature across the injury site.

Schwann cells react robustly to injury by secreting a number of growth factors to support glial and neuronal survival, as well as axon regrowth (Brosius Lutz and Barres, 2014). Molecular signaling mechanisms involved in this event have recently been reviewed by Glenn and Talbot and will not be discussed here in detail (Glenn and Talbot, 2013). A critical event in post nerve injury repair is the removal of myelin debris, which in the PNS in large part is accomplished by Schwann cells, along with blood-borne macrophages. During Wallerian degeneration, the removal of damaged axons and myelin allows for nerve fiber regrowth which are then myelinated by transdifferentiated Schwann cells (Arthur-Farraj et al., 2012). Until recently it was believed that in the injured nerve Schwann cells dedifferentiate to an immature, early development-like cellular phenotype before reestablishing contact with the regenerated axons. Recent studies examining the molecular phenotype of such cells however point to a transdifferentiation paradigm that represents a distinct repair phenotype (Arthur-Farraj et al., 2012). With regards to the quality of the newly-formed myelin there are some ambiguities in the field. Some studies indicate that the newly-formed myelin is significantly thinner than myelin formed during development, as judged from analysis of g ratios (Axon diameter/fiber diameter) (Schroder, 1972). It has also been observed that the remyelinated internodes are irregular with typically shorter and thinner myelin segments than would be expected based on nerve fiber diameter (Griffin and Thompson, 2008). Nonetheless, a recent study using genetic marking of remyelinating cells found prolonged maturation and refinement of regenerated myelin, which was evident for thickness as well as internodal length (Powers et al., 2013). This finding points to the inherent plasticity of myelinating Schwann cells, as well as the protracted timeline of functional recovery post nerve injury.

An understudied aspect of peripheral nerve injury is the contribution of unmyelinated Schwann cells to nerve repair. Nerve transection studies indicate Remak and synaptic Schwann cells alike respond during Wallerian degeneration by reentering the cell cycle and proliferation (Griffin and Thompson, 2008). These newly-formed Schwann cells can remyelinate demyelinated axons, a suggestion that is based on morphological observations (Griffin et al., 1987). Glia at the nerve terminal primarily respond by process extension, which serves to guide the regrowing axons to target tissue. Denervated Schwann cells also release growth factors in a fiber-type specific pattern, with unique signatures of sensory and motor fibers (Hoke et al., 2006). Understanding the mechanisms underlying the inherent

endogenous repair capacity of Schwann cells has allowed for strategies to promote efficient myelin repair in acute nerve injury paradigms, as well as in hereditary demyelinating disorders.

Myelin dysfunction in hereditary neuropathies

Distinct from the acute injury paradigms, myelin can be damaged chronically by progressive hereditary neuropathies such as Charcot-Marie-Tooth (CMT) diseases. CMTs affect up to 1 in 1,214 in the general population (Mathis et al., 2015) and involve progressive dysfunction of lower motor and sensory nerves leading to distal muscle weakness or atrophy, typically accompanied with reflex reduction, foot deformities and sensory impairments (Szigeti and Lupski, 2009). Based on genetic causes, CMT can be divided into nine groups (CMT1–6, CMTDI, CMTRI and CMTX), among which CMT1, CMT2 and CMTX are the most frequent subtypes (Mathis et al., 2015). Type I demyelinating CMTs can be further classified into six subtypes (CMT1A–F) according to genetic mutations (Szigeti and Lupski, 2009). CMT1A is the most prevalent form (70%) and typically linked with duplication of the peripheral myelin protein 22 (PMP22) gene (Szigeti and Lupski, 2009). CMT1B (6%–10%), the second most common form results from point mutations in myelin protein zero (*MPZ*) (Bird, 1993). According to a recent genetic testing of over 17,000 CMT patients, approximately 90% of affected individuals have mutations in just three genes: *PMP22, MPZ and GJB1*; with 56.7% of patients having PMP22 duplications and 21.9% with PMP22 deletions (DiVincenzo et al., 2014). Nonetheless, even within this group there is heterogeneity in disease phenotypes with regards to age of onset, severity and alteration in nerve conduction velocities (Shy et al., 2004). A related and relatively rare facial neuropathy that can be associated with CMT or multiple sclerosis is trigeminal neuralgia (Tomasello et al., 2008; Zhang and Meng, 2015), which frequently involves compression of the trigeminal nerve and probable localized myelin damage.

