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Myelin

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Preface

The successful publication of our previous book entitled *Schwann Cell Development and Pathology* (Kazunori Sango and Junji Yamauchi (eds) 2014), which has achieved more than 10,000-chapter downloads, motivated us toward the next project extending our research field to the brain, spinal cord, and peripheral nerves with a special focus on myelin. Keiichiro Susuki and Toru Ogata joined this project, and the four editors (Fig. 1) invited the expert investigators inside and outside Japan to submit review articles regarding recent topics of myelin in relation to their own research.

This book, composed of 4 parts with 24 chapters, presents the latest exciting advances in the understanding of the structure and function of myelin under normal

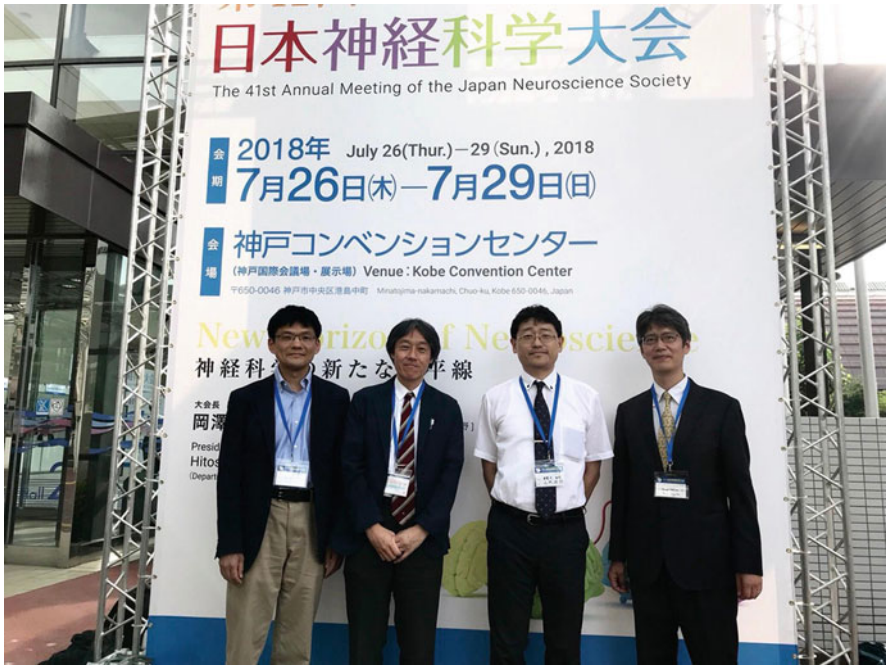

Fig. 1 Editors of this book; (from left to right) Keiichiro Susuki, Kazunori Sango, Junji Yamauchi and Toru Ogata at the 41st Annual Meeting of the Japan Neuroscience Society (July 28, 2018, Kobe)

Fig. 2 Attendee of the 4th Japan Myelin Meeting (July 14, 2018, Yokohama)

and pathological conditions. Detailed attention is paid to the findings and implications of recent research on the myelin-forming glial cells such as oligodendrocytes and Schwann cells.

In Part I edited by Yamauchi, the latest advances in the understanding of the mechanisms of myelination during development and regeneration are described.

In Part II edited by Susuki, specialized structures along the myelinated fibers, such as the nodes of Ranvier and Schmidt–Lanterman incisures, are fully discussed in association with their function and molecular composition.

In Part III edited by Ogata, various kinds of demyelinating diseases in the CNS, including multiple sclerosis, Pelizaeus–Merzbacher disease, traumatic brain and spinal cord injuries, and brain tumors of glial cell origin, are fully described from the basic and clinical viewpoints.

In Part IV edited by Sango, peripheral demyelinating neuropathies due to a variety of causes, e.g., genetic mutation (Charcot–Marie–Tooth diseases and amyloid polyneuropathy), immune and metabolic disorders, and the side effects of drugs, are introduced.

We are sure that up-to-date research topics with high-quality artworks introduced by expert investigators arouse the readers' interest in myelin biology. Discussion from the viewpoint of basic and clinical neuroscience makes the book educational for medical students and young clinicians. As editors, we give thanks to all the contributors for sparing their time for the book and Mitsubishi Tanabe Pharma Corporation for the financial support to hold Japan Glia Meeting and Japan Myelin Meeting (Fig. 2), where we have got an opportunity to fully discuss the recent topics

of myelin. We dedicate this book to the late Prof. Kazuhiro Ikenaka (1952–2018), a great scientist and supervisor who devoted his life to the development of research in the biology of glia and myelin.

Tokyo, Japan — Kazunori Sango
Saitama, Japan — Toru Ogata
Dayton, OH — Keiichiro Susuki
Tokyo, Japan — Junji Yamauchi

Contents

Part I

Myelination in Neural Development and Regeneration

Cellular Signal-Regulated Schwann Cell Myelination and Remyelination

1

Tomohiro Torii, Yuki Miyamoto, and Junji Yamauchi

Abstract

Increasing studies have demonstrated multiple signaling molecules responsible for oligodendrocytes and Schwann cells development such as migration, differentiation, myelination, and axo-glial interaction. However, complicated roles in these events are still poorly understood. This chapter focuses on well established intracellular signaling transduction and recent topics that control myelination and are elucidated from accumulating evidences. The underlying molecular mechanisms, which involved in membrane trafficking through small GTPase Arf6 and its activator cytohesins, demonstrate the crosstalk between well established intracellular signaling transduction and a new finding signaling pathway in glial cells links to physiological phenotype and essential role in peripheral nerve system (PNS). Since Arf family proteins affect the expression levels of myelin protein zero (MPZ) and Krox20, which is a transcription factor regulatory factor in early developmental stages of Schwann cells, Arf proteins likely to be key regulator for Schwann cells development. Herein, we discuss how intracellular signaling transductions in Schwann cells associate with myelination in CNS and PNS.

Keywords

Schwann cell · Myelination · Cytohein · Arf

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1.1 Introduction

In the peripheral nervous system, the axon is wrapped by multiple layers of myelin, which is made up of Schwann cells, and it protects and enhances electrical impulses (Nave and Werner 2014; Salzer 2015). Schwann cells mainly contribute to the production and maintenance of the myelin sheath, which is composed of lipids and proteolipids. Schwann cell myelination (possibly remyelination) is regulated by the axo–glial interaction, which plays a key role in physiological processing and is required for intracellular signaling pathways as well as differentiation and migration in previous studies (Salzer 2015; Bercury and Macklin 2015). It is well established that these associations also lead to activation of the neuregulin (NRG)-ErbB signal cascade and regulation of myelination (Chen et al. 2006) in Schwann cells during development. Over the past 20 years, many studies have generated knockout mice and transgenic mice to understand molecular mechanisms of Schwann cell myelination. Recent studies demonstrated that the constitutive active form of Akt causes hypermyelination in the central nervous system (CNS) and the peripheral nervous system (PNS) (Flores et al. 2008; Narayanan et al. 2009; Domènech-Estévez et al. 2016). These findings provided new insights into activated intracellular molecules and the downstream molecules that are necessary for Schwann cell myelination.

However, many studies have demonstrated that energy metabolism in oligodendrocytes contributes to the maintenance of axonal function and neuronal survival (Funfschilling et al. 2012). Glycolysis products such as pyruvate and lactate were shown to be transported from oligodendrocytes to axons via monocarboxylate transporters (MCTs) for neural function. A recent study demonstrated that myelin composed of Schwann cells also provides metabolites such as lactate to support axons in the PNS (Beirowski et al. 2014). Pooya et al. also described that the metabolic regulator LKB1 is essential for Schwann cell myelination, because mutant mice lacking LKB1 showed hypomyelination in the PNS (Pooya et al. 2014). Taken together, mitochondrial metabolism in Schwann cells is responsible for myelination, but detailed mechanisms are still unknown.

As described previously, many gene-deficient mice were generated to explain the molecular mechanisms of Schwann cell myelin formation and/or demyelination. An increasing number of studies using whole genome sequencing analysis and next-generation sequencing technology have also contributed to the identification of a gene mutation in patients with PNS disease, and to the determination of molecular pathogenesis of PNS diseases such as Charcot–Marie–Tooth (CMT) disease, which is a neurological disorder, and causes of peripheral neuropathy. These studies provide new insights into how the mechanism causes CMT disease, but these mechanisms of pathogenesis are still largely unknown. To improve the patient's condition, many researchers have investigated clinical treatments that are based on basic research.

1.1.1 Some Membrane Proteins and Lipids Are Composed of Myelin and Exist in Distinct Regions in Myelin

Four decades ago, it was shown that myelin has a relatively high lipid composition including cholesterol, phospholipids, galactolipids, and plasmalogens (Norton and Poduslo 1973). In compact myelin, a cholesterol-abundant membrane associated with proteolipid protein (PLP) is present in the CNS (Simons et al. 2000) and myelin protein zero (MPZ) is present in the PNS (Hasse et al. 2002). Although the study showed that mutant mice lacking PLP exhibited a decreased amount of cholesterol in myelin, the specific molecular mechanisms containing PLP and lipids/glycolipids remains unclear (Werner et al. 2013). Additionally, recent quantitative mass spectrometry data confirmed the relative abundance ratio of myelin-specific proteins in peripheral nerves (Patzig et al. 2011). These proteome analyzes helped to establish specific molecular mechanisms that regulate Schwann cell myelination and molecular pathogenesis. For example, Patzig et al. first characterized the cytoskeleton protein regulator, septin8, which is present in myelin and is responsible for myelination in the CNS (Patzig et al. 2016).

1.1.2 The Association Between Axon and Schwann Cells Regulate Myelination

The cell–cell and cell–extracellular matrix interactions play an important role in the development and organization of multicellular organisms. In the PNS, Schwann cells migrate along peripheral axons and are differentiated at a suitable area in sciatic nerves. During development, protein expression patterns are responsible for the association between axon and Schwann cells, and it is important to characterize the events to understand Schwann cell myelination and possible remyelination. Axon–Schwann cell interactions are mediated by transmembrane proteins such as NRG-ErbB (Chan et al. 2006; Yamauchi et al. 2008; Perlin et al. 2011; Salzer 2015), and we describe these interactions further.

1.1.3 Essential Role for Neuregulin-1 Type III in Schwann Cells Myelination and Remyelination

Many people hypothesized that distinct localization of the protein in Schwann cells is necessary for migration, differentiation, and myelination. NRG1, which is a member of the epidermal growth factor (EGF) superfamily and comprised of several alternative spliced variant isoforms, is well known as an activator of the ErbB family of tyrosine kinase receptor. NRG1 type III is expressed in the dorsal root ganglion (DRG) neurons and is present at a specific region in axons. The distinct subcellular localization and NRG1-ErbB signaling transduction are required for myelination in Schwann cells (Chen et al. 2006). NRG1-induced activation of ErbB2/3 acts as an activator for PI3K during myelination (Nave and Salzer 2006) and/or remyelination

and the actin dynamics regulator, cdc42, and its binding partner are involved in major Schwann cell migration pathways (Yamauchi et al. 2008). Additionally, mutant mice lacking NRG1 type III exhibit myelination thickness in brain, but not in the spinal cord and optic nerve (Taveggia et al. 2008), and also show delayed migration of Schwann cell precursors (Mityamoto et al. 2017). Taken together, NRG1-ErbB signaling promotes myelination in specific Schwann cell regions, but the signaling does not contribute to myelin maintenance.

However, Marca et al. characterized the NRG1 binding partner tumor necrosis factor-α-converting enzyme (TACE; also known as ADAM17), a negative regulator for Schwann cell myelination, because they observed that the MPZ expression levels were upregulated in Schwann cells when they were cocultured with TACE short hairpin RNA (shRNA)-expressing DRG neurons, and the loss of TACE promotes Schwann cell myelination in mutant mice lacking TACE, which is controlled by motor neuron-specific Mnx1 gene promoter (also known as HB9-Cre transgenic mice) (La Marca et al. 2011). They also demonstrated that TACE cleaves NRG1 type III and inhibits activation of Akt (Akt phosphorylation) that is involved in NRG1-dependent Schwann cell myelination signaling. Collectively, axo–glial interaction contributes to TACE activity, which is required for inhibition of Schwann cell myelination in the PNS. Previous research demonstrated that TACE/ADAM17 plays a role in oligodendrocyte myelination and remyelination in the CNS (Palazuelos et al. 2014, 2015). These findings help to understand the molecular mechanisms for myelination and also provide knowledge about signaling differences between the CNS and the PNS.

1.1.4 Small GTPases and Their Activator Are Essential for Schwann Cell Migration and Myelination

During the early stage of PNS development, neural crest cells migrate along axons and then differentiate to Schwann cell precursors to change cell shape for myelin ensheathment and myelination (Fig. 1.1A). Many studies investigating Schwann cell development have identified the required membrane transduction signals, such as the neuronal NRG1 ligand and the cognate receptors ErbB2 and ErbB3 on Schwann cells, and the signaling pathways that lead to the essential transcription factor cascade Sox10/Oct6/Krox20, as described in previous reports (Salzer 2015).

Small GTPases and various cells are known to regulate Schwann cell migration (Yamauchi et al. 2004). NRG1-induced ErbB2/3 activation and neurotrophin-3 (NT3)-induced TrkC receptor activation promote Schwann cell migration (Yamauchi et al. 2004; Hempstead 2005). Moreover, NRG1-mediated ErbB2/3 activation also leads to phosphorylation of Dock7 and activates cdc42/Rac1 signaling during Schwann cell migration (Fig. 1.1B(a)) (Yamauchi et al. 2008). However, Schwann cell migration is inhibited by brain-derived neurotrophic factor (BDNF), which plays an important role in neuroprotection and cell survival, acting through the neurotrophin receptor $p75^{NTR}$. Activation of the NT3/TrkC signaling pathway contributes to the inhibition of Schwann cell myelination and myelin protein

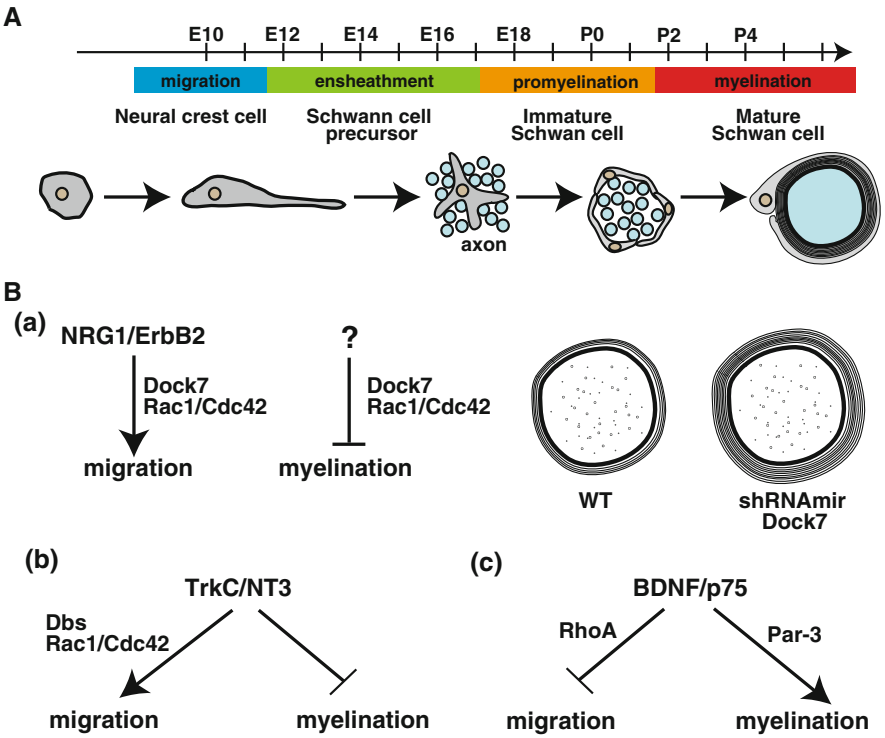

Fig. 1.1 Schwann cell lineage cells during PNS development. (**A**) Beginning approximately at E9.0, neural crest cells (NCCs) were identified using markers such as Sox9, Sox10, and Par3, and they start migrating in the sciatic nerve. GFAP and S100β expressions are increased in immature Schwann cells until approximately the postnatal days. After birth, increased expression of the transcription factors Knox20 and Oct6 cause Schwann cell myelination and Schwann cells begin to wrap around axons creating the myelin sheaths. (**B**) (**a**) The scheme for NRG1-induced Dock7 signal transduction in Schwann cells. The association between NRG1 and ErnB2/3 facilitates Schwann cell migration, acting through Dock7, and inhibits Schwann cells myelination. Dock7 shRNA transgenic mice exhibits hypermyelination at postnatal day 14 compared to control mice (Yamauchi et al. 2011). (**b**) During migration, NT3/TrkC induces activation of small GTPases GEF, but this signaling is not required for Schwann cell myelination. (**c**) BDNF/p75 promotes Schwann cell migration, but is not necessary for Schwann cell myelination. During PNS development, activation of these signaling pathways is dramatically changed

synthesis in Schwann cells (Fig. 1.1B(b)) (Chan et al. 2001). Taken together, these studies demonstrate that NT3/TrkC and BDNF/p75 signaling pathways compete with each other for Schwann cell development.

Guo et al. generated mutant mice lacking Rac1, which is controlled by the Dhh-Cre promoter, exhibit reduced myelin thickness in the sciatic nerves (Guo et al. 2012), and the small GTPase Rac1 was also shown to regulate Schwann cell myelination. They also detected that the phosphorylation levels of p21-activated kinase (Pak) and its effector merlin/neurofibromatosis type 2 (NF2), which control stabilization of E-cadherin-containing adhesion junctions (Lallemand et al. 2003),

were significantly decreased in these mice. E-cadherin signaling pathways also regulate Schwann cell myelination (Fannon et al. 1995; Crawford et al. 2008; Basak et al. 2015). The study demonstrated that reduced myelin in Rac1 KO mice was restored by the Merlin/NF2 mutant that exhibits Schwann cell hyperplasia (Giovannini et al. 1999), and forskolin treatment that leads to cAMP elevation and upregulation of E-cadherin in sciatic nerves. These findings suggest that Rac1 regulates Schwann cell myelination that is mediated by phosphorylation of Pak/Merlin and E-cadherin expression as well as Schwann cell migration (Yamauchi et al. 2004, 2005). Because E-cadherin facilitates NRG signaling (Basak et al. 2015), the pathway including Rac1 signaling may be associated with NRG1 and may contribute to Schwann cell myelination.

1.1.5 Intracellular Molecules Associated with Schwann Cell Myelination

Cell adhesion molecules play an important role in cell–cell interaction and can cause biological responses. An increasing amount of research has demonstrated that the association between Schwann cells and axons (axo–glial interaction) that mediate them that is essential for myelination in Schwann cells.