Considering the genetically heterogeneous nature of demyelinating neuropathies and the wide range of biological activities of the proteins involved, two distinct approaches for therapy development can be envisioned. In one approach, global signaling mechanisms of peripheral nerve biology are targeted, while in the second approach therapy design is based on the underlying molecular pathogenesis of the demyelination. After identification of specific gene defects linked with CMTs, laboratories in the field explored such disease mechanism-based therapeutic approaches. For instance, in autosomal dominant CMT1A, efforts were directed toward correcting the expression of the disease protein, PMP22 (Rangaraju and Notterpek, 2011; Scherer and Chance, 1995). In conditions where one copy of the gene is mutated, removal of the abnormal, often misfolded protein has been attempted through activation of protein quality control mechanisms, namely the chaperone and autophagy-lysosomal pathways (Rangaraju and Notterpek, 2011). For supplementation of factors known to be beneficial for peripheral nerve biology such as ascorbate or growth factors, or a combination of drugs in a polytherapeutic strategy, the compounds have been administeed sytemically or via gene therapy. Below we highlight examples of therapies explored in post-injury myelin repair paradigms and in hereditary demyelinating neuropathies.

Trophic factors

Neurotrophic factors play critical roles during development, as well as in the maintenance and repair of the PNS. In response to nerve injury, there is a rapid increase in the expression of nerve growth factor (NGF) and the p75 neurotrophin receptor in the distal segment, while brain-derived neurotrophic factor (BDNF), and neurotrophin-4 (NT4) are only elevated 2-weeks post-injury (Chan et al., 2001). In comparison, the levels of NT-3 are reduced soon after injury and recover during the repair phase. Developmental studies revealed a similar differential expression of BDNF and NT3, with BDNF promoting myelination while NT3 having an inhibitory effect (Chan et al., 2001). Axonally-derived trophic factors, particularly neuregulin-1 type III (NRG1) is essential in Schwann cell maturation, differentiation and proliferation via erbB receptors (Terenghi, 1999). Discovering and understanding this growth factor have led to preclinical studies in neuropathic rodents and offer promises for future clinical trials.

BDNF and NT3

Multiple studies indicate that BDNF acting through the p75 neurotrophin receptor has a positive influence on Schwann cell myelination (Chan et al., 2001). To explore the therapeutic potential of this finding, Xiao and colleagues engineered a small peptide mimetic of BDNF that still binds p75 (Xiao et al., 2013). Although this reagent has not been tested in injury or disease models, the synthetic peptide enhanced myelination by Schwann cells in vivo and in vitro suggesting the applicability of this approach for peripheral myelin repair. NT3 is a crucial autocrine factor for Schwann cells and promotes the survival of differentiated Schwann cells in the absence of axons (Meier et al., 1999). During development, the depletion of NT3 leads to motor neuropathy in mice due to impaired Schwann cell survival (Sahenk et al., 2008; Woolley et al., 2005), while the same intervention in adult neuropathic TrJ mice enhances myelination (Liu et al., 2007). NT3 was tested in a small pilot clinical study of CMT1A patients where treatment with NT3 three times a week for 6 months showed good tolerance and efficacy with enhancement of myelinated fibers (Sahenk et al., 2005). Although these pilot results from 4 CMT1A patients with NT3 look promising, challenges with the delivery and short half-life of neurotrophins will need to be solved prior to larger clinical trials (Jerath and Shy, 2015).

NRG1

Soluble neurregulin-1 (NRG1) is a neuron-glia modulator that is produced primarily in neurons and acts on Schwann cells via erbB tyrosine kinase receptors (Garratt et al., 2000). NRG1-erbB signaling is critical in regulating PNS myelin thickness during the entire lifespan (Garratt et al., 2000). After nerve injury, both axon- and Schwann cell-derived NRG1 are required for remyelination and axon regeneration, as suggested by findings in transgenic mice (Fricker et al., 2011; Stassart et al., 2013). The therapeutic potential of NRG1 was recently explored in CMT1A by crossbreeding neuropathic mice with transgenic NRG1 mice (Fledrich et al., 2014). Analyses of the double transgenic mice revealed pronounced improvement in the neuropathic phenotypes in the CMT1A mice, as determined by biochemical, morphological and physiological measurements. This study also demonstrates that treatment with soluble NRG1 at early postnatal ages (P6–18) provides

long-term benefits to affected rodents, supporting the suitability of NRG1 for consideration in human trials.

Small molecule therapies

ApoE mimetic

Apolipoprotein E (ApoE) was shown to be involved in peripheral nerve repair by promoting the recycling of cholesterol (Skene and Shooter, 1983). Expanding on this idea, studies by Li and colleagues created an ApoE-mimetic peptide and applied it to 8-week old mice in a nerve crush injury model (Li et al., 2010). As predicted, the ApoE mimetic promoted nerve regeneration post crush injury as measured by morphological and biochemical parameters and also enhanced myelination of the regenerated nerve fibers. The authors hypothesized that the beneficial effects of the ApoE mimetic were due to the positive influence of ApoE on cholesterol and lipid reutilization in the regenerating nerve. Based on in vitro studies they discovered that the ApoE mimetic enhanced the uptake of cholesterol-containing low-density lipoprotein particles by Schwann cells, which promotes remyelination. These findings recommend further testing of the described ApoE mimetic in peripheral demyelinating conditions where the Schwann cells are the primary culprit of the pathology.

Ascorbic acid

Ascorbic acid (vitamin C) was shown to improve myelination by rat Schwann cells co-cultured with neurons (Bunge et al., 1980) and subsequently identified as a critical factor for Schwann cell basal lamina assembly by linking hydroxyl-proline residues (Eldridge et al., 1987). Based on this knowledge, in 2004 ascorbic acid was tested in a CMT1A mouse model where positive results on ameliorating disease phenotype and reducing PMP22 expression were reported (Passage et al., 2004). These findings quickly led to several clinical trials with ascorbic acid, an FDA-approved drug. While Kaya *et al.* discovered that in vitro ascorbic acid suppresses PMP22 expression via reducing cAMP and adenylate cyclase activity (Kaya et al., 2007), in several double-blind clinical trials ascorbic acid was ineffective in ameliorating disease phenotypes (Burns et al., 2009; Lewis et al., 2013; Micallef et al., 2009; Pareyson et al., 2011; Verhamme et al., 2009). Potential explanations for the discrepancies between the animal and human studies include the outcome parameters used in the human studies, as well as the duration of the treatment regimen (Gess et al., 2015). It is possible that longer studies and more sensitive outcome measures would reveal benefits of ascorbate on neuropathy progression.

Progesterone antagonist

Similar to ascorbic acid, studies in cultured Schwann cells and in young-adult rats identified progesterone to stimulate P0 and PMP22 gene expression (Desarnaud et al., 1998; Melcangi et al., 1999). In 2003, Sereda and colleagues administrated progesterone to a PMP22-overproducing CMT1A rat and confirmed elevated PMP22 mRNA levels in sciatic nerves after treatment (Sereda et al., 2003). In contrast subcutaneous injection for 7 weeks of onapristone, an FDA-proved progesterone receptor antagonist, decreased PMP22 gene expression and improved myelination and motor performance (Sereda et al., 2003). Similarly, administration of onapristone in older CMT1A rats for 5 months reproduced

similar positive results in ameliorating disease phenotypes (Meyer zu Horste et al., 2007). Considering the potential side effects of anti-progesterone drugs, especially in women and children, no progesterone antagonists have been tested in neuropathic patients.