A signal transduction including adhesion G protein-coupled receptor (GPCR) Gpr126/adgrg6, which binds to laminin-211, is required for Schwann cell development and myelination (Monk et al. 2009). Monk et al. also reported that Oct6 and Knox20 expression levels are significantly reduced in Gpr126 mutant zebrafish. Moreover, Gpr126 also regulates remyelination of Schwann cells because tamoxifen-inducible Schwann cell-specific GPR126 knockout mice exhibit a delay in remyelination after injury compared to wild-type mice (Mogha et al. 2016). Another adhesion G protein-coupled receptor Gpr56 mutant zebrafish showed a reduction in myelinated axons and mature oligodendrocytes, and recent studies demonstrated that GPR56 regulates oligodendrocyte development in the CNS (Ackerman et al. 2015; Giera et al. 2015). Ackerman et al. also reported that GPR56-dependent oligodendrocyte development including oligodendrocyte precursor cell (OPC) proliferation is involved in activation of G proteins and small GTPase RhoA. Both GPR126 and laminin-211 facilitate cyclic adenosine monophosphate (cAMP) levels, which are necessary for myelination in Schwann cells, and the specific association between Schwann cells and axons that is mediated by GPR126/laminin-211 (Monk et al. 2009) and GPR126/collagen4 (Paavola et al. 2014) were characterized. Collectively, axo–glial association drives Grp126 activation and expression of transcription factors to stimulate Schwann cell myelination during development in PNS.

The tyrosine kinase Fyn is known to be a key molecule for maturation and initial stages of myelination in the CNS (Krämer-Albers and White 2011; Laursen et al. 2009; Umemori et al. 1994). The tyrosine kinase Fyn is also activated by the association between axons and oligodendrocytes, which is mediated by myelin-associated glycoprotein (MAG). Lipid raft and its downstream molecules are also

required for myelination (White and Krämer-Albers 2014) because mutant mice lacking Fyn and MAG showed hypomyelination (Biffiger et al. 2000). However, Fujita et al. demonstrated that signal transduction is necessary for Schwann cell myelination (Fujita et al. 1998), whereas Miyamoto et al. reported the other Fyn activation signaling contributes to Schwann cell myelination, as described below (Miyamoto et al. 2015). Thus, the Fyn-dependent myelination mechanism is thought to be different between the CNS and the PNS.

Nectin-like 4 (Necl4, also known as SynCAM4) is highly expressed in myelinating Schwann cell and is enriched at the axo–glia contact region (Spiegel et al. 2007) and at the Schmidt-Lanterman incisures in Schwann cells (Maurel et al. 2007). These studies also demonstrated that Necl4 specifically binds to Necl1 (SynCAM1)-Necl4, mediating the axo–glial interaction that contributes to Schwann cell myelination. However, these proteins are not required for initial contact between the axon and the Schwann cell (Spiegel et al. 2007).

These studies confirmed that multiple associations between Schwann cells and axons are essential for Schwann cell development and may regulate activation of the transmembrane protein and its downstream molecules at specific developmental stages.

1.1.6 Schwann Cell Myelination Is Required for Cytoskeleton Assembly and Disassembly

Generally, actin assembly and disassembly are regulated by the multiple pathways and are necessary for cell migration and cell morphological changes during development. The role of the Rho small GTPase family, which contributes to these events as described in many reviews (Hodge and Ridley 2016), has been well established. Rac GTPase and its downstream molecule the Wiskott-Aldrich syndrome protein (WASP)-WASP family verprolin homologous (WAVE) family WAVE2 control activation of the Arp2/3 complex, which directly binds to G-actin and promotes actin filament formation in various cells (Takenawa and Suetsugu 2007). In the PNS, Novak et al. generated mutant mice lacking N-WASP that is controlled by the Dhh-Cre promoter, which showed a myelination defect in the PNS (Novak et al. 2011). The mice exhibited reduced myelin thickness and motor abnormalities such as hypomotility, tremor, and progressive hind limb paralysis. Based on these findings, Novak et al. demonstrated that N-WASP contributes to membrane wrapping and myelination in Schwann cells. Another group also generated mutant mice lacking N-WASP controlled by the MPZ promoter and observed reduced myelin thickness (Jin et al. 2011). Collectively, N-WASP acts as an actin dynamic regulator in myelinating Schwann cell during PNS development.

F-actin is present in essentially all cytoplasmic compartments of myelinating Schwann cells: the Schmidt-Lanterman incisures, the periaxonal membrane, the paranodes, and the outer and inner mesaxon and paranodal loops (Trapp et al. 1989), which are sites of morphogenesis during myelination. Inhibition of actin assembly blocks myelination in coculture models (Fernandez-Valle et al. 1997).

Because actin-related protein Arp2/3-specific inhibitor CK-666 significantly suppresses myelin ensheathment, Arp2/3 activation is necessary for myelin ensheathment in oligodendrocytes (Zuchero et al. 2015). Additionally, Zuchero et al. suggested that the interaction between the actin dynamics regulator cofilin and MBP is necessary for depolymerization of actin during myelination. Similarly, Nawaz et al. used mutant mice lacking cofilin1 and demonstrated that cofilin is included in NRG1 that is induced in signaling transduction during Schwann cell myelination (Nawaz et al. 2015). In the PNS and the CNS, cofilin-mediated actin disassembly, which also promotes actin turnover, plays an important role in myelin wrapping (Nawaz et al. 2015; Zuchero et al. 2015).

Mutations in the FRABIN/FGD4 gene encoding the FRABIN/FGD4 protein, which is identified as a specific cdc42 GDP/GTP nucleotide exchange factor (GEF), cause the peripheral neuropathy CMT type 4(CMT4H) disease (Delague et al. 2007; Stendel et al. 2007; Horn et al. 2012). Horn et al. demonstrated that Fgd4-deficient mice exhibit excessively folded myelin (outfolding) that causes demyelinating neuropathy in the PNS. FGD-dependent cdc42 activation is required for Schwann cell myelin ensheathment and radical sorting as reported by Guo et al. (2013). However, it is still unclear why an FGD mutant leads to focally folded myelin in the PNS.

1.1.7 Cytoskeleton-Associated Proteins Regulate Schwann Cell Myelination

Another cytoskeleton component microtubule function is to organize cell morphology. Microtubules and their binding partners provide neuronal vesicles with axonal transport and trafficking along axons, but it is still unknown if these proteins serve a key role in Schwann cell myelination. Some studies showed that the regulatory mechanisms for myelination in the CNS and the PNS are regulated by microtubule and/or actin-associated proteins such as kinesin-3 family member 13b (Kif13b) (Noseda et al. 2016), Kif1b (Lyons et al. 2009), and septin-8 (Patzig et al. 2016). Kif13b was characterized as a motor protein and it is transported along microtubules in various cells. Noseda et al. generated MPZ driven Kif13b conditional knockout mice, and they observed reduced myelin thickness in the sciatic nerve in these mice compared to control mice, which suggests that Kif13b positively regulates Schwann cell myelination through p38 mitogen-activated protein kinase (MAPK) phosphorylation in Schwann cells. They also characterized Kif13b as a negative regulator for oligodendrocyte myelination using mutant mice lacking Kif13b under the control of the 2–3-cyclic nucleotide 3-phosphodiesterase (CNPase) promoter (Noseda et al. 2016). The study demonstrated that molecular mechanisms including Kif13b/p38 MAP kinase, which contributes to myelin formation, are different between the CNS and the PNS.

Septins were identified as GTP-binding proteins and the septin family is associated with cell plasma membranes, microtubules, and actin filaments. Many studies demonstrated that the septin family proteins are crucial for neuronal polarity.

For example, septin6 (SEPT6) and septin7 (SEPT7) form a complex and its interaction promotes the remodeling of actin and microtubules in the branching axon (Hu et al. 2012). Although septin8 (SEPT8) binds to vesicle-associated membrane protein 2 (VAMP2), which is the main component of synaptic vesicles in neurons, and also regulates SNARE complex formation and neurotransmitter release (Ito et al. 2009), it was largely unknown whether or not these proteins are responsible for Schwann cell function. A previous study demonstrated that septins are expressed in Schwann cells and are highly enriched at paranodal loops and microvilli in non-compact myelin compartments (Buser et al. 2009). Patzig et al. first confirmed that SEPT8 is present in the adaxonal compartment of myelin in oligodendrocytes and associates with oligodendrocyte myelination (Patzig et al. 2016). They also generated mutant mice lacking SEPT8 and reported the mice revealed myelin outfoldings in the CNS. It is still largely unknown if these molecular mechanisms contribute to myelin formation in the PNS.

1.1.8 The Polarity Protein Par-3 and Its Binding Partners Regulate Schwann Cell Myelination

As described above, Schwann cell myelination is required for activated cell polarity signaling pathways that is regulated by the polarity protein complex Par-3/Par-6/aPKC and these proteins are enriched in a distinct area of myelinating Schwann cells (Chan et al. 2006; Taveggia and Salzer 2007). Additionally, these intracellular signaling pathways control cell–cell contact mediated at tight junctions in epithelial cells and are conserved among several cells. During initiation of myelination, Par-3 also binds to p75NTR at the axo–glial junction in Schwann cells and regulates Schwann cell myelination (Chan et al. 2006).

A recent study demonstrated that the polarity protein Pals1/Mpp5, which is a binding partner of Par3, also regulates polarization and radial sorting in Schwann cells during early development of the PNS. Pals1-deficient mice exhibited delayed myelination and impairment of axonal radial sorting. Although radial sorting contributes to proper Schwann cell myelination, Pals1 does not affect Schwann cell myelin thickness (Zollinger et al. 2015).

Chan et al. demonstrated that Par3 colocalizes with Necl-4, which regulates myelination as described above, in the adaxonal region of the Schwann cell (Chan et al. 2006; Perlin and Talbot 2007). It has been established that Par3 proteins also interact with many intracellular proteins including cell adhesion molecules and intracellular molecules such as small GTPases cdc42/Rac1 that regulate cell migration (Pegtel et al. 2007). Tep et al. confirmed that Rac1 activation is mediated by the BDNF-induced Par3 signaling pathway, which contributes to Schwann cell myelination in zebrafish (Tep et al. 2012).

1.1.9 Activation of Arf6 Is Required for Schwann Cell Migration and Myelination

It was well established that cytohesin-1, which is the guanine nucleotide exchange factor (GEF) for the small GTPases Arf6 and/or Arf1 in mammalian cells (Casanova 2007), regulates cell migration (Quast et al. 2009). Cytohesin-1 knockout mice exhibits decreased myelin thickness in sciatic nerve, and recent studies identified and characterized cytohesin-1 as a positive regulator for Schwann cell myelination (Yamauchi et al. 2012) and migration (Yamauchi et al. 2012; Miyamoto et al. 2013). To confirm this phenotype, they also generated cytohesin-1 transgenic mice, which are controlled by the MPZ promoter, and revealed that overexpression of cytohesin-1 enhances Arf6 activation and Schwann cell myelination in sciatic nerves in transgenic mice (Torii et al. 2013). Generally, Arf6 regulates endocytosis, which is mediated through phospholipase D (PLD), phosphatidylinositol 4-phosphate 5-kinase (PIP5K), and membrane trafficking in various cells (D'Souza-Schorey and Chavrier 2006). There have been no previous studies showing that Schwann cell myelination is mediated by Arf6-dependent PLD and PIP5K activation.

Another Arf6GEF cytohesin-2/ARNO also controls cell migration (Goldfinger et al. 2006; Torii et al. 2010), acting through intracellular molecules such as the small GTPase Rac that regulates actin dynamics and cell migration (Sadok and Marshall 2014). Torii et al. generated mutant mice lacking cytohesin-2, which is controlled by the MPZ promoter. These mutant mice revealed a significant reduction in myelin thickness in the sciatic nerve (Torii et al. 2015). While the distribution and function of cytohesin-1 and activated Arf6 have been established in Schwann cells, the difference in function between cytohesin-1 and cytohesin-2 suggests that important differences may exist between the roles played by polarity proteins in PNS myelin. Although cytohesin-1/-2-mediated Arf6 activation is responsible for Schwann cell myelination and migration, these molecular mechanisms in the cell remain largely unknown.

An increasing amount of evidence has demonstrated that Arf6 and its activator Arf6GEF are associated with Rac1-mediated actin dynamics/cytoskeleton rearrangement during membrane trafficking (Boshans et al. 2000; Franco et al. 1999; Radhakrishna et al. 1999; Santy and Casanova 2001). Moreover, Marchesin et al. (2015) explained that Arf6 also facilitates the formation of the Arp2/3 and WAVE complex that interacts with the activated Rac1/IRSp53 complex to modulate actin dynamics. Taken together with these studies, cytohesin mediated-activation of Arf6 may promote Schwann cell myelination, acting though Rac1/WAVE2 signaling pathway in the PNS.

1.1.10 PI3K/Akt/mTOR Signal Pathway Is Necessary for Schwann Cell Myelination

As described above, Akt activation is necessary for myelination and it is involved in myelination signaling transduction. Akt is known to be a downstream signaling

element of beta1 integrin (Barros et al. 2009), insulin-like growth factor 1 (IGF-1), and ErbB2/ErbB3 during oligodendrocyte myelination in the CNS. Additionally, receptor tyrosine-protein kinase ErbB2/ErbB3 regulates activation of phosphoinositide 3-kinase (PI3K) and produce phosphatidylinositol (3,4,5)-triphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2). PIP3 recruits Akt to the plasma membrane and controls its enzyme activity in the region. Goebbels et al. generated mutant mice lacking phosphatase and tensin homologue deleted on chromosome 10 (PTEN) under the control of the CNPase promoter, which showed myelin outfoldings and tomacula formation in the PNS (Goebbels et al. 2012), and thus they confirmed the Akt negative regulator PTEN also contributes to Schwann cell myelination. Akt, mTOR, and S6 are also significantly activated and phosphorylated in these mice compared to control mice. Because the signal transduction pathway including these proteins is involved in myelin formation, their findings also suggest that inhibition of PTEN is necessary for myelin formation, and that activated PI3 kinase and Akt lead to excessive myelin membrane growth. Moreover, they observed that disruption of PTEN does not affect myelin thickness in sciatic nerves of PTEN knockout mice. Consistent with these mechanisms, PTEN was shown to act as a negative regulator to regulate myelin formation in the CNS and the PNS.

1.1.11 Ga6-Tyro3 Signaling Pathway Is Included in Schwann Cell Myelination

Gas6, which is a specific ligand for the Tyro3, Axl, Mer (TAM) family of receptor protein tyrosine kinases such as Tyro3 (also known as Brt, Dtk, Rse, Sky, and Tif), Axl (also known as Ark, Tyro7, and Ufo), and Mer (also known as Eyk, Nym, and Tyro12), is necessary for myelination in the CNS (Binder et al. 2008). These intracellular signal transduction pathways also facilitate remyelination after both cuprizone-induced (Binder et al. 2011) and lysolecithin-induced (Goudarzi et al. 2016) demyelination. Three TAMs are also expressed in the brain during postnatal development, specifically, in the white matter, which consists of myelinated axons (Prieto et al. 2000). These studies are helping to identify the signals that are associated with myelination in oligodendrocytes and Schwann cells.

The TAM family members are type I receptor-tyrosine kinases, and each is composed of an Ig-like domain, a fibronectin type 3 (FN3) domain, and a protein tyrosine kinase domain (Godowski et al. 1995). It is well established that TAMs control inflammatory responses, cell proliferation, cell survival, and phagocytosis in cells of various types (Fig. 1.2). They also mediate extracellular-to-intracellular signal transduction (Godowski et al. 1995). As their endogenous ligands, the TAMs recognize protein S (PROS1) and Gas6, both of which are widely expressed in the central nervous system (CNS) after birth (Prieto et al. 1999). A previous study indicated that activated Gas6 and its receptor Tyro3 signaling regulate myelination in Schwann cells (Miyamoto et al. 2015). Expression of MPZ, which is specifically expressed in Schwann cells, was significantly decreased in Tyro3 null mice.

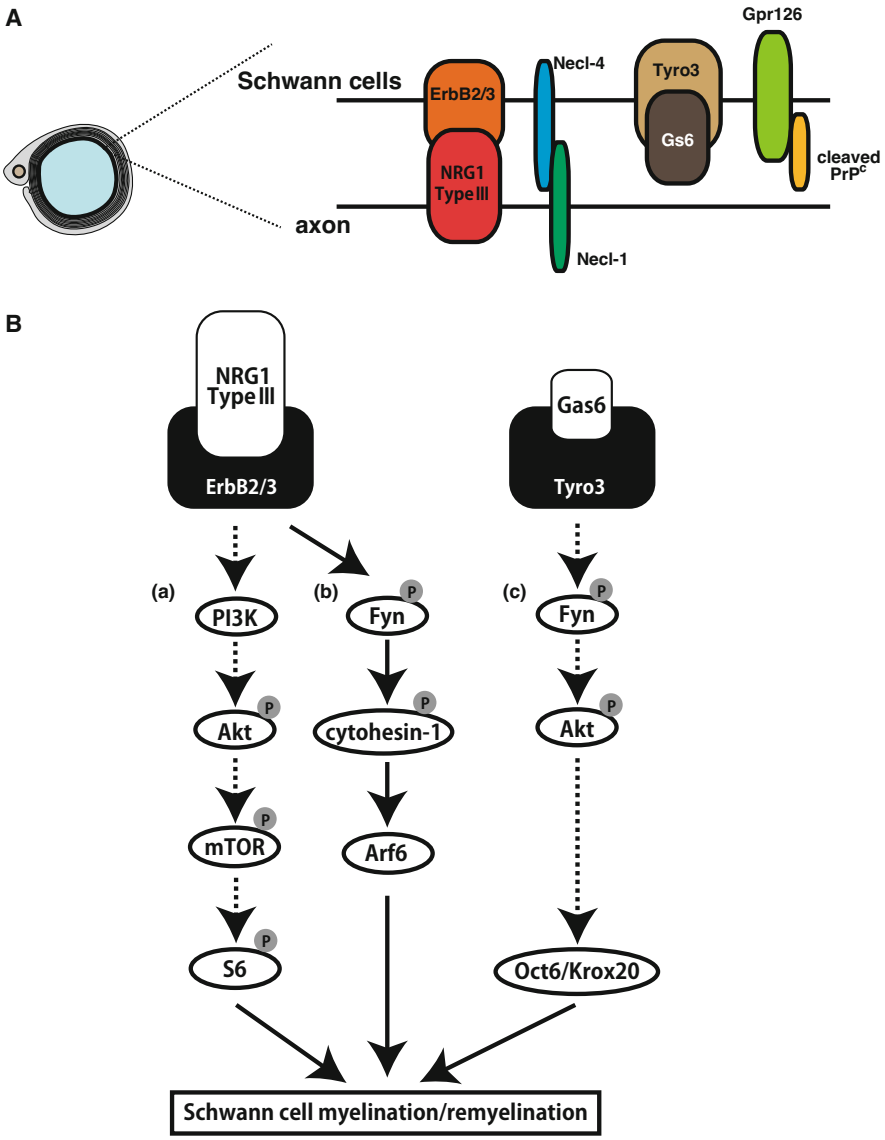

Fig. 1.2 Schwann cell myelination is regulated by several intracellular signaling transduction pathways. (**A**) The association between axo–Schwann cells are mediated by NRG1/ErbB2/3 and Necl1-Necl4. The specific interactions are responsible for Schwann cells myelination. Gas6-Tyro3 and Grp126 signaling facilitate Schwann cells myelination during PNS development. (**B**) (**a**) NRG1-induced PI3K/Akt/mTOR signaling pathway is responsible for Schwann cell myelination. (**b**) NRG1/ErbB2/3 signaling regulates Fyn phosphorylation and activation of its downstream molecule cytohesin-1/Arf6 in myelinating Schwann cells. (**c**) Fyn also is activated by Gas6/Tyro3 signaling and controls Schwann cells myelination, acting through Akt and Oct6/Knox20