Curcumin

Curcumin, a natural compound derived from curry spice turmeric, is known for its pleiotropic effects which include anti-oxidant and anti-inflammatory properties. In two distinct CMT1 rodent models, curcumin was tested for its potential influence on ER-stress via enhancement of chaperone expression (Khajavi et al., 2005). Oral administration of curcumin was effective in ameliorating neuropathic symptoms, including enhanced myelination, and improved motor performance in PMP22 mutant Trembler J mice (Khajavi et al., 2007). Similarly, utilizing R98C MPZ mutant mice, it was reported that curcumin mitigates peripheral neuropathy through alleviating ER stress (Patzko et al., 2012). In independent studies, curcumin relieved neuropathic pain induced by toxins or diabetes (Al Moundhri et al., 2013; Babu et al., 2015; Joshi et al., 2013; Zhao et al., 2014). While these positive reports in rodents are encouraging, various studies indicate that curcumin has a poor bioavailability, which limits its use in controlled clinical trials.

Rapamycin

Another natural compound with pleotropic activity is rapamycin (RM) that has the ability to influence the fate of misfolded proteins within cells, primarily through the induction of autophagy (Santos et al., 2011). RM is considered as a calorie restriction mimetic that promotes stress-induced pathways without reducing food intake and extends lifespan in a variety of species (Harrison et al., 2009). In explant cultures from neuropathic mice, the application of RM significantly enhanced myelination by an autophagy-dependent mechanism (Rangaraju et al., 2010). RM was also effective in facilitating the processing of PMP22 and reducing the levels of ubiquitinated protein aggregates. In subsequent studies RM was administered to neuropathic mice either by dietary supplementation or i.p. injections (Nicks et al., 2014). In both paradigms, peripheral nerves of RM-treated neuropathic mice showed significant improvements in myelin, however without benefit to neuromuscular function (Nicks et al., 2014). One explanation for this functional outcome might involve the distinct response of nerve and muscle tissue to the inhibition of mTOR (Quy et al., 2013). This study emphasizes the need for comprehensive assessment of neuromuscular function when potential drugs for PNS myelin repair are tested, particularly when systemic administration is necessary.

Chaperone enhancement

Besides the proteasome and autophagy-lysosomal pathways, chaperones function to assure the quality of protein folding and processing. Mutations in small chaperones are known to cause type II, axonal neuropathies pointing to the involvement of these molecules in PNS biology (Jerath and Shy, 2015). Heat shock protein 70 (HSP70) interacts with MBP, and in demyelinating neuropathies it is found along with PMP22 in cytosolic protein aggregates (Fortun et al., 2007). The entrapment of chaperones in protein aggregates led to attempts to enhance chaperone production in multiple neurodegenerative disease models through inhibition of HSP90 (Lindberg et al., 2015). Indeed, the induction of HSP70 and other

chaperones prevents the aggregation of the mutated or overproduced PMP22 and is beneficial for myelination (Chittoor-Vinod et al., 2015; Rangaraju et al., 2008). Interestingly, modulation of chaperones is also beneficial in rodent models of diabetic neuropathies (Ma et al., 2015), which supports the notion that this pathway is suitable for development in PNS disorders and injury.

Polytherapeutic approach for CMT1A

While several of the above mentioned compounds have pleiotropic affects, so far none of them led to significant improvements in neuropathic patients with myelin defects. In a recent systems biology-based approach a novel combination of three repurposed drugs, namely baclofen, naltrexone and sorbitol (named PXT3003) were tested in CMT1A animal models and in a phase 2 clinical trial (Attarian et al., 2014; Chumakov et al., 2014). Prior to the clinical trial, PXT3003 was studied in three distinct experimental models; including myelinating cocultures from CMT1A rats, transgenic PMP22 overproducing rats and in mouse nerve crush injury (Chumakov et al., 2014). In each of these experimental paradigms, the three combined drugs proved efficacies in improving myelination and ameliorating neuropathic phenotype, including lowering PMP22 message levels (Chumakov et al., 2014). Significantly, PXT3003 was shown to be safe, with good tolerability in a one-year long clinical trial with 80 adult CMT1A patients (Attarian et al., 2014). Although based on neuropathy assessment scale, only a relatively modest improvement (8–12%) was observed, this trial is the most promising treatment so far for hereditary demyelination (Attarian et al., 2014; Ekins et al., 2015). Electrophysiological data from this study suggests that PTX3003 slows disease course in the patients through improving myelin and protecting axons. Because of the positive results from the Phase II clinical trial (Attarian et al., 2014), Pharnext is launching an international, multi-center, randomized, double blind, placebo-controlled Phase III study with PTX3003. Since PTX3003 was effective in improving myelination in the nerve crush injury model (Chumakov et al., 2014) it is possible that this investigational Pleodrug will be beneficial in both chronic and acute demyelination paradigms.