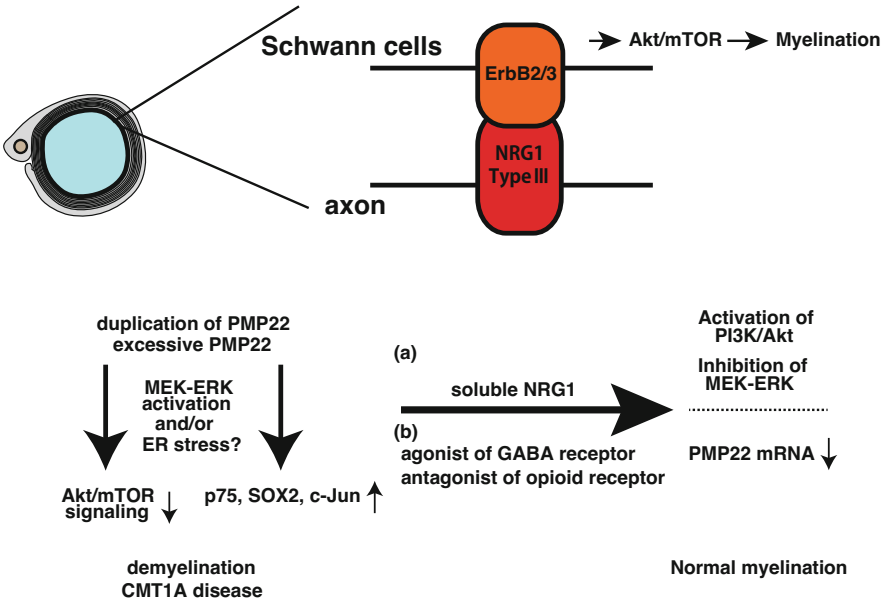

Fig. 1.3 Molecular pathogenesis of CMT1A and establishment of clinical treatment for patients with CMT1A disease. NRG1-ErbB is necessary for Schwann cell myelination, acting through mTOR/Akt and have been confirmed in many studies. PMP22 duplication cause CMT1A disease, however, the disease was improved by soluble NRG1 treatment (**a**) and/or some drugs (**b**)

Moreover, Oct6 and Knox20 expression levels also decreased in 1-day postnatal sciatic nerves from these mice, but there was no significant difference Oct6 and Knox20 expression levels in 6-day-old and 12-day-old sciatic nerves from Tyro3 null and control mice. Based on these data, the authors suggested that Tyro3 and its binding partner Fyn regulate activation of Akt signal transduction pathways that participate in Schwann cell myelination. These studies demonstrated that the Gas6-TAM family tyrosine kinase participate in myelination and remyelination as well as in inflammation (Rothlin et al. 2015) (Fig. 1.3).

1.1.12 Molecular Mechanisms of Pathogenesis in PNS Disorders

Several PNS disease model mice have been generated and analyzed (Meyer et al. 2006). The pathology of CMT disease has also been reported in many studies. For example, overexpression of the peripheral myelin protein 22 (PMP22), which is caused by a duplication in the PMP22 gene on chromosome 17, leads to the most common inherited CMT neuropathy, CMT1A disease (Pareyson and Marchesi 2009; Rossor et al. 2013).

Fledrich et al. showed that a transgenic rat overexpressing PMP22 exhibited reduced myelinated axons in the PNS and demonstrated that the neuropathic

phenotype was significantly improved by neuregulin-1 treatment, which regulates Schwann cell differentiation and acts through PI3K-Akt signaling transduction (Fledrich et al. 2014). Moreover, they confirmed that mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase (ERK) activity is upregulated and there is also decreased activation of the PI3K-Akt signaling pathway in the PNS in the CMT1A model rat. p75, Sox2 (Etxaniz et al. 2014), and c-Jun, which were identified and characterized as immaturity markers, are increased in the sciatic nerve of these rats. Additionally, D'Urso et al. confirmed that excess PMP22 accumulates in the endoplasmic reticulum and Golgi compartments in myelinating Schwann cells (D'Urso et al. 1998). However, CMT1E is associated with a unique point mutation in peripheral myelin protein 22 (PMP22), which acts as a dominant-negative mutant and may inhibit Schwann cell myelination. Thus, it has been hypothesized that the endoplasmic reticulum (ER) stress may lead to CMT disease. However, the molecular pathogenesis in patients with CMT1E is still largely unknown.

Fabrizi et al. demonstrated that a point mutation in MPZ causes a demyelinating neuropathy and results in CMT1B disease (Fabrizi et al. 2000). An increasing number of studies have reported that various mutants of MPZ were identified in patients with CMT1B disease (Kochański et al. 2003; Iida et al. 2012). Additionally, patients with CMT1B reveal focally folded myelin. However, it remains unclear whether MPZ mutants are the molecular mechanism that causes focally folded myelin.

1.1.13 Prospective Clinical Treatment for Patients with PNS Disease

Patients with CMT and CMT-related disorders show progressive degeneration of the peripheral nerves. A growing number of studies have identified more than 60 genes that are involved in causing these diseases, and their molecular pathogenesis have been described (Bouhy and Timmerman 2013). To date, researchers have focused on identifying drugs that can induce myelination and/or remyelination using these CMT model mice. Treatment with neurotrophin-3 (NT3) was shown to promote remyelination in the sciatic nerve in CMT1A model mice. Additionally, clinical treatment using a combination of drugs including an agonist for the neurotransmitter gamma aminobutyric acid (GABA) receptor and an antagonist of the opioid receptor improve CMT1A disease symptoms, because PMP22 mRNA expression levels in the patients were reduced by the treatment. This treatment may maintain normal conditions of PI3K/Akt/mTOR signaling and it did not affect MPZ mRNA expression. In the future, the treatment will be available for clinical treatment of patients with CMT1A disease.

As stated above, recent studies have shown that Gas6-Tyro3 signaling is necessary for Fyn activation and Schwann cell myelination (Miyamoto et al. 2015). These findings indicate the likelihood that controlling Gas6-Tyro3 signaling may enable a new therapy for CMT that would improve the quality of life for CMT patients. Given that these enzymes constitute one of the most important classes of therapeutic

targets, special emphasis is placed on the MEK1/2-ERK signaling pathway through the Gas6-TAM signaling pathway.

References

Ackerman SD, Garcia C, Piao X, Gutmann DH, Monk KR (2015) The adhesion GPCR Gpr56 regulates oligodendrocyte development via interaction with Gα12/13 and RhoA. Nat Commun 6:6122

Barros CS, Nguyen T, Spencer KS, Nishiyama A, Colognato H, Muller U (2009) Beta1 integrins are required for normal CNS myelination and promote AKT-dependent myelin outgrowth. Development 136:2717–2724

Basak S, Desai DJ, Rho EH, Ramos R, Maurel P, Kim HA (2015) E-cadherin enhances neuregulin signaling and promotes Schwann cell myelination. Glia 63:1522–1536

Beirowski B, Babetto E, Golden JP, Chen YJ, Yang K, Gross RW, Patti GJ, Milbrandt J (2014) Metabolic regulator LKB1 is crucial for Schwann cell-mediated axon maintenance. Nat Neurosci 17:1351–1361

Bercury KK, Macklin WB (2015) Dynamics and mechanisms of CNS myelination. Dev Cell 32:447–458

Biffiger K, Bartsch S, Montag D, Aguzzi A, Schachner M, Bartsch U (2000) Severe hypomyelination of the murine CNS in the absence of myelin-associated glycoprotein and Fyn tyrosine kinase. J Neurosci 20:7430–7437

Binder MD, Cate HS, Prieto AL, Kemper D, Butzkueven H, Gresle MM, Cipriani T, Jokubaitis VG, Carmeliet P, Kilpatrick TJ (2008) Gas6 deficiency increases oligodendrocyte loss and microglial activation in response to cuprizone-induced demyelination. J Neurosci 28:5195–5206

Binder MD, Xiao J, Kemper D, Ma GZ, Murray SS, Kilpatrick TJ (2011) Gas6 increases myelination by oligodendrocytes and its deficiency delays recovery following cuprizone-induced demyelination. PLoS One 6:e17727

Boshans RL, Szanto S, van Aelst L, D'Souza-Schorey C (2000) ADP-ribosylation factor 6 regulates actin cytoskeleton remodeling in coordination with Rac1 and RhoA. Mol Cell Biol 20:3685–3694

Bouhy D, Timmerman V (2013) Animal models and therapeutic prospects for Charcot-Marie-Tooth disease. Ann Neurol 74:391–396

Buser AM, Eme B, Werner HB, Nave KA, Schareren-Wiemers N (2009) The septin cytoskeleton in myelinating glia. Mol Cell Neurosci 40:156–166

Casanova JE (2007) Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. Traffic 8:1476–1485

Chan JR, Cosgaya JM, Wu YJ, Shooter EM (2001) Neurotrophins are key mediators of the myelination program in the peripheral nervous system. Proc Natl Acad Sci U S A 98:14661–14668

Chan JR, Jolicoeur C, Yamauchi J, Elliott J, Fawcett JP, Ng BK, Cayouette M (2006) The polarity protein Par-3 directly interacts with p75NTR to regulate myelination. Science 314 (5800):832–836

Chen S, Velardez MO, Warot X, Yu ZX, Miller SJ, Cros D, Corfas G (2006) Neuregulin 1-erbB signaling is necessary for normal myelination and sensory function. J Neurosci 26:3079–3086

Crawford AT, Desai D, Gokina P, Basak S, Kim HA (2008) E-cadherin expression in postnatal Schwann cells is regulated by the cAMP-dependent protein kinase a pathway. Glia 56:1637–1647

D'Souza-Schorey C, Chavrier P (2006) ARF proteins: roles in membrane traffic and beyond. Nat Rev Mol Cell Biol 7:347–358

Delague V, Jacquier A, Hamadouche T, Poitelon Y, Baudot C, Boccaccio I, Chouery E, Chaouch M, Kassouri N, Jabbour R, Grid D, Mégarbané A, Haase G, Lévy N (2007) Mutations

in FGD encoding the Rho GDP/GTP exchange factor FRABIN cause autosomal recessive Charcot-Marie-Tooth type 4H. Am J Hum Genet 81:1–16

Domènech-Estévez E, Baloui H, Meng X, Zhang Y, Deinhardt K, Dupree JL, Einheber S, Chrast R, Salzer JL (2016) Akt regulates axon wrapping and myelin sheath thickness in PNS. J Neurosci 36:4506–4521

D'Urso D, Prior R, Greiner-Petter R, Gabreëls-Festen AA, Müller HW (1998) Overloaded endoplasmic reticulum-Golgi compartments, a possible pathomechanism of peripheral neuropathies caused by mutations of the peripheral myelin protein PMP22. J Neurosci 18:731–740

Etxaniz U, Pérez-San Vicente A, Gago-López N, García-Dominguez M, Iribar H, Aduriz A, Pérez-López V, Burgoa I, Irizar H, Muñoz-Culla M, Vallejo-lllarramendi A, Leis O, Matheu A, Martin AG, Otaegui D, López-Mato MP, Gutilérrez-Rivera A, MacLellan R, Izeta A (2014) Neural-competent cells of adult human dermis belong to the Schwann lineage. Stem Cell Reports 3:774–788

Fabrizi GM, Taioli F, Cavallaro T, Rigatelli F, Simonati A, Mariani G, Perrone P, Rizzuto N (2000) Focally folded myelin in Charcot-Marie-Tooth neuropathy type 1B with Ser49Leu in the myelin protein zero. Acta Neuropathol 100:299–304

Fannon AM, Sherman DL, Ilyina-Gragerova G, Brophy PJ, Friedrich VL Jr, Colman DR (1995) Novel E-cadherin-mediated adhesion in peripheral nerve: Schwann cell architecture is stabilized by autotypic adherens junctions. J Cell Biol 129:189–202

Fernandez-Valle C, Gorman D, Gomez AM, Bunge MB (1997) Actin plays a role in both changes in cell shape and gene-expression associated with Schwann cell myelination. J Neurosci 17:241–250

Fledrich R, Stassart RM, Klink A, Rasch LM, Prukop T, Haag L, Cresnik D, Kungl T, Abdelaal TA, Keric N, Stadelmann C, Brück W, Nave KA (2014) Soluble neurogulin-1 modulates disease pathogenesis in rodent models of Charcot-Marie-Tooth disease 1A. Nat Med 20:1055–1061

Flores AI, Narayanan SP, Morse EN, Shick HE, Yin X, Kidd G, Avila RL, Kirschner DA, Macklin WB (2008) Constitutively active Akt induces enhanced myelination in the CNS. J Neurosci 28:7174–7183

Franco M, Peters PJ, Boretto J, van Donselaar E, Neri A, D'Souza-Schorey C, Chavrier P (1999) EFA6, a sec7 domain-containing exchange factor for ARF6, coordinates membrane recycling and actin cytoskeleton organization. EMBO J 16:5445–5454

Fujita N, Kemper A, Dupree J, Nakayasu H, Bartsch U, Schachner M, Maeda N, Suzuki K, Popko B (1998) The cytoplasmic domain of the large myelin-associated glycoprotein isoform is needed for proper CNS but not peripheral nervous system myelination. J Neurosci 18:1970–1978

Fünfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Möbius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes CT, Frahm J, Goebbels S, Nave KA (2012) Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature 485(7399):517–521

Giera S, Deng Y, Luo R, Ackerman SD, Mogha A, Monk KR, Ying Y, Jeong SJ, Manabu M, Bialas A, Chang BS, Stevens B, Corfas G, Piao X (2015) The adhesion G protein-coupled receptor GPR56 is a cell-autonomous regulator of oligodendrocyte development. Nat Commun 6:6121

Giovannini M, Robanus-Maandag E, Niwa-Kawakita M, van der Valk M, Woodruff JM, Goutebroze L, Mérel P, Berns A, Thomas G (1999) Schwann cell hyperplasia and tumors in transgenic mice expressing a naturally occurring mutant NF2 protein. Genes Dev 13:978–986

Godowski PJ, Mark MR, Chen J, Sadick MD, Raab H, Hammonds RG (1995) Reevaluation of the roles of protein S and gas6 as ligands for the receptor tyrosine kinase Rse/Tyro 3. Cell 82:355–358

Goebbels S, Oltrogge JH, Wolfer S, Wieser GL, Nientiedt T, Pieper A, Ruhwedel T, Sereda MW, Nave KA (2012) Genetic disruption of PTEN in a novel mouse model of tomaculous neuropathy. EMBO Mol Med 4:486–499

Goldfinger LE, Ptak C, Jeffery ED, Shabanowitz J, Hunt DF, Ginsberg MH (2006) RLIP (RalBP1) is an R-Ras effector that mediates adhesion-dependent Rac activation and cell migration. J Cell Biol 174:877–888

Goudarzi S, Rivera A, Butt AM, Hafizi S (2016) Gas6 promotes oligodendrogenesis and myelination in adult central nervous system and after lysolecithin-induced demyelination. ASN Neuro 14. pii: 1759091416668430

Guo L, Moon C, Niehaus K, Zheng Y, Ratner N (2012) Rac1 controls Schwann cell myelination through cAMP and NF2/merlin. J Neurosci 32:17251–17261

Guo L, Moon C, Zheng Y, Ratner N (2013) Cdc42 regulates Schwann cell radial sorting and myelin sheath folding through NF2/merlin-dependent and independent signaling. J Neurosci 61:1906–1921

Hasse B, Bosse F, Muller HW (2002) Proteins of peripheral myelin are associated with glycosphingolipid/cholesterol-enriched membranes. J Neurosci Res 69:227–232

Hempstead BL (2005) Coupling neurotrophins to cell migration through selective guanine nucleotide exchange factor activation. Proc Natl Acad Sci U S A 102:5645–5646

Hodge RG, Ridley AJ (2016) Regulating Rho GTPases and their regulators. Nat Rev Mol Cell Biol 17:496–510

Horn M, Baumann R, Pereira JA, Sidiropoulos PN, Somandin C, Welzl H, Stendel C, Lühman T, Wessig C, Toyka KV, Relvas JB, Senderek J, Suter U (2012) Myelin is dependent on the Charcot-Marie-Tooth Type 4H disease culprit protein FRABIN/FGD4 in Schwann cells. Brain 135:3567–3583

Hu J, Bai X, Bowen JR, Dolat L, Korobova F, Yu W, Baas PW, Svitkina T, Gallo G, Spiliotis ET (2012) Septin-driven coordination of actin and microtubule remodeling regulates the collateral branching of axons. Curr Biol 22:1109–1115

Iida M, Koike H, Ando T, Sugiura M, Yamamoto M, Tanaka F, Sobue G (2012) A novel MPZ mutation in Charcot-Marie-Tooth disease type 1B with focally folded myelin and multiple entrapment neuropathies. Neuromuscul Disord 22:166–169

Ito H, Atsuzawa K, Morishita R, Usuda N, Sudo K, Iwamoto I, Mizutani K, Katoh-Semba R, Nozawa Y, Asano T, Nagata K (2009) Sept8 controls the binding of vesicle-associated membrane protein 2 to synaptophysin. J Neurochem 108:867–880

Jin F, Dong B, Georgiou J, Jiang Q, Zhang J, Bharioke A, Qiu F, Lommel S, Feltri ML, Wrabetz L, Roder JC, Eyer J, Chen X, Peterson AC, Siminovitch KA (2011) N-WASp is required for Schwann cell cytoskeletal dynamics, normal myelin gene expression and peripheral nerve myelination. Development 138:1329–1337

Kochański A, Drac H, Jedrzejowska H, Hausmanowa-Petrusewicz I (2003) Focally folded myelin in Charcot-Marie-Tooth type 1B disease is associated with Asn131Lys mutation in myelin protein zero gene: short report. Eur J Neurol 10:547–549

Krämer-Albers EM, White R (2011) From axon-glial signalling to myelination: the integrating role of oligodendroglial Fyn kinase. Cell Mol Life Sci 68:2003–2012

La Marca R, Cerri F, Horiuchi K, Bachi A, Feltri ML, Wrabetz L, Biobel CP, Quattrini A, Salzer JL, Taveggla C (2011) TACE (ADAM17) inhibits Schwann cell myelination. Nat Neurosci 14:857–865

Lallemand D, Curto M, Saotome I, Giovannini M, MacClatchey AI (2003) NF2 deficiency promotes tumorigenesis and metastasis by destabilizing adherens junctions. Genes Dev 17:1090–1100

Laursen LS, Chan CW, French-Constant C (2009) An integrin-contactin complex regulates CNS myelination by differential Fyn phosphorylation. J Neurosci 29:9174–9185

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

Marchesin V, Montagnac G, Chavrier P (2015) ARF6 promotes the formation of Rac1 and WAVE-dependent ventral F-actin rosettes in breast cancer cells in response to epidermal growth factor. PLoS One 10:e0121747

Maurel P, Einheber S, Galinska J, Thaker P, Lam I, Rubin MB, Scherer SS, Murakami Y, Gutmann DH, Salzer JL (2007) Nectin-like proteins mediate axon Schwann cell interactions along the internode and are essential for myelination. J Cell Biol 178:861–874

Meyer Z, Hörste G, Nave KA (2006) Animal models of inherited neuropathies. Curr Opin Neurol 19:464–473

Mityamoto Y, Torii T, Tanoue A, Kawahara K, Arai M, Tsumura H, Ogata T, Nagao M, Terada N, Yamamoto M, Takashima S, Yamauchi J (2017) Neuregulin-1 type III knockout mice exhibit delayed migration of Schwann cell precursors. Biochem Biophys Res Commun 486:506–513

Miyamoto Y, Torii T, Nakamura K, Takashima S, Sanbe A, Tanoue A, Yamauchi J (2013) Signaling through Arf6 guanine-nucleotide exchange factor cytohesin-1 regulates migration in Schwann cells. Cell Signal 25:1379–1387

Miyamoto Y, Torii T, Takada S, Ohno N, Saitoh Y, Nakamura K, Ito A, Ogata T, Terada N, Tanoue A, Yamauchi J (2015) Involvement of the Tyro3 receptor and its intracellular partner Fyn signaling in Schwann cell myelination. Mol Biol Cell 26:3489–3503

Mogha A, Harty BL, Carlin D, Joseph J, Sanchez NE, Suter U, Piao X, Cavalli V, Monk KR (2016) Gpr126/Adgrg6 has Schwann cell autonomous and nonautonomous function in peripheral nerve injury and repair. J Neurosci 36:12351–12367

Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C, Moens CB, Talbot WS (2009) A G protein-coupled receptor is essential for Schwann cells to initiate myelination. Science 325:1402–1405

Narayanan SP, Flores AI, Wang F, Macklin WB (2009) Akt signals through the mammalian target of rapamycin pathway to regulate CNS myelination. J Neurosci 29:6860–6870

Nave KA, Salzer JL (2006) Axonal regulation of myelination by neuregulin 1. Curr Opin Neurobiol 16:492–500

Nave KA, Werner HB (2014) Myelination of the nervous system: mechanisms and function. Annu Rev Cell Dev Biol 30:503–533

Nawaz S, Sánchez P, Schmitt S, Snaidero N, Mitkovski M, Velte C, Brückner BR, Alexopoulos I, Czopka T, Jung SY, Rhee JS, Janshoff A, Witke W, Schaap IAT, Lyons DA, Simons M (2015) Actin filament turnover drives leading edge growth during myelin sheath formation in the central nervous system. Dev Cell 34:139–151

Norton WT, Poduslo SE (1973) Myelination in rat brain: changes in myelin composition during brain maturation. J Neurochem 21:759–773

Noseda R, Guerrero-Valero M, Alberizzi V, Previtali SC, Sherman DL, Palmisano M, Huganir RL, Nave KA, Cuenda A, Feltri ML, Brophy PJ, Bolino A (2016) Kif13b regulates PNS and CNS myelination through the Dlg1 scaffold. PLoS Biol 14:2100–2440

Novak N, Bar V, Sabanay H, Frechter S, Jaegle M, Snapper SB, Meijer D, Peles E (2011) N-WASP is required for membrane wrapping and myelination by Schwann cells. J Cell Biol 192:243–250

Paavola KJ, Sidik H, Zuchero JB, Eckart M, Talbot WS (2014) Type 4 collagen is an activating ligand for the adhesion G protein-coupled receptor GPR126. Sci Signal 7:ra76

Palazuelos J, Crawford HC, Klingener M, Sun B, Karelis J, Raines EW, Aguirre A (2014) TACE/ADAM17 is essential for oligodendrocyte development and CNS myelination. J Neurosci 34:11884–11896

Palazuelos J, Klingener M, Raines EW, Crawford HC, Aguirre A (2015) Oligodendrocyte regeneration and CNS remyelination require TACE/ADAM17. J Neurosci 35:12241–12247

Pareyson D, Marchesi C (2009) Diagnosis, natural history, and management of Charcot-Marie-Tooth disease. Lancet Neurol 8:654–667

Patzig J, Jahn O, Wichert SP, de Monasterio-Schrader P, Rosfa S, Kuharev L, Yan K, Bormuth I, Bremer J, Aguzzi A, Orfaniotou F, Hesse D, Schwab MH, Möbius W, Nave KA, Werner HB (2011) Quantitative and integrative proteome analysis of peripheral nerve myelin identifies novel myelin proteins and candidate neuropathy loci. J Neurosci 31:16369–16386

Patzig J, Erwig MS, Tenzer S, Kusch K, Dibaj P, Möbius W, Goebbels S, Schaeren-Wiemers N, Nave KA, Werner HB (2016) Septin/anillin filaments scaffold central nervous system myelin to accelerate nerve conduction. elife 5. pii:e17119

Pegtel DM, Ellenbroek SI, Mertens AE, van der Kammen RA, de Rooij J, Collard JG (2007) The Par-Tiam1 complex controls persistent migration by stabilizing microtubule-dependent front-rear polarity. Curr Biol 17:1623–1634

Perlin JR, Talbot WS (2007) Putting the glue in glia: Necls mediate Schwann cell–axon adhesion. J Cell Biol 178:721

Perlin JR, Lush ME, Stephens WZ, Piotrowski T, Talbot WS (2011) Neuronal neuregulin 1 type III directs Schwann cell migration. Development 138:4639–4648

Pooya S, Liu X, Kumar VB, Anderson J, Imai F, Zhang W, Ciraolo G, Ratner N, Setchell KD, Yoshida Y, Jankowski MP, Dasgupta B (2014) The tumour suppressor LKB1 regulates myelination through mitochondrial metabolism. Nat Commun 5:4993

Prieto AL, Weber JL, Tracy S, Heeb MJ, Lai C (1999) Gas6, a ligand for the receptor protein-tyrosine kinase Tyro-3, is widely expressed in the central nervous system. Brain Res 816:646–661

Prieto AL, Weber JL, Lai C (2000) Expression of the receptor protein-tyrosine kinases Tyro-3, Axl, and Mer in the developing rat central nervous system. J Comp Neurol 425:295–314

Quast T, Tappertzhofen B, Schild C, Grell J, Czeloth N, Förster R, Alon R, Fraemohs L, Dreck K, Weber C, Lämmermann T, Sixt M, Kolanus W (2009) Cytohesin-1 controls the activation of RhoA and modulates integrin-dependent adhesion and migration of dendritic cells. Blood 113:5801–5810

Radhakrishna H, Al-Awar O, Khachikian Z, Donaldson JG (1999) Rac ruffling suggests a role for membrane trafficking in cortical actin rearrangements. J Cell Sci 112:855–866

Rossor AM, Polke JM, Houlden H, Reilly MM (2013) Clinical implications of genetic advances in Charcot-Marie-Tooth disease. Nat Rev Neurol 9:562–571

Rothlin CV, Carreara-Silva EA, Bosurgi L, Ghosh S (2015) TAM receptor signaling in immune homeostasis. Annu Rev Immunol 33:355–391

Sadok A, Marshall CJ (2014) Rho GTPases: masters of cell migration. Small GTPases 5:e29710

Salzer JL (2015) Schwann cell myelination. Cold Spring Harb Perspect Biol 7:a020529

Santy LC, Casanova JE (2001) Activation of ARF6 by ARNO stimulates epithelial cell migration through downstream activation of both Rac1 and phospholipase D. J Cell Biol 154:599–610

Simons M, Kramer EM, Thiele C, Stoffel W, Trotter J (2000) Assembly of myelin by association of proteolipid protein with cholesterol- and galactosylceramide-rich membrane domains. J Cell Biol 151:143–154

Spiegel I, Adamsky K, Eshed Y, Milo F, Sabanay H, Sarig-Nadir O, Horresh I, Scherer SS, Rasband MN, Peles E (2007) A central role for Necl4 (SynCAM4) in Schwann cell-axon interaction and myelination. Nat Neurosci 10:861–869

Stendel C, Roos A, Deconinck T, Pereira J, Castagner F, Niemann A, Kirscher J, Korinthenberg R, Ketelsen UP, Battaloglu E, Parman Y, Nicholson G, Ouvrier R, Seeger J, De Jonghe P, Weis J, Krüttgen A, Rudnik-Schöneborn S, Bergmann C, Suter U, Zerres K, Timmerman V, Relvas JB, Senderek J (2007) Peripheral nerve demyelination caused by a mutant Rho GTPase guanine nucleotide exchange factor, frabin/FGD4. Am J Hum Genet 81:158–164

Takenawa T, Suetsugu S (2007) The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. Nat Rev Mol Cell Biol 8:37–48

Taveggia C, Salzer JL (2007) PARsing the events of myelination. Nat Neurosci 10:17–18

Taveggia C, Thaker P, Petrylak A, Caporaso GL, Toews A, Falls DL, Einheber S, Salzer JL (2008) Type III neuregulin-1 promotes oligodendrocyte myelination. Glia 56:284–293

Tep C, Kim ML, Opincariu LI, Limpert AS, Chan JR, Appel B, Carter BD, Yoon SO (2012) Brain-derived neurotrophic factor (BDNF) induces polarized signaling of small GTPase (Rac1) protein at the onset of Schwann cell myelination through partitioning-defective 3 (Par3) protein. J Biol Chem 287:1600–1608

Torii T, Miyamoto Y, Sanbe A, Nishimura K, Yamauchi J, Tanoue A (2010) Cytohesin-2/ARNO, through its interaction with focal adhesion adaptor protein paxillin, regulates preadipocyte migration via the downstream activation of Arf6. J Biol Chem 285:24270–24281

Torii T, Miyamoto Y, Onami N, Tsumura H, Nemoto N, Kawahara K, Kato M, Kotera J, Nakamura K, Tanoue A, Yamauchi J (2013) In vivo expression of the Arf6 guanine-nucleotide exchange factor cytohesin-1 in mice exhibits enhanced myelin thickness in nerves. J Mol Neurosci 51:522–531

Torii T, Ohno N, Miyamoto Y, Kawahara K, Saitoh Y, Nakamura K, Takashima S, Sakagami H, Tanoue A, Yamauchi J (2015) Arf6 guanine-nucleotide exchange factor cytohesin-2 regulates myelination in nerves. Biochem Biophys Res Commun 460:819–825

Trapp BD, Andrews SB, Cootauco C, Quarles R (1989) The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes. J Cell Biol 109:2417–2426

Umemori H, Sato S, Yagi T, Aizawa S, Yamamoto T (1994) Initial events of myelination involve Fyn tyrosine kinase signalling. Nature 367:572–576

Werner HB, Krämer-Albers EM, Strenzke N, Saher G, Tenzer S, Ohno-Iwashita Y, De Monasterio-Schrader P, Möbius W, Moser T, Griffiths IR, Nave KA (2013) A critical role for the cholesterol-associated proteolipids PLP and M6B in myelination of the central nervous system. Glia 61:567–586

White R, Krämer-Albers EM (2014) Axon-glia interaction and membrane traffic in myelin formation. Front Cell Neurosci 7:284

Yamauchi J, Chan JR, Shooter EM (2004) Neurotrophins regulate Schwann cell migration by activating divergent signaling pathways dependent on Rho GTPases. Proc Natl Acad Sci U S A 101:8774–8779

Yamauchi J, Miyamoto Y, Tanoue A, Shooter EM, Chan JR (2005) Ras activation of a Rac1 exchange factor, Tiam1, mediates neurotrophin-3-induced Schwann cell migration. Proc Natl Acad Sci U S A 102:14889–14894

Yamauchi J, Miyamoto Y, Jonah CR, Tanoue A (2008) ErbB2 directly activates the exchange factor Dock7 to promote Schwann cell migration. J Cell Biol 181:351–365

Yamauchi J, Miyamoto Y, Hamasaki H, Sanbe A, Kusakawa S, Nakamura A, Tsumura H, Maeda M, Nemoto N, Kawahara K, Torii T, Tanoue A (2011) The atypical Guanine-nucleotide exchange factor, Dock7, negatively regulates Schwann cell differentiation and myelination. J Neurosci 31:12579–12592

Yamauchi J, Miyamoto Y, Torii T, Takashima S, Kondo K, Kawahara K, Nemoto N, Chan JR, Tsujimoto G, Tanoue A (2012) Phosphorylation of cytohesin-1 by Fyn is required for initiation of myelination and the extent of myelination during development. Sci Signal 5:ra69

Zollinger DR, Chang KJ, Baalman K, Kim S, Rasband MN (2015) The polarity protein Pals1 regulates radial sorting of axon. J Neurosci 35:10474–10484

Zuchero JB, Fu MM, Sloan SA, Ibrahim A, Olson A, Zaremba A, Dugas JC, Wienbar S, Caprariello AV, Kantor C, Leonoudakis D, Lariosa-Willingham K, Kronenberg G, Gertz K, Soderling SH, Miller RH, Barres BA (2015) CNS myelin wrapping is driven by actin disassembly. Dev Cell 34:152–167

Regulatory Mechanism of Peripheral Nerve Myelination by Glutamate-Induced Signaling

2

Toshiyuki Araki

Abstract

Regulation of differentiation and proliferation of Schwann cells is an essential part of the regulation of peripheral nerve development, degeneration, and regeneration. ZNRF1, a ubiquitin ligase, is expressed in undifferentiated/repair Schwann cells, directs glutamine synthetase to proteasomal degradation, and thereby increase glutamate levels in Schwann cell environment. Glutamate elicits subcellular signaling in Schwann cells via mGluR2 to modulate Neuregulin-1/ErbB2/3 signaling and thereby promote undifferentiated phenotype of Schwann cell.

Keywords

ZNRF1 ubiquitin ligase · Oxidative stress · Neuregulin-1 · ErbB2/3 · Phosphorylation · Differentiation · Subcellular signaling · Glutamate · mGluR2

2.1 Introduction

Schwann cells are the glial cells in the peripheral nervous system and ensheath neuronal axons to form myelin, which serves as insulator required for fast electrical conduction, as well as to protect and nurture neuronal axons. Schwann cells are known to be derived from neural crest cells during development (Jessen and Mirsky 2005). Migration and maturation of Schwann cells during development are achieved in close connection with neuronal axons. In rodents, pro-myelinating Schwann cells, or immature Schwann cells, characterized by induced expression of molecules including neural cell adhesion molecule (NCAM), p75 neurotrophin receptor

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(p75NTR), and glial fibrillary acidic protein (GFAP) develop from neural crest-derived Schwann cell precursors at around embryonic day 15–17 (Jessen et al. 2015). They keep proliferating in contact with axons as immature Schwann cells, and also differentiate into non-myelinating Schwann cells eventually forming Remak bundles or promyelinating Schwann cells that make one-to-one contact relationship with axons to eventually express myelin proteins including myelin protein zero (P0) and myelin basic protein (MBP) and form myelin sheath. Damage to a peripheral nerve triggers characteristic change of phenotypes of Schwann cells located distal to the injury site (Fawcett and Keynes 1990). The myelinating and non-myelinating Schwann cells distal to nerve injury undergo a large-scale change in gene expression to dedifferentiate to show a phenotype similar to immature Schwann cells (also called Bungner cells or repair cells) (Jessen and Mirsky 2016). These cells can proliferate without axonal contact, and help support nerve regeneration, which is a distinguishing characteristic of peripheral nerves from central nerves. They eventually redifferentiate into myelinating Schwann cells and non-myelinating Schwann cells in regenerated nerves.

Understanding of regulatory mechanisms of switching Schwann cell phenotypes should lead to a detailed understanding of physiological myelination and development of a therapeutic approach against demyelinating neuropathy. The vastly different phenotypes of Schwann cells observed during development as well as after nerve injury are defined by a tightly regulated expression of specific molecules (Salzer 2008). Such molecules include transcription factors important for orchestrated expression of molecules, and cell surface molecules important for signaling, attachment, and sorting of Schwann cells in the environment. We have previously performed gene expression profiling studies of post-injury peripheral nerve. In the course of those studies, we identified that glutamine synthetase (GS) is degraded in dedifferentiated Schwann cells in the peripheral nerve after injury. In a follow-up study, we recently reported the role of glutamate in the peripheral nerve. In this chapter, we mainly focus on the regulatory mechanism of peripheral myelination during development and remyelination during regeneration with special reference to the series of our studies on glutamate metabolism.

2.2 ZNRF1 E3 Ubiquitin Ligase-Dependent Degradation of GS

To clarify injury-induced phenotypic changes of Schwann cells at molecular level, we performed gene expression profile analysis using injured peripheral nerve distal to the injury site (Araki et al. 2001; Nagarajan et al. 2002). We were able to classify dysregulated genes into four groups, with expression induction peaking 1, 4, 7, and 14 days after injury. Those peaking at day 1 include immediate early genes and those expressed in blood-borne cells. The genes peaking at day 4 contain cell proliferation-related genes, reflecting the Schwann cell proliferation peaking around 4 days after injury in this model. Many of the genes involved in promoting nerve regeneration are found among genes most highly induced 1–2 weeks after injury in Schwann cells. Among the dysregulated genes, we identified Zinc-RING finger 1 (ZNRF1) as a

gene whose expression is induced after injury in Schwann cells, peaking at 7 days after injury (Araki et al. 2001).

ZNRF1 gene encodes a 228-amino acid residue polypeptide. ZNRF1 has a myristoylation signal consensus sequence at its N-terminus, suggesting that ZNRF1 protein is anchored to the plasma membrane (Araki et al. 2001). ZNRF1 has a RING finger motif at its C-terminal region, suggesting that ZNRF1 has E3 ubiquitin ligase activity, and indeed we were able to show the E3 activity of ZNRF1 in an in vitro ubiquitination assay (Araki and Milbrandt 2003). Therefore, we sought to identify the enzymatic target for ZNRF1 ubiquitin ligase activity. By screening mouse brain proteins that associate with ZNRF1, we identified glutamine synthetase (GS) (Saitoh and Araki 2010).