Alternative therapies for myelin repair

Life style choices, such as diet and exercise are known to have pronounced influence on health. These two variables have been investigated in multiple aging paradigms, but there has been limited work on exploring the effects of diet and/or exercise in peripheral myelin repair.

Caloric restriction

In a previous study we examined nerves from rats aged from 8–38 months old and found that in animals kept on life-long calorie restricted diet myelin architecture, such as internodal and nodal organization was greatly preserved as compared to animals fed ad libitum (Rangaraju et al., 2009). Across lifespan, we also discovered a pronounced reduction in markers of oxidative damage in myelinated nerves of diet-restricted rodents (Opalach et al., 2010). In agreement with these aging studies, a five month long intermittent fasting regimen was highly beneficial in preserving peripheral nerve myelin and neuromuscular

function in TrJ neuropathic mice (Madorsky et al., 2009). In fact, morphological and biochemical analyses of sciatic nerves from diet restricted rodents revealed significant improvements in myelin thickness and myelin protein expression. Based on literature searches it appears that peripheral nerve injury repair studies have not been performed in animals on calorie restricted diet, however beneficial effects of intermittent, every-other-day feeding have been observed post-spinal cord injury (Jeong et al., 2011; Plunet et al., 2008). The beneficial effects of calorie restriction in part are the results of enhanced activity of endogenous protein quality control mechanisms, including the autophagy and chaperone pathways (Lee and Notterpek, 2013).

Exercise

While exercise is widely viewed as a valuable intervention for neuromuscular function, there are few studies on the benefits of exercise on nerve remyelination, either post-injury or in hereditary myelin disease. In a recent nerve transection study, exercise alone or in combination with electrical stimulation visibly promoted axon growth; however myelination was not assessed (Gordon and English, 2015). Interestingly these studies revealed a gender effect on the types of exercise used, with continues treadmill training benefitting male mice, while an interval training regimen being more advantageous in females. In another combinatorial post nerve transection treatment paradigm, treadmill exercise was paired with Schwann cell grafts (Goulart et al., 2014). In comparison to just repair of the injury, when Schwann cells with or without treadmill training were added, there were more regenerated fibers with myelin and the overall myelin area of the regenerated segment was increased. This study elegantly shows the added benefit of exercise on remyelination in comparison to Schwann cell transplantation alone. To date, the benefits and risks of exercise have not been systematically evaluated in neuropathic patients. A recent review of the literature focusing on CMT neuropathies have found that while exercise has benefits for strength and function, more studies are necessary to determine the optimal exercise modality and intensity for people with CMT (Sman et al., 2015).

Biomaterial engineering

Because of the accessibility of peripheral nerves for biopsy, and the relatively simple procedures to establish Schwann cell cultures, there are a large number of reports in the literature using these cells for the repair of myelin in the CNS (Lavdas et al., 2008). In the PNS, besides myelin repair, Schwann cells provide a permissive environment for nerve regeneration, which is the primary goal post-injury. There are multiple sources for generating Schwann cells, which include autologous cell grafts or cells differentiated from stem cells or reprogrammed somatic cells (Radtke et al., 2011). These cells can be used as vehicles to deliver genes or factors that promote functional nerve repair. In addition, there have been significant advances in the development of biomaterials that not only support neurite regeneration but can also hold transplanted cells in situ (Marquardt and Sakiyama-Elbert, 2013). Cells transplanted with or without supportive biomaterial, or the biomaterial itself can serve as release vehicles for permissive factors. Essentially there are limitless combinations of materials, cell types, and supportive factors that can be engineered and explored for localized myelin repair in the PNS. In hereditary demyelinating neuropathies,

when multiple nerves are affected throughout the body, systemic delivery of molecules and compounds is of interest using nanotechnology or alternative targeting technologies. Below we highlight some examples of cell transplantation strategies, with or without genetic engineering.