GS is the enzyme which converts glutamate into glutamine (Cooper 1988). In the central nervous system (CNS), GS is known to be expressed exclusively in astrocytes and is frequently used as a marker for identification of astrocytes (Albrecht et al. 2007). In CNS, glutamate is generated in neurons and serves as an excitatory neurotransmitter. Astrocytes take up released glutamate from synaptic cleft via glutamate transporter to terminate neurotransmission. Glutamate uptake into astrocytes also contributes to decrease excitotoxicity caused by excess glutamate derived from injury to the brain or abnormal excitation activity of neurons (Coulter and Eid 2012). In the peripheral nervous system, on the other hand, GS protein was reported to be expressed in Schwann cells and satellite cells in the peripheral ganglia (Miller et al. 2002), but functional significance of GS in PNS was unknown, since glutamate is not a neurotransmitter in the PNS.

In the PNS, we found that GS mRNA is constantly expressed both in myelinating and in non-myelinating (repair) Schwann cells, while GS protein is highly expressed only in myelinating Schwann cells (Saitoh and Araki 2010). This corresponds to the ZNRF1-dependent GS degradation in non-myelinating (repair) Schwann cells and suggests that GS expression levels are regulated mostly post-translationally by ZNRF1-dependent proteasomal degradation. To understand the functional significance of GS in the PNS, we performed ZNRF1 overexpression in primary cultured Schwann cells and applied the cells on "in vitro myelination" experiments. "in vitro myelination experiment" is a culture model of myelination, in which DRG neurons and Schwann cells are cocultured, followed by induction of myelination by ascorbic acid application, and is often used for functional evaluation of genes and compounds affecting Schwann cell differentiation (Bunge et al. 1980). We found that GS overexpressing Schwann cells showed increased myelination in the in vitro myelination culture compared to wild-type Schwann cells. These results suggested that GS may have a function to regulate myelination, in addition to its apparent role for supplying nutrition to neuronal axons by metabolizing amino acids.

In response to nerve injury, Schwann cells in the segment distal to the injury site dedifferentiate from myelinating Schwann cells to repair cells or immature Schwann cell-like cells (Jessen and Mirsky 2016). We observed that, during this phenotypic transition, oxidative stress levels increase in Schwann cells, presumably due to vast changes of metabolic status inside the Schwann cells (Saitoh and Araki 2010). Proteins coordinating divalent cations are known to be labile to posttranslational

modification by oxidative stress (Sherman and Brophy 2005). GS protein requires magnesium ion for maintaining structural stability, and when oxidized (carbonylated), GS is known to lose enzymatic activity by structural changes (Oliver et al. 1990). We showed that GS is carbonylated in dedifferentiated Schwann cells, which suggests that loss of structural integrity of GS in dedifferentiated Schwann cells due to the increased oxidative stress and subsequently degraded in the proteasome (Saitoh and Araki 2010). This is particularly interesting, because, as mentioned previously, ascorbic acid, a potent reducing agent, has long been used as an inducer of myelination in the in vitro myelination experiment. Ascorbic acid was also shown to induce myelination in a mouse model of Charcot–Marie–Tooth disease type 1 (Passage et al. 2004). Our findings may be regarded as another supportive evidence that oxidative environment may be supportive to dedifferentiation and proliferation of Schwann cells (Hinder et al. 2012), while antioxidant application is promotive to myelination (Sekido et al. 2004; Hyung et al. 2015).

2.3 Effect of Glutamate on Schwann Cells

Our observation of increased myelination with GS-overexpressing Schwann cells in culture suggested that GS activity is not only necessary for metabolizing amino acid but also related to Schwann cell differentiation. As described above, GS is an enzyme converting glutamate to glutamine, and thus increased GS activity by GS overexpression in Schwann cells should cause decrease in glutamate and increase in glutamine levels. We explored which of these two changes affects Schwann cell phenotype, and found that increased glutamate level in culture increases Schwann cell proliferation, while increased/decreased glutamine levels seemed indifferent to Schwann cell phenotype, suggesting that GS affect Schwann cell phenotype via affecting glutamate levels (Saitoh and Araki 2010). To clarify how glutamate affects Schwann cell phenotype, we employed a series of glutamate agonists/antagonists on cultured Schwann cells and compared the phenotypic changes with that observed by glutamate application. From this experiment, we found that metabotropic glutamate receptor (mGluR) agonists, and mGluR2/3-specific agonists, in particular, were able to mimic the glutamate-dependent Schwann cell proliferation, and mGluR2/3-specific antagonists inhibited the glutamate-dependent Schwan cell proliferation. Since Schwann cells expressed mGluR2 but not mGluR3, and RNAi-mediated downregulation of mGluR2 inhibited the glutamate-dependent Schwann cell proliferation, mGluR2 was identified to be responsible for glutamate-induced Schwann cell proliferation. Schwann cell proliferation is often mediated by Erk phosphorylation as subcellular signaling. In the case of glutamate-induced Schwann cell proliferation, we also observed increased Erk phosphorylation levels in response to glutamate. Interestingly, we also found that mGluR2-mediated glutamate effect requires Neurogulin-1 (Nrg1)-elicited Erk phosphorylation signaling. We observed that PKI166 (pharmacological Nrg1-specific antagonist) can completely shut down the glutamate-dependent Schwan cell proliferation. Nrg1, a potent Schwann cell mitogen, takes ErbB2/ErbB3 heterodimer on Schwann cell plasma membrane as the

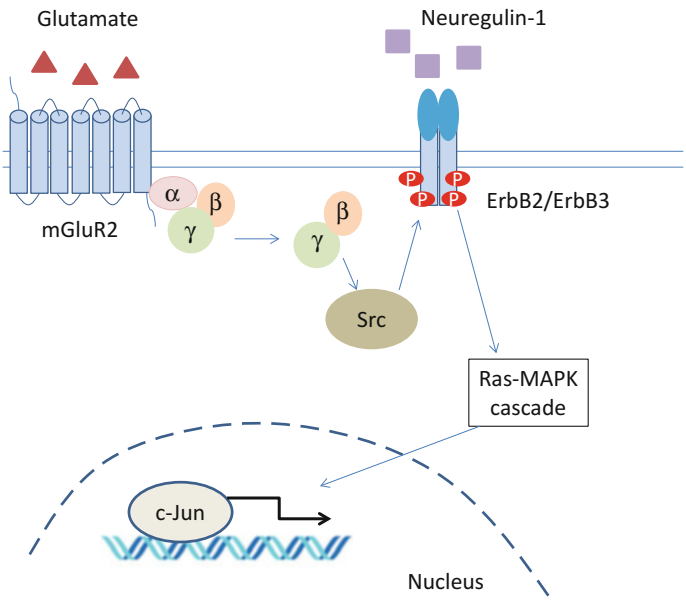

Fig. 2.1 Schematic diagram of glutamate-elicited subcellular signaling in Schwann cells. Glutamate binds to mGluR2 to dissociate Gβγ. Gβγ promotes Src-dependent phosphorylation of ErbB2. Neuregulin1-elicited Erk phosphorylation is potentiated by the glutamate/mGluR2-elicited ErbB2 phosphorylation to regulate gene transcription to promote proliferation/undifferentiated phenotype of Schwann cells via expression of the transcription factors, including c-jun

signaling complex (Newbern and Birchmeier 2010). Nrg1-ErbB2/3 signaling is also known to be able to mediate differentiation signal by increasing Akt phosphorylation, but we found that glutamate stimulation did not change Akt phosphorylation status in Schwann cell. This differential effect may be mediated by ErbB2 phosphorylation profile observed by glutamate stimulation; we found that tyrosine residues 1221 and 1222 of ErbB2 particularly increased by glutamate stimulation compared to other possible phosphorylation sites. Metabotropic glutamate receptors are G-protein-coupled receptors (GPCR) (Kim et al. 2008). By analyzing the involvement of candidate molecules for GPCR-elicited subcellular signaling, we identified that mGluR2 signals via Gβγ, Src, but not Gα, phosphatidylinositol 3-kinase (Fig. 2.1).

Nrg1/ErbB2/3 signaling pathway is known to play a variety of different roles depending on stages of nerve development, degeneration, and regeneration in the PNS (Newbern and Birchmeier 2010; Boerboom et al. 2017). The signaling pathway regulates Schwann cell proliferation, migration, and survival, as well as differentiation/myelination (Syroid et al. 1996; Morris et al. 1999; Leimeroth et al. 2002; Taveggia et al. 2005; Chen et al. 2006; Freidin et al. 2009). Nrg1 is regarded as an important inducer of Erk activation after peripheral nerve injury in Schwann cells (Napoli et al. 2012). The Erk activation by phosphorylation leads to demyelination and Schwann cell dedifferentiation (Syed et al. 2010). On the other hand, Nrg1/

ErbB2/3 signaling is also implicated in remyelination after injury. Nrg1 type III, a transmembrane isoform of Nrg1, is known to elicit signals to determine myelin thickness during development and after nerve injury via ErbB2/3 receptor signaling in Schwann cells (Taveggia et al. 2005; Michailov et al. 2004; Fricker et al. 2011). The differential signaling of Nrg1/ErbB2/3 is presumably achieved by different environmental contexts, which causes interaction/coexistence of Nrg1/ErbB2/3 with other signaling pathways. For instance, the tyrosine phosphatase Shp2 (PTPN11) may be an essential modulator of Nrg1/ErbB2/3 signaling, since Nrg1-evoked proliferation and migration is abolished in the absence of Shp2 (Grossmann et al. 2009). The glutamate-elicited signaling we identified may be regarded as a mild modulator of Nrg1/ErbB2/3 signaling during development as well as degeneration of peripheral nerve. Glutamate released from Schwann cells and axons may serve as a modulator for Nrg1 to direct ErbB2/3 signaling to Schwann cell dedifferentiation and proliferation.

2.4 Role of Glutamate Signaling on Peripheral Nerve Myelination

During degeneration, and regeneration, differentiation and dedifferentiation signals orchestrate for the progression of phenotypic transition of Schwann cells. For instance, in the progression of myelination, dedifferentiation-promoting signals are suppressed (Jessen et al. 2015). In the case of glutamate-mGluR2-elicited signaling in Schwann cells, downregulation of glutamate-mGluR2-elicited signaling by RNAi-mediated downregulation of mGluR2 expression or mGluR2 antagonist application induced expression of Krox20, a key transcription factor for promoting Schwann cell myelination, while it reduced expression of c-Jun, which promotes immature Schwann cell phenotype (Saitoh et al. 2016). These data revealed that glutamate-mGluR2-elicited signaling orchestrates differentiating and dedifferentiating signals of Schwann cells. This may explain the apparent discrepancy between the relatively weak changes in Erk phosphorylation or expression levels of Krox20 and c-Jun, and clear transition of Schwann cell phenotype by glutamate-mGluR2-elicited signaling. Orchestrated changes of differentiating and dedifferentiating signals may play a more important role than dramatic changes of expression levels of transcription factors for the transition of Schwann cell phenotypes.

mGluR2 function for peripheral nerve myelination was confirmed by using animal models (Saitoh et al. 2016). Local administration of anmGluR2 antagonist to the injured nerve (the segment distal to the injury site) showed weak inhibition of the progression of demyelination. Local administration of glutamate to normal peripheral nerve, on the other hand, resulted in clear induction of dedifferentiation of Schwann cells (initiation of proliferation and demyelination around the administration site). The weak effect of mGluR2 signal inhibition may be because nerve injury elicits a variety of signals that lead to demyelination and the mGluR2 signal may be related to only a part of them.

Of note, a recent report showed that glutamate signal through NMDA-type ionotropic glutamate receptor on Schwan cell to induce transient induction of phosphorylation of Erk and Akt, and promotes Schwann cell migration (Campana et al. 2017). An important difference between ionotropic and metabotropic glutamate receptor signaling is the required glutamate concentration; ~100 micromolar is sufficient for ionotropic receptor activation, while milimolar range of glutamate is necessary for metabotropic receptor. The difference in the time-course of the glutamate effect and required glutamate concentration range suggests ionotropic and metabotropic glutamate receptors may transmit independent signals to Schwann cells. Indeed, while we have found that mGluR2-elicited signaling is independent of AKT activity, NMDAR inhibition was shown not only to downregulate ERK phosphorylation but also to inhibit AKT activation as well (Campana et al. 2017). We also presume that there were difference in key experimental conditions between our work and others, because, for instance, we did not see the lack of Erk phosphorylation in the basal conditions (without stimulation by glutamate or neurotrophin) in culture shown in the previous report, unless in the presence of an ErbB3 antagonist. Such difference in the experimental conditions may explain the different observations, at least in part.

2.5 Role of Glutamate Signaling Regulation by ZNRF1

The studies we described in this chapter showed that induced expression of ZNRF1 in injured nerve Schwann cells degrades GS which loses enzymatic activity by oxidative stress increased in response to dedifferentiation of Schwann cells, and thereby promotes immature phenotype of Schwann cells. In this context, oxidative stress serves as a subcellular signal to induce the phenotypic transition of Schwann cells. Ascorbic acid, a well-known reducing reagent, is often used as an inducer of myelination in the in vitro myelination model with DRG neuron and Schwann cell coculture. Ascorbic acid was also shown to be effective as a drug for suppressing disease phenotype of an animal model for hereditary peripheral nerve demyelinating neuropathy (Passage et al. 2004), and previous reports suggested some potential mechanism of beneficial actions based on its reducing activity (Saifi et al. 2003). Regulation of ZNRF1-dependent GS degradation may be a part of the mechanisms that explain how oxidative environment promotes dedifferentiation and reducing environment promotes differentiation of Schwann cells.

Based on the hopeful consequence in the animal model, beneficial effect of ascorbic acid was expected in human patients of hereditary demyelinating neuropathy, but clinical trials of high-dose ascorbic acid showed no improvement of myelination in human (Gess et al. 2015). Oxidative stress is regarded to be responsible, at least in part, for the pathogenesis of a variety of neurological disorders. However, radical scavengers and reducing reagents were often shown to be ineffective against such disorders (Ahmadinejad et al. 2017). This may be explained by the difference between human and animal models, and possibly be because these antioxidative compounds cannot reach to the site responsible for pathogenesis in

human. When radical scavengers and reducing reagents are ineffective, a possible strategy is to intervene signaling pathway or mechanism that is related to oxidative stress but not the oxidation itself. For this purpose, the signaling mechanism elicited by increased oxidative stress needs to be clarified. In this sense, our studies may be able to contribute to the development of a therapeutic approach against peripheral nerve demyelinating diseases, for example, by inhibiting mGluR signaling.

References

Ahmadinejad F, Geir Moller S, Hashemzadeh-Chaleshtori M, Bidkhori G, Jami MS (2017) Molecular mechanisms behind free radical scavengers function against oxidative stress. Antioxidants (Basel) 6(3)

Albrecht J, Sonnewald U, Waagepetersen HS, Schousboe A (2007) Glutamine in the central nervous system: function and dysfunction. Front Biosci 12:332–343

Araki T, Milbrandt J (2003) ZNRF proteins constitute a family of presynaptic E3 ubiquitin ligases. J Neurosci 23(28):9385–9394

Araki T, Nagarajan R, Milbrandt J (2001) Identification of genes induced in peripheral nerve after injury. Expression profiling and novel gene discovery. J Biol Chem 276(36):34131–34141

Boerboom A, Dion V, Chariot A, Franzen R (2017) Molecular mechanisms involved in Schwann cell plasticity. Front Mol Neurosci 10:38

Bunge MB, Williams AK, Wood PM, Uitto J, Jeffrey JJ (1980) Comparison of nerve cell and nerve cell plus Schwann cell cultures, with particular emphasis on basal lamina and collagen formation. J Cell Biol 84(1):184–202

Campana WM, Mantuano E, Azmoon P, Henry K, Banki MA, Kim JH et al (2017) Ionotropic glutamate receptors activate cell signaling in response to glutamate in Schwann cells. FASEB J 31(4):1744–1755

Chen S, Velardez MO, Warot X, Yu ZX, Miller SJ, Cros D et al (2006) Neuregulin 1-erbB signaling is necessary for normal myelination and sensory function. J Neurosci 26(12):3079–3086

Cooper A (1988) Glutamine synthetase. In: Kvamme E (ed) Glutamine and glutamate in mammals. CRC, Boca Raton, pp 7–31

Coulter DA, Eid T (2012) Astrocytic regulation of glutamate homeostasis in epilepsy. Glia 60 (8):1215–1226

Fawcett JW, Keynes RJ (1990) Peripheral nerve regeneration. Annu Rev Neurosci 13:43–60

Freidin M, Asche S, Bargiello TA, Bennett MV, Abrams CK (2009) Connexin 32 increases the proliferative response of Schwann cells to neuregulin-1 (Nrg1). Proc Natl Acad Sci U S A 106 (9):3567–3572

Fricker FR, Lago N, Balarajah S, Tsantoulas C, Tanna S, Zhu N et al (2011) Axonally derived neuregulin-1 is required for remyelination and regeneration after nerve injury in adulthood. J Neurosci 31(9):3225–3233

Gess B, Baets J, De Jonghe P, Reilly MM, Pareyson D, Young P (2015) Ascorbic acid for the treatment of Charcot-Marie-Tooth disease. Cochrane Database Syst Rev (12):CD011952

Grossmann KS, Wende H, Paul FE, Cheret C, Garratt AN, Zurborg S et al (2009) The tyrosine phosphatase Shp2 (PTPN11) directs Neuregulin-1/ErbB signaling throughout Schwann cell development. Proc Natl Acad Sci U S A 106(39):16704–16709

Hinder LM, Vincent AM, Burant CF, Pennathur S, Feldman EL (2012) Bioenergetics in diabetic neuropathy: what we need to know. J Peripher Nerv Syst 17(Suppl 2):10–14

Hyung S, Yoon Lee B, Park JC, Kim J, Hur EM, Francis Suh JK (2015) Coculture of primary motor neurons and Schwann cells as a model for in vitro myelination. Sci Rep 5:15122

Jessen KR, Mirsky R (2005) The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci 6(9):671–682

Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. J Physiol 594(13):3521–3531

Jessen KR, Mirsky R, Lloyd AC (2015) Schwann cells: development and role in nerve repair. Cold Spring Harb Perspect Biol 7(7):a020487

Kim CH, Lee J, Lee JY, Roche KW (2008) Metabotropic glutamate receptors: phosphorylation and receptor signaling. J Neurosci Res 86(1):1–10

Leimeroth R, Lobsiger C, Lussi A, Taylor V, Suter U, Sommer L (2002) Membrane-bound neuregulin1 type III actively promotes Schwann cell differentiation of multipotent progenitor cells. Dev Biol 246(2):245–258

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C et al (2004) Axonal neuregulin-1 regulates myelin sheath thickness. Science 304(5671):700–703

Miller KE, Richards BA, Kriebel RM (2002) Glutamine-, glutamine synthetase-, glutamate dehydrogenase- and pyruvate carboxylase-immunoreactivities in the rat dorsal root ganglion and peripheral nerve. Brain Res 945(2):202–211

Morris JK, Lin W, Hauser C, Marchuk Y, Getman D, Lee KF (1999) Rescue of the cardiac defect in ErbB2 mutant mice reveals essential roles of ErbB2 in peripheral nervous system development. Neuron 23(2):273–283

Nagarajan R, Le N, Mahoney H, Araki T, Milbrandt J (2002) Deciphering peripheral nerve myelination by using Schwann cell expression profiling. Proc Natl Acad Sci U S A 99 (13):8998–9003

Napoli I, Noon LA, Ribeiro S, Kerai AP, Parrinello S, Rosenberg LH et al (2012) A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration in vivo. Neuron 73(4):729–742

Newbern J, Birchmeier C (2010) Nrg1/ErbB signaling networks in Schwann cell development and myelination. Semin Cell Dev Biol 21(9):922–928

Oliver CN, Starke-Reed PE, Stadtman ER, Liu GJ, Carney JM, Floyd RA (1990) Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. Proc Natl Acad Sci U S A 87 (13):5144–5147

Passage E, Norreel JC, Noack-Fraissignes P, Sanguedolce V, Pizant J, Thirion X et al (2004) Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. Nat Med 10(4):396–401

Saifi GM, Szigeti K, Snipes GJ, Garcia CA, Lupski JR (2003) Molecular mechanisms, diagnosis, and rational approaches to management of and therapy for Charcot-Marie-Tooth disease and related peripheral neuropathies. J Investig Med 51(5):261–283

Saitoh F, Araki T (2010) Proteasomal degradation of glutamine synthetase regulates Schwann cell differentiation. J Neurosci 30(4):1204–1212

Saitoh F, Wakatsuki S, Tokunaga S, Fujieda H, Araki T (2016) Glutamate signals through mGluR2 to control Schwann cell differentiation and proliferation. Sci Rep 6:29856

Salzer JL (2008) Switching myelination on and off. J Cell Biol 181(4):575–577

Sekido H, Suzuki T, Jomori T, Takeuchi M, Yabe-Nishimura C, Yagihashi S (2004) Reduced cell replication and induction of apoptosis by advanced glycation end products in rat Schwann cells. Biochem Biophys Res Commun 320(1):241–248

Sherman DL, Brophy PJ (2005) Mechanisms of axon ensheathment and myelin growth. Nat Rev Neurosci 6(9):683–690

Syed N, Reddy K, Yang DP, Taveggia C, Salzer JL, Maurel P et al (2010) Soluble neuregulin-1 has bifunctional, concentration-dependent effects on Schwann cell myelination. J Neurosci 30 (17):6122–6131

Syroid DE, Maycox PR, Burrola PG, Liu N, Wen D, Lee KF et al (1996) Cell death in the Schwann cell lineage and its regulation by neuregulin. Proc Natl Acad Sci U S A 93(17):9229–9234

Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S et al (2005) Neuregulin-1 type III determines the ensheathment fate of axons. Neuron 47(5):681–694

Cytoskeletal Signal-Regulated Oligodendrocyte Myelination and Remyelination

3

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Abstract

Myelination and remyelination in the central nervous system (CNS) are essential for rapid conduction of action potentials and for appropriate neuronal communications supporting higher brain functions. Myelination is dependent on developmental stage and is controlled by neuronal axons–oligodendrocyte (OL) signaling. Numerous studies of the initial myelination and remyelination stages in the CNS have demonstrated several key cytoskeletal signals in axons and OLs. In this review, we focus on cytoskeletal signal-regulated OL myelination and remyelination, with particular attention to neuronal Notch proteins, bidirectional Eph/ephrin signaling, OL integrin and cadherin superfamily proteins, OL actin rearrangement, and OL tyrosine kinase Fyn substrate proteins during the initial myelination and remyelination stages in the CNS.

Keywords

CNS · OPC · Oligodendrocyte (OL) · Myelination · Remyelination · Cytoskeletal signal · PSA-NCAM · EAE · MS · Eph · Ephrin · CSPG · Integrin · Cadherin · Actin · N-WASP · Arp2/3 · WAVE · Fyn · FAK · Sgk1 · Ndrg1 · LINC · ECM

Abbreviations

Arp2/3	Actin-related protein 2/3
CDC42	Cell division control protein 42 homolog
CNS	Central nervous system
CSPG	Chondroitin sulfate proteoglycan

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DCC	Deleted in colorectal cancer
Dsc	Desmocollin
Dsg	Desmoglein
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix
FAK	Focal adhesion kinase
GTPase	Guanosine triphosphatase
ILK	Integrin-linked kinase
IPP	ILK-PINCH-parvin
LINC	Linker of nucleoskeleton and cytoskeleton
LINGO-1	Leucine-rich repeat and immunoglobulin-like domain-containing protein 1
MS	Multiple sclerosis
Ndrg1	N-myc downstream-regulated gene 1
N-WASP	Neural Wiskott-Aldrich syndrome protein
OL	Oligodendrocyte
OPC	Oligodendroglial progenitor cell
PINCH	Particularly interesting new cysteine-histidine-rich protein
PSA-NCAM	Polysialylated neural cell adhesion molecule
PTK	Protein tyrosine kinase
Rac1	RAS-related C3 botulinus toxin substrate 1
RhoA	Ras homolog gene family, member A
RhoGAP	Rho GTPase-activating proteins
Sgk1	Serum glucocorticoid-regulated kinase
ST8SIA2	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2
SYNE1	Synaptic nuclear envelope protein 1
WAVE	WASP family verprolin homologous protein 1

3.1 Introduction

It has been reported that the bulk of central nervous system (CNS) myelination in humans occurs prior to approximately 20years of age (Lebel et al. 2008; Dubois et al. 2014; Mitew et al. 2014). However, recent studies also suggested that CNS myelination in humans occurs throughout our lives (Bartzokis et al. 2012; Young et al. 2013; Mitew et al. 2014; Herbert and Monk 2017). The generation and regeneration of CNS myelin by oligodendrocytes (OLs) is important for obtaining rapid conduction of action potentials and appropriate neuronal communications to support higher brain functions (Nave and Werner 2014; Samanta and Salzer 2015; Almeida and Lyons 2017; Forbes and Gallo 2017; Franklin and Ffrench-Constant 2017). Numerous studies have examined various signaling pathways of CNS myelination and remyelination in vivo and in vitro. These studies have shown that to produce initial myelination and remyelination in the CNS, OLs need to generate a multitude of processes and to locate suitable axons. These functions involve

dynamic changes in OL cytoskeletal functions. In this review, we focus on key cytoskeletal signals involving Notch proteins, bidirectional Eph/ephrin signaling, OL integrin and cadherin superfamily proteins, OL actin rearrangements, and OL tyrosine kinase Fyn substrate proteins, during initial CNS myelination and remyelination.

3.2 Neural Cell Adhesion Molecule (NCAM) Signaling

Downregulation of neuronal polysialylated neural cell adhesion molecule (PSA-NCAM) expression developmentally initiates the onset of myelination (Charles et al. 2000; Decker et al. 2000; Fewou et al. 2007; Jakovcevski et al. 2007, 2009). Furthermore, not only oligodendroglial progenitor cell (OPC) expressed NCAM but also ST8SIA2 polysialyltransferase in OPC, one of the NCAM-modifying proteins, has effects on OL differentiation and remyelination after cuprizone-induced demyelination (Werneburg et al. 2017).

3.3 Notch Signaling Pathways

Numerous studies have demonstrated that Notch signaling pathways are important for the initiation of myelination. These reports showed that developmental downregulation of several neuronal Notch-related proteins coincides with the onset of myelination. In the developmental stage, Jagged-1 signaling via the Notch1 receptor is known to inhibit OPC differentiation (Wang et al. 1998; Hu et al. 2003; Watkins et al. 2008). However, Jagged-1 signaling via Notch1 receptor may have no function in remyelination in the adult stages (Stidworthy et al. 2004). However, other discrepant reports have indicated that Jagged-1 signaling via Notch1 receptors is involved in remyelination of experimental autoimmune encephalomyelitis (EAE)-demyelinated mice (Seifert et al. 2007).

LINGO-1 associates with the OL Nogo receptor, also known as the reticulon 4 receptor. LINGO-1 is a negative regulator of OPC differentiation, and downregulation of LINGO-1 promoted myelination in mice in the developmental stage and remyelination in MS model mice (Mi et al. 2004, 2005, 2013; Bove and Green 2017; Shao et al. 2017). A recent report suggested that miRNA regulation is also involved in myelination and remyelination in the CNS. One type of miRNA that targets LINGO-1 mRNA, miR-219, might be able to regulate CNS myelination and remyelination (Wang et al. 2017).

3.4 Eph/Ephrin Signaling and β1 Integrin Pathways

The expression of receptors in the Eph/ephrin family is upregulated during OPC differentiation, and ephrin-B subfamily expression in OPC is involved in cell migration (Prestoz et al. 2004; Cohen 2005). In the developmental stages,

bidirectional Eph/ephrin signaling have been found to regulate integrin activation and to control cell adhesion (Huynh-Do et al. 1999, 2002; Miao et al. 2000; Bourgin et al. 2007; Arvanitis et al. 2013). Furthermore, it has been reported that an interaction between OL β1 integrin receptor and neuronal laminin α2 promotes process formation and myelination (Câmara et al. 2009; Colognato et al. 2002, 2007; Hu et al. 2009). EphA signals from neuronal ephrin-A to OLs induce process retraction and inhibit CNS myelination in an integrin-independent manner (Linneberg et al. 2015). EphB signals from neuronal ephrin-B to OLs in the CNS only inhibit myelination by regulating β1 integrin activation and laminin binding (Linneberg et al. 2015). However, reverse signaling from OL ephrin-B to neuronal EphA4 or B1 induces CNS myelination (Linneberg et al. 2015).

Initial process extension of OPC is important for laminin-β1 integrin receptor signaling in the CNS. It is well known that after brain injury, glial scars may occur in the white matter. Glial scars formed by chondroitin sulfate proteoglycans (CSPGs) and CSPCs on OPC are associated with the downregulation of β1 integrin functions and competitively inhibit remyelination in the CNS (Sun et al. 2017).

3.5 Actin Cytoskeleton

The β1 integrin activation in OLs during myelination induces Fyn dephosphorylation and activation of one of the focal adhesion proteins, ILK (Liang et al. 2004). It has been reported that ILK has the potential for regulation of the OL actin cytoskeleton and therefore of OL morphogenesis (O'Meara et al. 2013, 2016; Michalski et al. 2016; Hussain and Macklin 2017). This ILK activation in OLs recruits obligate binding partners PINCH and α-pervin, and this IPP complex is itself activated. Furthermore, the activated IPP complex induces actin polymerization (Bauer et al. 2009; Ghatak et al. 2013) and microtubule assembly (Wickström et al. 2010; Akhtar and Streuli 2013).

Actin needs to remodel dynamically during myelination in the region of process protrusion (Blanchoin et al. 2014; Samanta and Salzer 2015). A polymerizing and nucleating factor, the paxillin—N-WASP—Arp2/3 complex, is recruited in focal adhesion regions (Bacon et al. 2007; O'Meara et al. 2013, 2016). Furthermore, WAVE1, a member of the WASP family, is also recruited at the same site and causes activation of actin polymerization during CNS myelination (Kim et al. 2006; Havrylenko et al. 2015). FAK-induced Cdc42 and Rac1 activate and modulate microtubule assembly (Chun et al. 2003; Kim et al. 2008; Forrest et al. 2009).

Recent reports identified novel mechanisms of OL actin regulation by the molecular components of the linker of nucleoskeleton and cytoskeleton (LINC) complex (such as SYNE1) (Dammer et al. 2013; Hernandez et al. 2016). The LINC–actin cytoskeleton complex mediates signals from nuclear components for cytoplasmic G-actin/F-actin regulation during OL differentiation and myelination (Hernandez et al. 2016).

3.6 Cadherin Super Family

Stressed mice with elevated plasma corticosterone levels showed upregulation and activation of Sgk1 in mature OLs. Active Sgk1 causes phosphorylation of Ndrg1 and phospho-Ndrg1 increases the expression of crucial adhesion molecules such as N-cadherin, α-catenin, and β-catenin in OLs (Miyata et al. 2011). This activation of the Sgk1 cascade results in morphological changes in the OLs of nerve fiber bundles (Miyata et al. 2015a, b, 2016; Tohyama et al. 2015).

The traditional and desmosomal cadherin superfamily, Dsg1, Dsg2, and Dsc1, was found to be expressed in the corpus callosum of the mouse brain, and a subtype of Dsg1, Dsg1c, is expressed in OLs. Furthermore, Dsg1 proteins are localized around the plasma membrane regions of OLs, and electron microscopic analysis failed to detect desmosome-like structures, for example, keratin intermediate filaments, within the inner dense plaque (Miyata et al. 2015a).

3.7 Fyn Activation

The non-receptor tyrosine kinase Fyn is a well-known extracellular signaling protein involved in the dynamic nature of OLs and in myelination (Umemori et al. 1994, 1999; Sperber et al. 2001; Miyamoto et al. 2008; Laursen et al. 2009; Krämer-Albers and White 2011; Wake et al. 2011; Peckham et al. 2016). Fyn is involved in multiple myelination pathways as a downstream effector. In OPCs, activated Fyn functions as a RhoA inhibitor by activating p190/p250 RhoGAP and leads to excess OPC process and branch formation (Wolf et al. 2001; Taniguchi et al. 2003; Liang et al. 2004; Kippert et al. 2007; Baer et al. 2009). Activated Fyn also mediates bindings of neuronal L1 ligand and OL contactin, or netrin-1 and the DCC receptor, inducing initiation of CNS myelination (Laursen et al. 2009; Rajasekharan et al. 2009).

FAK (as known as PTK2) tyrosine is phosphorylated by activated Fyn, and this FAK phosphorylation induces cdc42 and Rac1 activation (Hoshina et al. 2007; Rajasekharan et al. 2009). FAK is one of the main regulators of integrin–ECM signaling during initial CNS myelination (Parsons 2003; Mitra et al. 2005; Mitra and Schlaepfer 2006; Lafrenaye and Fuss 2010). Furthermore, integrin–ECM signaling is important for OL differentiation and CNS myelination (Forrest et al. 2009; Eyermann et al. 2012). FAK could control the efficiency and proper timing of myelination during the initial myelination stages in the CNS (Forrest et al. 2009), and FAK could inhibit process formation in the fibronectin-rich ECM (Lafrenaye and Fuss 2010).

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References

Akhtar N, Streuli CH (2013) An integrin-ILK-microtubule network orients cell polarity and lumen formation in glandular epithelium. Nat Cell Biol 15:17–27

Almeida RG, Lyons DA (2017) On myelinated axon plasticity and neuronal circuit formation and function. J Neurosci 37:10023–10034

Arvanitis DN, Behar A, Tryoen-Toth P, Bush JO, Jungas T, Vitale N, Davy A (2013) Ephrin B1 maintains apical adhesion of neural progenitors. Development 140:2082–2092

Bacon C, Lakics V, Machesky L, Rumsby M (2007) N-WASP regulates extension of filopodia and processes by oligodendrocyte progenitors, oligodendrocytes, and Schwann cells – implications for axon ensheathment at myelination. Glia 55:844–858

Baer AS, Syed YA, Kang SU, Mitteregger D, Vig R, Ffrench-Constant C, Franklin RJ, Altmann F, Lubec G, Kotter MR (2009) Myelin-mediated inhibition of oligodendrocyte precursor differentiation can be overcome by pharmacological modulation of Fyn-RhoA and protein kinase C signalling. Brain 132:465–481

Bartzokis G, Lu PH, Heydari P, Couvrette A, Lee GJ, Kalashyan G, Freeman F, Grinstead JW, Villablanca P, Finn JP, Mintz J, Alger JR, Altshuler LL (2012) Multimodal magnetic resonance imaging assessment of white matter aging trajectories over the lifespan of healthy individuals. Biol Psychiatry 72:1026–1034

Bauer NG, Richter-Landsberg C, Ffrench-Constant C (2009) Role of the oligodendroglial cytoskeleton in differentiation and myelination. Glia 57:1691–1705

Blanchoin L, Boujemaa-Paterski R, Sykes C, Plastino J (2014) Actin dynamics, architecture, and mechanics in cell motility. Physiol Rev 94:235–263

Bourgin C, Murai KK, Richter M, Pasquale EB (2007) The EphA4 receptor regulates dendritic spine remodeling by affecting beta1-integrin signaling pathways. J Cell Biol 178:1295–1307

Bove RM, Green AJ (2017) Remyelinating pharmacotherapies in multiple sclerosis. Neurotherapeutics 14:894–904

Câmara J, Wang Z, Nunes-Fonseca C, Friedman HC, Grove M, Sherman DL, ffrench-Constant C (2009) Integrin-mediated axoglial interactions initiate myelination in the central nervous system. J Cell Biol 185:699–712

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

Chun SJ, Rasband MN, Sidman RL, Habib AA, Vartanian T (2003) Integrin-linked kinase is required for laminin-2-induced oligodendrocyte cell spreading and CNS myelination. J Cell Biol 163:397–408

Cohen RI (2005) Exploring oligodendrocyte guidance: to boldly go where no cell has gone before. Cell Mol Life Sci 62:505–510

Colognato H, Baron W, Avellana-Adalid V, Relvas JB, Evercooren ABV, Georges-Labouesse E, Ffrench-Constant C (2002) CNS integrins switch growth factor signalling to promote target-dependent survival. Nat Cell Biol 4:833–841

Colognato H, Galvin J, Wang Z, Relucio J, Nguyen T, Harrison D, Yurchenco PD, Ffrench-Constant C (2007) Identification of dystroglycan as a second laminin receptor in oligodendrocytes, with a role in myelination. Development 134:1723–1736

Dammer EB, Duong DM, Diner I, Gearing M, Feng Y, Lah JJ, Levey AI, Seyfried NT (2013) Neuron enriched nuclear proteome isolated from human brain. J Proteome Res 12:3193–3206

Decker L, Avellana-Adalid V, Nait-Oumesmar B, Durbec P, Baron-Van Evercooren A (2000) Oligodendrocyte precursor migration and differentiation: combined effects of PSA residues, growth factors, and substrates. Mol Cell Neurosci16:422–439

Dubois J, Dehaene-Lambertz G, Kulikova S, Poupon C, Hüppi PS, Hertz-Pannier L (2014) The early development of brain white matter: a review of imaging studies in fetuses, newborns and infants. Neuroscience 276:48–71

Eyermann C, Czaplinski K, Colognato H (2012) Dystroglycan promotes filopodial formation and process branching in differentiating oligodendroglia. J Neurochem 120:928–947

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

Forbes TA, Gallo V (2017) All wrapped up: environmental effects on myelination. Trends Neurosci 40:572–587

Forrest AD, Beggs HE, Reichardt LF, Dupree JL, Colello RJ, Fuss B (2009) Focal adhesion kinase (FAK): a regulator of CNS myelination. J Neurosci Res 87:3456–3464

Franklin RJM, Ffrench-Constant C (2017) Regenerating CNS myelin – from mechanisms to experimental medicines. Nat Rev Neurosci 18:753–769