Cell transplantations

There are numerous sources for stem cells that in turn can be differentiated into Schwann cells (for review, see Ren et al., 2012). Mesenchymal stem cells (MSCs) obtained from bone marrow, or olfactory ensheathing cells, are two frequently used cell types that show promising results in supporting peripheral nerve regeneration in rodents (Radtke et al., 2011). Human umbilical cord-derived mesenchymal stem cells similarly can be differentiated into myelin-forming Schwann cells, offering another option for translational studies (Matsuse et al., 2010). With emergence of the induced pluripotent stem (iPS) cell technology, human somatic cells such as dermal fibroblast now offer yet another source for autologous Schwann cells. The unique self-renewal capacity of iPS cells provides an unlimited supply of pluripotent stem cells for research, and patient-specific iPS cell lines would eliminate some ethical concerns. To date, there are only few reports on successful differentiation of Schwann cells from iPS cells and in most cases the cells were only characterized for glial gene expression, rather than myelination (Ma et al., 2015). The only publication showing MBP expression by human iPSC-derived Schwann cells was obtained in myelinating cocultures with rat sensory DRG neurons (Liu et al., 2012). Therefore, it is unclear as to what percentage of stem or iPS cell-derived Schwann cells turns into myelin-forming and non-myelinating Schwann cells. While the advances in cell engineering are exciting, further characterization and optimization of the myelination capacity of stem cell-derived Schwann cells will be required before human applications. The bridging of cell engineering with biomaterial technologies provides an exciting opportunity for moving this field forward.

Engineered nerve conduits

While autologous nerve transplants are still considered as the gold standard to repair transected peripheral nerves, this procedure has undesirable side effects associated with loss of function at the donor site. Therefore, the development and optimization of nerve conduits that support quick and accurate regeneration of peripheral nerves is of clinical importance. With advances in biomaterial engineering current effort is focused on optimizing the implanted material to provide physical and topographical cues to the regenerating axons, in a manner that is similar to decellularized nerve grafts (Marquardt and Sakiyama-Elbert, 2013). Such decellularized grafts then can be engineered to deliver cultured cells and factors to support nerve regeneration and remyelination. This strategy using bone mesenchymal stem cells and chondroitinase ABC was shown to significantly improve nerve regeneration, as well as myelination, including myelin thickness (Wang et al., 2012). In a recent study, autologous nerve grafts and micro-structured collagen scaffolds seeded with Schwann cells were directly compared for their ability to support nerve repair (Bozkurt et al., 2016). This report indicates that the addition of Schwann cells to the engineered scaffold benefitted myelination, although nerve regeneration was just as efficient without the glia. With the mentioned advances in cell and biomaterial engineering, it is anticipated that individuals

with acute nerve injuries or with chronic painful conditions, such as trigeminal neuralgia, will have more options for therapy.

Gene therapies

Targeted gene manipulation

As mentioned above, transplanted cells with or without genetic modification can deliver trophic factors for nerve and myelin repair. Gene therapy that directly delivers genetic material into somatic cells is applicable for hereditary demyelinating neuropathies that involve gene deletion or loss of function mutations (Jerath and Shy, 2015). In giant axonal neuropathy (GAN), an inherited disease due to loss of function of the gigaxonin protein, researchers delivered adeno-associated virus containing normal GAN gene, which restored cytoskeleton homeostasis in primary skin fibroblasts from patients and in GAN mice (Mussche et al., 2013). In a mouse model of CMTX, Sargiannidou et al. injected a lentiviral vector carrying *GJB1* gene into the sciatic nerve of 2-month-old Cx32-deficient mice (Sargiannidou et al., 2015) and found 50% Schwann cells began to express Cx32 and the ratio of abnormal myelin sheath was significantly reduced. This approach has not been explored in HNPP caused by the deletion of the PMP22 gene, although patients with rare forms of homozygous PMP22 deletion could benefit from a similar strategy (Al-Thihli et al., 2008). In disorders associated with gain-of-function or gene duplication, specific sequences of inhibitory small RNAs such as double-stranded RNAs, small interfering RNAs (siRNA) and microRNAs (miRNAs) can be used to down-regulate the expression of target genes (Shy, 2006). For instance, we discovered that miR-29a can reduce PMP22 expression via a specific miRNA binding sequence found in the 3’-UTR of the PMP22 gene (Verrier et al., 2009). While this finding has clear translational value for treating CMT1A, targeted delivery of the inhibitory RNAs to Schwann cells poses a challenge.