Ghatak S, Morgner J, Wickström SA (2013) ILK: a pseudokinase with a unique function in the integrin-actin linkage. Biochem Soc Trans 41:995–1001

Havrylenko S, Noguera P, Abou-Ghali M, Manzi J, Faqir F, Lamora A, Guérin C, Blanchoin L, Plastino J (2015) WAVE binds Ena/VASP for enhanced Arp2/3 complex-based actin assembly. Mol Biol Cell 26:55–65

Herbert AL, Monk KR (2017) Advances in myelinating glial cell development. Curr Opin Neurobiol 42:53–60

Hernandez M, Patzig J, Mayoral SR, Costa KD, Chan JR, Casaccia P (2016) Mechanostimulation promotes nuclear and epigenetic changes in oligodendrocytes. J Neurosci 36:806–813

Hoshina N, Tezuka T, Yokoyama K, Kozuka-Hata H, Oyama M, Yamamoto T (2007) Focal adhesion kinase regulates laminin-induced oligodendroglial process outgrowth. Genes Cells 12:1245–1254

Hu QD, Ang BT, Karsak M, Hu WP, Cui XY, Duka T, Takeda Y, Chia W, Sankar N, Ng YK, Ling EA, Maciag T, Small D, Trifonova R, Kopan R, Okano H, Nakafuku M, Chiba S, Hirai H, Aster JC, Schachner M, Pallen CJ, Watanabe K, Xiao ZC (2003) F3/contactin acts as a functional ligand for notch during oligodendrocyte maturation. Cell 115:163–175

Hu J, Deng L, Wang X, Xu XM (2009) Effects of extracellular matrix molecules on the growth properties of oligodendrocyte progenitor cells in vitro. J Neurosci Res 87:2854–2862

Hussain R, Macklin WB (2017) Integrin-linked kinase (ILK) deletion disrupts oligodendrocyte development by altering cell cycle. J Neurosci 37:397–412

Huynh-Do U, Stein E, Lane AA, Liu H, Cerretti DP, Daniel TO (1999) Surface densities of ephrin-B1 determine EphB1-coupled activation of cell attachment through alphavbeta3 and alpha5beta1 integrins. EMBO J 18:2165–2173

Huynh-Do U, Vindis C, Liu H, Cerretti DP, McGrew JT, Enriquez M, Daniel TO (2002) Ephrin-B1 transduces signals to activate integrin-mediated migration, attachment and angiogenesis. J Cell Sci 115:3073–3081

Jakovcevski I, Mo Z, Zecevic N (2007) Down-regulation of the axonal polysialic acid-neural cell adhesion molecule expression coincides with the onset of myelination in the human fetal forebrain. Neuroscience 149:328–337

Jakovcevski I, Filipovic R, Mo Z, Rakic S, Zecevic N (2009) Oligodendrocyte development and the onset of myelination in the human fetal brain. Front Neuroanat 3:5

Kim HJ, DiBernardo AB, Sloane JA, Rasband MN, Solomon D, Kosaras B, Kwak SP, Vartanian TK (2006) WAVE1 is required for oligodendrocyte morphogenesis and normal CNS myelination. J Neurosci 26:5849–5859

Kim H, Shin J, Kim S, Poling J, Park HC, Appel B (2008) Notch-regulated oligodendrocyte specification from radial glia in the spinal cord of zebrafish embryos. Dev Dyn 237:2081–2089
Kippert A, Trajkovic K, Rajendran L, Ries J, Simons M (2007) Rho regulates membrane transport in the endocytic pathway to control plasma membrane specialization in oligodendroglial cells. J Neurosci 27:3560–3570
Krämer-Albers EM, White R (2011) From axon-glial signalling to myelination: the integrating role of oligodendroglial Fyn kinase. Cell Mol Life Sci 68:2003–2012
Lafrenaye AD, Fuss B (2010) Focal adhesion kinase can play unique and opposing roles in regulating the morphology of differentiating oligodendrocytes. J Neurochem 115:269–282
Laursen LS, Chan CW, ffrench-Constant C (2009) An integrin-contactin complex regulates CNS myelination by differential Fyn phosphorylation. J Neurosci 29:9174–9185
Lebel C, Walker L, Leemans A, Phillips L, Beaulieu C (2008) Microstructural maturation of the human brain from childhood to adulthood. NeuroImage 40:1044–1055
Liang X, Draghi NA, Resh MD (2004) Signaling from integrins to Fyn to Rho family GTPases regulates morphologic differentiation of oligodendrocytes. J Neurosci 24:7140–7149
Linneberg C, Harboe M, Laursen LS (2015) Axo-Glia interaction preceding CNS myelination is regulated by bidirectional Eph-Ephrin signaling. ASN Neuro 7. pii: 1759091415602859
Mi S, Lee X, Shao Z, Thill G, Ji B, Relton J, Levesque M, Allaire N, Perrin S, Sands B, Crowell T, Cate RL, McCoy JM, Pepinsky RB (2004) LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat Neurosci 7:221–228
Mi S, Miller RH, Lee X, Scott ML, Shulag-Morskaya S, Shao Z, Chang J, Thill G, Levesque M, Zhang M, Hession C, Sah D, Trapp BD, He Z, Jung V, McCoy JM, Pepinsky RB (2005) LINGO-1 negatively regulates myelination by oligodendrocytes. Nat Neurosci 8:745–751
Mi S, Pepinsky RB, Cadavid D (2013) Blocking LINGO-1 as a therapy to promote CNS repair: from concept to the clinic. CNS Drugs 27:493–503
Miao H, Burnett E, Kinch M, Simon E, Wang B (2000) Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation. Nat Cell Biol 2:62–69
Michalski JP, Cummings SE, O'Meara RW, Kothary R (2016) Integrin-linked kinase regulates oligodendrocyte cytoskeleton, growth cone, and adhesion dynamics. J Neurochem 136:536–549
Mitew S, Hay CM, Peckham H, Xiao J, Koenning M, Emery B (2014) Mechanisms regulating the development of oligodendrocytes and central nervous system myelin. Neuroscience 276:29–47
Mitra SK, Schlaepfer DD (2006) Integrin-regulated FAK-Src signaling in normal and cancer cells. Curr Opin Cell Biol 18:516–523
Mitra SK, Hanson DA, Schlaepfer DD (2005) Focal adhesion kinase: in command and control of cell motility. Nat Rev Mol Cell Biol 6:56–68
Miyamoto Y, Yamauchi J, Tanoue A (2008) Cdk5 phosphorylation of WAVE2 regulates oligodendrocyte precursor cell migration through nonreceptor tyrosine kinase Fyn. J Neurosci 28:8326–8337
Miyata S, Koyama Y, Takemoto K, Yoshikawa K, Ishikawa T, Taniguchi M, Inoue K, Aoki M, Hori O, Katayama T, Tohyama M (2011) Plasma corticosterone activates SGK1 and induces morphological changes in oligodendrocytes in corpus callosum. PLoS One 6:e19859
Miyata S, Yoshikawa K, Taniguchi M, Ishikawa T, Tanaka T, Shimizu S, Tohyama M (2015a) Sgk1 regulates desmoglein 1 expression levels in oligodendrocytes in the mouse corpus callosum after chronic stress exposure. Biochem Biophys Res Commun 464:76–82
Miyata S, Hattori T, Shimizu S, Ito A, Tohyama M (2015b) Disturbance of oligodendrocyte function plays a key role in the pathogenesis of schizophrenia and major depressive disorder. Biomed Res Int 2015:492367
Miyata S, Taniguchi M, Koyama Y, Shimizu S, Tanaka T, Yasuno F, Yamamoto A, Iida H, Kudo T, Katayama T, Tohyama M (2016) Association between chronic stress-induced structural abnormalities in Ranvier nodes and reduced oligodendrocyte activity in major depression. Sci Rep 6:23084

Nave KA, Werner HB (2014) Myelination of the nervous system: mechanisms and functions. Annu Rev Cell Dev Biol 30:503–533

O'Meara RW, Michalski JP, Anderson C, Bhanot K, Rippstein P, Kothary R (2013) Integrin-linked kinase regulates process extension in oligodendrocytes via control of actin cytoskeletal dynamics. J Neurosci 33:9781–9793

O'Meara RW, Cummings SE, Michalski JP, Kothary R (2016) A new in vitro mouse oligodendrocyte precursor cell migration assay reveals a role for integrin-linked kinase in cell motility. BMC Neurosci 17:7

Parsons JT (2003) Focal adhesion kinase: the first ten years. J Cell Sci 116:1409–1416

Peckham H, Giuffrida L, Wood R, Gonsalvez D, Ferner A, Kilpatrick TJ, Murray SS, Xiao J (2016) Fyn is an intermediate kinase that BDNF utilizes to promote oligodendrocyte myelination. Glia 64:255–269

Prestoz L, Chatzopoulou E, Lemkine G, Spassky N, Lebras B, Kagawa T, Thomas JL (2004) Control of axonophilic migration of oligodendrocyte precursor cells by Eph-ephrin interaction. Neuron Glia Biol 1:73–83

Rajasekharan S, Baker KA, Horn KE, Jarjour AA, Antel JP, Kennedy TE (2009) Netrin 1 and Dcc regulate oligodendrocyte process branching and membrane extension via Fyn and RhoA. Development 136:415–426

Samanta J, Salzer JL (2015) Myelination: actin disassembly leads the way. Dev Cell 34:129–130

Seifert T, Bauer J, Weissert R, Fazekas F, Storch MK (2007) Notch1 and its ligand Jagged1 are present in remyelination in a T-cell- and antibody-mediated model of inflammatory demyelination. Acta Neuropathol 113:195–203

Shao Z, Lee X, Huang G, Sheng G, Henderson CE, Louvard D, Sohn J, Pepinsky B, Mi S (2017) LINGO-1 Regulates oligodendrocyte differentiation through the cytoplasmic gelsolin signaling pathway. J Neurosci 37:3127–3137

Sperber BR, Boyle-Walsh EA, Engleka MJ, Gadue P, Peterson AC, Stein PL, Scherer SS, McMorris FA (2001) A unique role for Fyn in CNS myelination. J Neurosci 21:2039–2047

Stidworthy MF, Genoud S, Li WW, Leone DP, Mantei N, Suter U, Franklin RJ (2004) Notch1 and Jagged1 are expressed after CNS demyelination, but are not a major rate-determining factor during remyelination. Brain 127:1928–1941

Sun Y, Deng Y, Xiao M, Hu L, Li Z, Chen C (2017) Chondroitin sulfate proteoglycans inhibit the migration and differentiation of oligodendrocyte precursor cells and its counteractive interaction with laminin. Int J Mol Med 40:1657–1668

Taniguchi S, Liu H, Nakazawa T, Yokoyama K, Tezuka T, Yamamoto T (2003) P250GAP, a neural RhoGAP protein, is associated with and phosphorylated by Fyn. Biochem Biophys Res Commun 306:151–155

Tohyama M, Miyata S, Hattori T, Shimizu S, Matsuzaki S (2015) Molecular basis of major psychiatric diseases such as schizophrenia and depression. Anat Sci Int 90:137–143

Umemori H, Sato S, Yagi T, Aizawa S, Yamamoto T (1994) Initial events of myelination involve Fyn tyrosine kinase signalling. Nature 367:572–576

Umemori H, Kadowaki Y, Hirosawa K, Yoshida Y, Hironaka K, Okano H, Yamamoto T (1999) Stimulation of myelin basic protein gene transcription by Fyn tyrosine kinase for myelination. J Neurosci 19:1393–1397

Wake H, Lee PR, Fields RD (2011) Control of local protein synthesis and initial events in myelination by action potentials. Science 333:1647–1651

Wang S, Sdrulla AD, diSibio G, Bush G, Nofziger D, Hicks C, Weinmaster G, Barres BA (1998) Notch receptor activation inhibits oligodendrocyte differentiation. Neuron 21:63–75

Wang H, Moyano AL, Ma Z, Deng Y, Lin Y, Zhao C, Zhang L, Jiang M, He X, Ma Z, Lu F, Xin M, Zhou W, Yoon SO, Bongarzone ER, Lu QR (2017) miR-219 cooperates with miR-338 in myelination and promotes myelin repair in the CNS. Dev Cell 40:566–582

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

Werneburg S, Fuchs HLS, Albers I, Burkhardt H, Gudi V, Skripuletz T, Stangel M, Gerardy-Schahn R, Hildebrandt H (2017) Polysialylation at early stages of oligodendrocyte differentiation promotes myelin repair. J Neurosci 37:8131–8141

Wickström SA, Lange A, Hess MW, Polleux J, Spatz JP, Krüger M, Pfaller K, Lambacher A, Bloch W, Mann M, Huber LA, Fässler R (2010) Integrin-linked kinase controls microtubule dynamics required for plasma membrane targeting of caveolae. Dev Cell 19:574–588

Wolf RM, Wilkes JJ, Chao MV, Resh MD (2001) Tyrosine phosphorylation of p190 RhoGAP by Fyn regulates oligodendrocyte differentiation. J Neurobiol 49:62–78

Young KM, Psachoulia K, Tripathi RB, Dunn S-J, Cossell L, Attwell D, Tohyama K, Richardson WD (2013) Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. Neuron 77:873–885

Activity-Dependent Myelination

4

Daisuke Kato and Hiroaki Wake

Abstract

Oligodendrocyte form myelin around the axons to regulate the conduction velocity. Myelinated axons are composed of white matter to act as cables to connect distinct brain regions. Recent human MRI studies showed that the signal from white matter change in the people with special skills such as taxi driver, piano player, and juggling. The change of the white matter suggested that (1) The plasticity of myelination depends on neuronal activity (activity-dependent myelination) and (2) White matter plasticity is essential for brain functions. In this session, we discussed that how the un-electrical components, oligodendrocytes, and its precursor cells receive the signal from electrically active neurons and differentiate, proliferate, and myelinate the axons to modulate the activity of neuronal circuits, ultimately affect on their behaviors. In this review, we highlight the physiological functions of oligodendrocyte and their neuronal activity-dependent functions and thus show new insight for their contribution to brain functions.

Keywords

Glial cells · Oligodendrocytes · Oligodendrocyte progenitor cells · Myelination

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Abbreviations

AIS	Axonal initial segment
AMPAR	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ATP	Adenosine triphosphate
CNS	Central nervous system
DRG	Dorsal root ganglion
fMRI	Functional magnetic resonance imaging
GABAR	Gamma-aminobutyric acid receptor
GFP	Green fluorescent protein
MBP	Myelin basic protein
mGluR	Metabotropic glutamate receptor
NMDAR	*N*-methyl-D-aspartate receptor
NRG1	Neuregulin1
OPCs	Oligodendrocyte progenitor cells
Pdgfra	Alpha receptor for platelet-derived growth factor
TTX	Tetrodotoxin

4.1 Introduction

Oligodendrocytes are glial cells, which form myelin around axons, regulating conduction velocity. Myelinated bundles exist underneath the superficial layer of the cortex and act as cables to connect distant cortical regions. Therefore, oligodendrocytes are thought to play a crucial role in information processing (Nave 2010; Nave and Werner 2014). Attention has been recently focused on oligodendrocyte precursor cells (OPCs) due to their ability to proliferate and generate oligodendrocytes, even in the adult brain (Hill and Nishiyama 2014). OPCs also form synapse-like structures in several brain regions to modify neuronal circuits (Bergles et al. 2000). OPCs proliferation and differentiation are regulated by neural activity. Therefore, differentiated oligodendrocytes modulate information processing by regulating conduction velocity. Here, we review both well-known and recently uncovered using two-photon microscopy physiological functions of oligodendrocyte and their precursor cells.

Pio Del Rio-Hortega first described oligodendrocytes and classified them into four types, based on morphology and distribution (Garcia-Marin et al. 2007). The main function of oligodendrocytes is to form myelin around neuronal axons, thereby regulating the central nervous system (CNS) conduction velocity. The bundles of myelinated axons make up the white matter in the brain, which acts as cables to connect distant cortical regions, therefore playing a crucial role in information processing (Nave 2010; Nave and Werner 2014). Recent reports show that oligodendrocyte progenitor cells (OPCs; e.g., NG2 cells, which express specific antigens and NG2 chondroitin sulfate) are motile cells. They proliferate and differentiate into

oligodendrocytes, or myelinating cells, even in the mature brain (Hughes et al. 2013). They also form synaptic structures with presynaptic terminals as a part of neural circuitry (Bergles et al. 2000). Moreover, oligodendrocytes form axon–glial interactions to differentiate and preferentially form myelin around electrically active axons by local translation of myelin basic protein (MBP) (Wake et al. 2011). In this section, we discuss the basic physiological properties of oligodendrocytes, OPCs, and myelination.

4.2 OPCs and Oligodendrocytes

OPCs express specific markers, such as NG2 chondroitin sulfate proteoglycan and the alpha receptor for platelet-derived growth factor (Pdgfra) (Nishiyama et al. 2009). OPCs originate from the ventral germinal zones of medial and lateral ganglionic eminences, expressing the transcription factors, Nkx2.1 and Gsh2, in the CNS (Hill and Nishiyama 2014). Subsequently, Emx1-expressing OPCs originate from the dorsal ventricular zone during late embryonic stages (Kessaris et al. 2006). After birth, OPCs are generated from the dorsal ventricular zone in the developing phase (Menn et al. 2006) and also generated from OPCs even in the adult brain (Zhu et al. 2008; Kang et al. 2010; Young et al. 2013).

The formation and maintenance of the myelin sheath during development and in adult required the several step of differentiation and also various signals, such as neuregulin1 (NRG1)/ErbB receptors signaling (Taveggia et al. 2010), Jagged Notch1 signaling (Piaton et al. 2010; Taveggia et al. 2010), and Nectin-like protein (Pereira et al. 2012). NRG1 type III, one of the 16 NRG1 isoforms (Nave and Salzer 2006), is cleaved by the proteolytic enzyme beta-secretase 1 (BACE1), followed by ErbB signaling activation for peripheral nervous system (PNS) myelination (Michailov et al. 2004; Taveggia et al. 2005). In addition to the PNS, NRG1/ErbB signaling also contributes to CNS myelinogenesis. NRG1 type III knockout mice exhibited CNS hypomyelination (Taveggia et al. 2008, 2010), while mice overexpressing the dominant-negative form of the ErbB receptor displayed impaired oligodendrocyte differentiation and reduced OPC number, along with hypomyelination (Kim et al. 2003).