Gene delivery to the PNS

In addition to correcting specific gene defects, gene therapy can be used to enhance the expression of therapeutic molecules such as trophic factors. For example, the therapeutic potential of intravenous NT3 administration is limited by its short serum half-life, which led to studies with a systemic AAV1 gene delivery vector (Sahenk et al., 2014). This approach appears to be effective in TrJ mice where the levels of NT-3 were maintained in muscle and alleviated neuropathic syndromes (Sahenk et al., 2014). In addition to virus-based vectors, nanoparticles provide an alternative strategy for delivering small genetic material such as siRNA to the PNS. Encapsulated siRNAs in lipid nanoparticles can cross the blood-brain and blood-nerve barrier and have been successfully delivered to the nervous system (Bruun et al., 2015; Gomes et al., 2015; Shyam et al., 2015). Mittnacht and colleagues employed chitosan-based siRNA nanoparticles in combination with microstructured implants to promote nerve regeneration by reducing the expression of inhibitory RhoA molecules (Mittnacht et al., 2010). This innovative approach visibly demonstrates the advantages of a nanobiofunctionalized implant that allowed local neurite outgrowth even in an inhibitory environment. In our attempt to deliver molecules specifically to Schwann cells, we engineered phospholipid-based liposomes that selectively enter Schwann cells when injected via the tail vein (Lee et al., 2013). Cell culture experiments indicate that this particle escapes

the endogenous cellular degradative mechanisms, possibly providing sustained compound release within Schwann cells. Because of the accessibility of peripheral nerves to therapeutic modulation, it is expected that with further advances in biomaterial and cell engineering additional innovative approaches for peripheral myelin repair will be forthcoming.

Myelin repair in toxin-induced neuropathies

While not a commonly encountered problem, PNS myelin can be directly damaged by toxin or drug exposure. The majority of toxin-induced peripheral neuropathies are associated with axonopathies, as a result of insult to the axonal cytoskeleton or mitochondria (Diezi et al., 2013). The number of research publications concerning PNS gliotoxins is limited, although understanding the effects of local anesthetics on Schwann cells is of significance. A recent report tested a number of commonly used local anesthetics and identified ropivacaine as a drug that upon prolonged exposure and/or at high concentrations causes significant demyelination and Schwann cell death (Yang et al., 2011). Recreational use of nitrous oxide, a frequently used mild anesthetic during dental procedures, was also linked with myelinopathy in a case report (Hsu et al., 2012). Tellurium is likely the most characterized gliotoxin that is known to interfere with the cholesterol biosynthesis pathway and lead to pronounced myelin damage in both the CNS and PNS (Morell and Toews, 1996). In general, most toxin-induced neuropathies are reversible once the culprit is identified and exposure is discontinued. In some instances, such as nitrous oxide myelinopathy, vitamin B12 treatment was initiated which in combination with drug abstinence led to full recovery of the patient within two months (Hsu et al., 2012). We similarly found reversible myelin damage associated with dichloroacetate (DCA)-mediated mitochondrial damage in myelinating cocultures (Felitsyn et al., 2008). The favorable outcome of toxin-induced peripheral myelinopathies after drug removal is another reflection of Schwann cell plasticity that allows the PNS to repair itself post-injury.

Conclusion

While the PNS is amenable to experimental manipulation and is responsive to therapy, further progress in our understanding of Schwann cell biology and improvements in cell and tissue engineering will be required to offer effective therapy options to individuals with local or systemic peripheral myelin damage. Currently patients with CMTs or trigeminal neuralgia have limited options to alleviate symptoms, which typically include surgical approaches. Slowing down or reversing the myelin damage either through targeted cell therapy or specific recommendations for life style changes such as diet and/or exercise is of great interest to affected individuals. We hope that the knowledge learned from acute nerve injury repair paradigms will be beneficial for developing effective therapies for chronic PNS myelin disorders.

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