OPCs form synapse-like structures with presynaptic elements and receive direct neuronal inputs from pyramidal cells in the hippocampal CA3 region (Bergles et al. 2000), cerebellum (Lin et al. 2005), and white matter (Kukley et al. 2007). Presynaptic neurotransmitters act on receptors located on OPCs, which regulates their proliferation and differentiation through voltage-dependent Ca^{2+} channels (Gallo et al. 2008; Hill and Nishiyama 2014). These data suggest that proliferation and differentiation of OPCs and myelination can be regulated by neuronal impulses (Wake et al. 2011; Hill and Nishiyama 2014). Traditionally, experiments have reduced neural activity with the application of tetrodotoxin (TTX). Reduction of neural activity by intraocular TTX injection suppressed OPCs proliferation in the optic nerve, suggesting an effect on OPCs function (Barres and Raff 1993). Several molecules are released after neural activity in both vesicular-dependent (Kukley

et al. 2007; Ziskin et al. 2007) and non-vesicular-dependent way (Fields and Burnstock 2006). Using a coculture system of dorsal root ganglion (DRG) neurons and OPCs, ATP was released from axons in response to electrical stimulation, promoting OPCs differentiation and the formation of myelin sheath (Stevens et al. 2002). Moreover, axonal release of ATP induced the secretion of the cytokine leukemia inhibitory factor (LIF) from astrocytes, which can promote OPCs differentiation (Ishibashi et al. 2006).

Several in vivo studies have demonstrated activity-dependent OPCs proliferation and differentiation. Electrical stimulation of the rat corticospinal tract promoted OPCs proliferation and differentiation through NMDAR (Li et al. 2010). Voluntary exercise (i.e., wheel running) promoted OPCs differentiation (Simon et al. 2011) and motor behavior also facilitates OPCs proliferation and differentiation (McKenzie et al. 2014; Xiao et al. 2016). OPCs in the developing barrel cortex receive glutamatergic projections from the thalamus. Sensory deprivation (i.e., whisker removal during developmental stages) resulted in the reduction of thalamocortical inputs on OPCs, thus inhibiting OPCs proliferation and altering OPCs distribution in the barrel cortex (Mangin et al. 2012). Furthermore, in Thy1-ChR2 mice, neural activity via optogenetic stimulation regulated OPCs proliferation, differentiation, and myelination (Gibson et al. 2014). These findings indicate that neural activity controls OPCs proliferation and differentiation, which can then modify neural activity through axonal myelin formation by differentiated oligodendrocytes. Recent studies using two-photon microscopy imaging of the GFP-labeled, membrane-anchored form of NG2 demonstrated that OPCs extend highly ramified, motile processes, and maintain cell density by mediating local proliferation, consequently revealing the spatial and morphological properties of OPCs proliferation and differentiation (Hughes et al. 2013).

The axonal initial segment (AIS) is a characteristic region of axons that is the initiation site of action potentials. AIS has an important role in information processing by regulating action potentials. Prolonged depolarization with high concentrations of extracellular K^+ induced changes in the AIS position in cultured hippocampal neurons (Grubb and Burrone 2010). Neural activity reductions induced by ocular TTX injections decreased the number of myelinated axon segments (Demerens et al. 1996); in contrast, the repetitive propagation of action potentials led to increments in myelin formation (Wurtz and Ellisman 1986). Moreover, activity-dependent axonal secretion of neurotransmitters is required for myelin sheath maintenance (Hines et al. 2015) and controls the myelin segment number of individual oligodendrocytes (Mensch et al. 2015) in vivo. These findings suggest that myelin and axon morphologies are dependent on neural activity, but that the underlying cellular mechanisms of these changes remain unclear. Recently, several mechanisms of these changes are implicated (Fields 2015). For example, axonal glutamate release from DRG neurons increased local MBP synthesis specifically at cholesterol-rich microdomains between DRG neurons and OPCs via Fyn kinase (Wake et al. 2011) and neuregulin and BDNF signaling induced NMDAR-dependent myelination of active axons (Lundgaard et al. 2013), which are crucial mechanisms of activity-dependent myelination (Fig. 4.1).

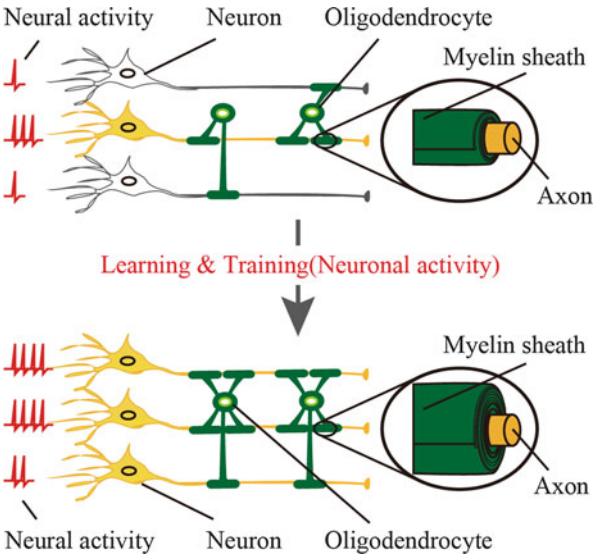

Fig. 4.1 Activity-dependent myelination. Individual oligodendrocytes simultaneously myelinate different axons and preferentially form myelin sheaths around the electrically active axon. Increase of neural activity associated with learning and training promotes myelination and thickening of myelin sheaths, which increase the conduction velocity and thus involve in information processing

Accumulating evidence indicates that neuronal activity is induced by behavior and that learning and experience promote myelination in vivo (Purger et al. 2016). The prefrontal cortex is a specialized brain region involved in emotional and cognitive behavior. It is well known that social isolation during development results in morphological and transcriptional oligodendrocyte changes, consequently disrupting myelin formation (Liu et al. 2012; Makinodan et al. 2012). However, it is not still known how the disruption in myelinogenesis occurs. Recent reports indicate that social isolation in adult mice leads to reduced sociability and locomotor activity, decreasing myelin-related gene products and myelin thickness in the prefrontal cortex, along with chromatin changes (Liu et al. 2012). Social isolation during the critical period also affects myelination and behavioral deterioration in adults through the NRG1/ErbB3 signaling pathway (Makinodan et al. 2012). In the sensory cortex, longitudinal in vivo two-photon imaging reveals that sensory enrichment dramatically increases oligodendrocytes generation, but does not affect myelin sheath remodeling (Hughes et al. 2018). Research using both functional magnetic resonance imaging (fMRI) and quantitative immunohistochemistry implicates the contribution of forelimb motor learning to changes in white matter structure and myelin formation (Sampaio-Baptista et al. 2013). Moreover, axon myelination by oligodendrocytes differentiated from OPCs was necessary to achieve motor skill learning (McKenzie et al. 2014; Xiao et al. 2016), indicating that active myelination induced neural circuit maturation, subsequently facilitating learning.

In addition to rodent studies, recent MRI studies in humans have shown that there is increased signaling in white matter in response to training, such as juggling (Scholz et al. 2009), indicating the presence of activity-dependent myelination and white matter plasticity in the adult brain (Fields 2011). Thus, accumulating evidence underscores the importance of myelin in information processing and suggests that a

disruption in these processes could be implicated in some psychiatric and neurological disorders. In fact, differences in myelin-related genes were detected using a genome-wide analysis of individuals with schizophrenia (Hakak et al. 2001). In addition, white matter lesions detected by MRI were associated with cognitive function in elderly people (Bennett and Madden 2014). These findings suggest clarification is required for the regulation of activity-dependent myelination and its contribution to psychiatric and neurological diseases resulting from the disruption of active myelin formation (Nave and Ehrenreich 2014).

There are controversial studies regarding activity-dependent myelination. In oligodendrocyte cultures, myelin-related proteins were produced, and myelin sheath-like structures were formed (Dubois-Dalcq et al. 1986). OPCs can also form myelin around axons fixed with paraformaldehyde (Rosenberg et al. 2008) and around nanofibers (Lee et al. 2012), indicating that OPCs differentiation and myelination are possible in the absence of the dynamic signaling between axons and OPCs.

4.3 Conclusions

In this review, we have summarized the physiological roles of oligodendrocyte with a new perspective and dysfunction of oligodendrocyte and precursor cells implicated in disease pathogenesis of neurological and psychiatric disorders. These findings suggest that glial cells are not just supportive cells for neurons but actively involved in contributing to disease progression. However, further experiments are needed to unravel the precise mechanism that dysfunction of glial cells contributes to the development of the disease.

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References

Barres BA, Raff MC (1993) Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. Nature 361:258–260

Bennett IJ, Madden DJ (2014) Disconnected aging: cerebral white matter integrity and age-related differences in cognition. Neuroscience 276:187–205

Bergles DE, Roberts JD, Somogyi P, Jahr CE (2000) Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 405:187–191

Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C (1996) Induction of myelination in the central nervous system by electrical activity. Proc Natl Acad Sci U S A 93:9887–9892

Dubois-Dalcq M, Behar T, Hudson L, Lazzarini RA (1986) Emergence of three myelin proteins in oligodendrocytes cultured without neurons. J Cell Biol 102:384–392
Fields RD (2011) Imaging learning: the search for a memory trace. Neuroscientist 17:185–196
Fields RD (2015) A new mechanism of nervous system plasticity: activity-dependent myelination. Nat Rev Neurosci 16:756–767
Fields RD, Burnstock G (2006) Purinergic signalling in neuron-glia interactions. Nat Rev Neurosci 7:423–436
Gallo V, Mangin JM, Kukley M, Dietrich D (2008) Synapses on NG2-expressing progenitors in the brain: multiple functions? J Physiol 586:3767–3781
Garcia-Marin V, Garcia-Lopez P, Freire M (2007) Cajal's contributions to glia research. Trends Neurosci 30:479–487
Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, Inema I, Miller SE, Bieri G, Zuchero JB, Barres BA, Woo PJ, Vogel H, Monje M (2014) Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. Science 344:1252304
Grubb MS, Burrone J (2010) Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. Nature 465:1070–1074
Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, Haroutunian V, Fienberg AA (2001) Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci U S A 98:4746–4751
Hill RA, Nishiyama A (2014) NG2 cells (polydendrocytes): listeners to the neural network with diverse properties. Glia 62:1195–1210
Hines JH, Ravanelli AM, Schwindt R, Scott EK, Appel B (2015) Neuronal activity biases axon selection for myelination in vivo. Nat Neurosci 18:683–689
Hughes EG, Kang SH, Fukaya M, Bergles DE (2013) Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nat Neurosci 16:668–676
Hughes EG, Orthmann-Murphy JL, Langseth AJ, Bergles DE (2018) Myelin remodeling through experience-dependent oligodendrogenesis in the adult somatosensory cortex. Nat Neurosci 21:696–706
Ishibashi T, Dakin KA, Stevens B, Lee PR, Kozlov SV, Stewart CL, Fields RD (2006) Astrocytes promote myelination in response to electrical impulses. Neuron 49:823–832
Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE (2010) NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron 68:668–681
Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD (2006) Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci 9:173–179
Kim JY, Sun Q, Oglesbee M, Yoon SO (2003) The role of ErbB2 signaling in the onset of terminal differentiation of oligodendrocytes in vivo. J Neurosci 23:5561–5571
Kukley M, Capetillo-Zarate E, Dietrich D (2007) Vesicular glutamate release from axons in white matter. Nat Neurosci 10:311–320
Lee S, Leach MK, Redmond SA, Chong SY, Mellon SH, Tuck SJ, Feng ZQ, Corey JM, Chan JR (2012) A culture system to study oligodendrocyte myelination processes using engineered nanofibers. Nat Methods 9:917–922
Li Q, Brus-Ramer M, Martin JH, McDonald JW (2010) Electrical stimulation of the medullary pyramid promotes proliferation and differentiation of oligodendrocyte progenitor cells in the corticospinal tract of the adult rat. Neurosci Lett 479:128–133
Lin SC, Huck JH, Roberts JD, Macklin WB, Somogyi P, Bergles DE (2005) Climbing fiber innervation of NG2-expressing glia in the mammalian cerebellum. Neuron 46:773–785
Liu J, Dietz K, DeLoyht JM, Pedre X, Kelkar D, Kaur J, Vialou V, Lobo MK, Dietz DM, Nestler EJ, Dupree J, Casaccia P (2012) Impaired adult myelination in the prefrontal cortex of socially isolated mice. Nat Neurosci 15:1621–1623

Lundgaard I, Luzhynskaya A, Stockley JH, Wang Z, Evans KA, Swire M, Volbracht K, Gautier HO, Franklin RJ, Attwell D, Karadottir RT (2013) Neuregulin and BDNF induce a switch to NMDA receptor-dependent myelination by oligodendrocytes. PLoS Biol 11:e1001743

Makinodan M, Rosen KM, Ito S, Corfas G (2012) A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science 337:1357–1360

Mangin JM, Li P, Scafidi J, Gallo V (2012) Experience-dependent regulation of NG2 progenitors in the developing barrel cortex. Nat Neurosci 15:1192–1194

McKenzie IA, Ohayon D, Li H, de Faria JP, Emery B, Tohyama K, Richardson WD (2014) Motor skill learning requires active central myelination. Science 346:318–322

Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A (2006) Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci 26:7907–7918

Mensch S, Baraban M, Almeida R, Czopka T, Ausborn J, El Manira A, Lyons DA (2015) Synaptic vesicle release regulates myelin sheath number of individual oligodendrocytes in vivo. Nat Neurosci 18:628–630

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave KA (2004) Axonal neuregulin-1 regulates myelin sheath thickness. Science 304:700–703

Nave KA (2010) Myelination and support of axonal integrity by glia. Nature 468:244–252

Nave KA, Ehrenreich H (2014) Myelination and oligodendrocyte functions in psychiatric diseases. JAMA Psychiat 71:582–584

Nave KA, Salzer JL (2006) Axonal regulation of myelination by neuregulin 1. Curr Opin Neurobiol 16:492–500

Nave KA, Werner HB (2014) Myelination of the nervous system: mechanisms and functions. Annu Rev Cell Dev Biol 30:503–533

Nishiyama A, Komitova M, Suzuki R, Zhu X (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat Rev Neurosci 10:9–22

Pereira JA, Lebrun-Julien F, Suter U (2012) Molecular mechanisms regulating myelination in the peripheral nervous system. Trends Neurosci 35:123–134

Piaton G, Gould RM, Lubetzki C (2010) Axon-oligodendrocyte interactions during developmental myelination, demyelination and repair. J Neurochem 114:1243–1260

Purger D, Gibson EM, Monje M (2016) Myelin plasticity in the central nervous system. Neuropharmacology 110:563–573

Rosenberg SS, Kelland EE, Tokar E, De la Torre AR, Chan JR (2008) The geometric and spatial constraints of the microenvironment induce oligodendrocyte differentiation. Proc Natl Acad Sci U S A 105:14662–14667

Sampaio-Baptista C, Khrapitchev AA, Foxley S, Schlagheck T, Scholz J, Jbabdi S, DeLuca GC, Miller KL, Taylor A, Thomas N, Kleim J, Sibson NR, Bannerman D, Johansen-Berg H (2013) Motor skill learning induces changes in white matter microstructure and myelination. J Neurosci 33:19499–19503

Scholz J, Klein MC, Behrens TE, Johansen-Berg H (2009) Training induces changes in white-matter architecture. Nat Neurosci 12:1370–1371

Simon C, Gotz M, Dimou L (2011) Progenitors in the adult cerebral cortex: cell cycle properties and regulation by physiological stimuli and injury. Glia 59:869–881

Stevens B, Porta S, Haak LL, Gallo V, Fields RD (2002) Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36:855–868

Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, Xu X, Esper RM, Loeb JA, Shrager P, Chao MV, Falls DL, Role L, Salzer JL (2005) Neuregulin-1 type III determines the ensheathment fate of axons. Neuron 47:681–694

Taveggia C, Thaker P, Petrylak A, Caporaso GL, Toews A, Falls DL, Einheber S, Salzer JL (2008) Type III neuregulin-1 promotes oligodendrocyte myelination. Glia 56:284–293

Taveggia C, Feltri ML, Wrabetz L (2010) Signals to promote myelin formation and repair. Nat Rev Neurol 6:276–287

Wake H, Lee PR, Fields RD (2011) Control of local protein synthesis and initial events in myelination by action potentials. Science 333:1647–1651

Wurtz CC, Ellisman MH (1986) Alterations in the ultrastructure of peripheral nodes of Ranvier associated with repetitive action potential propagation. J Neurosci 6:3133–3143

Xiao L, Ohayon D, McKenzie IA, Sinclair-Wilson A, Wright JL, Fudge AD, Emery B, Li H, Richardson WD (2016) Rapid production of new oligodendrocytes is required in the earliest stages of motor-skill learning. Nat Neurosci 19:1210–1217

Young KM, Psachoulia K, Tripathi RB, Dunn SJ, Cossell L, Attwell D, Tohyama K, Richardson WD (2013) Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. Neuron 77:873–885

Zhu X, Bergles DE, Nishiyama A (2008) NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development 135:145–157

Ziskin JL, Nishiyama A, Rubio M, Fukaya M, Bergles DE (2007) Vesicular release of glutamate from unmyelinated axons in white matter. Nat Neurosci 10:321–330

Heterogeneity of Oligodendrocytes and Their Precursor Cells

5

Chikako Hayashi and Nobuharu Suzuki

Abstract

While oligodendrocytes have been thought to be homogenous, a number of reports have indicated evidences of the heterogeneity of oligodendrocytes and their precursor cells, OPCs. Almost a century ago, Del Río Hortega found three and four types of oligodendrocytes with regions where they exist and their morphologies, respectively. Interfascicular oligodendrocytes are one of the three regional dependent types and are the most typical oligodendendroglial cells that myelinate axonal fibers in the white matter tracts. In the other two, perineuronal oligodendrocyes function as reserve cells for remyelination and regulate neuronal excitability, whereas perivascular oligodendrocytes may play a role in metabolic support of axons. Among the four morphological categories, type I and II oligodendrocytes form many myelin sheaths on small-diameter axons and specific signal is required for the myelination of small-diameter axons. Type III and IV oligodendrocytes myelinate a few number of axons/or one axon, whose diameters are large. A recent comprehensive gene expression analysis with single-cell RNA sequencing identifies six different populations in mature oligodendrocytes and only one population in OPCs. However, OPCs are not uniformed developmentally and regionally. Further, the capacity of OPC differentiation depends on the environments and conditions of the tissues. Taken together, oligodendrocytes and OPCs are diverse as the other cell types in the CNS. The orchestration of these cells with their specialized functions is critical for proper functioning of the CNS.

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Keywords
Oligodendrocyte · Oligodendrocyte precursor cell (OPC) · Heterogeneity

5.1 Introduction

Oligodendrocytes have been thought that they consist of a homogenous population, although many neuronal subtypes are morphologically and functionally identified. However, almost a century ago, Del Río Hortega already well-described regional and morphological differences of oligodendrocytes in the CNS (Del Río Hortega 1922, 1928). He found three distinct localizations of oligodendrocytes: (1) interfascicular oligodendrocytes that exist in between axonal fiber tracts; (2) perineuronal oligodendrocytes, alternatively called satellite oligodendrocytes that are localized just beside neuronal cell bodies; and (3) perivascular oligodendrocytes that attach blood vessels (Fig. 5.1). Further, he categorized four different subtypes of oligodendrocytes (type I–IV) with morphology and the size and number of axons that they myelinate. Recently, there has been a quantity of evidences that indicate the heterogeneous populations of oligodendrocytes and their progenitor cells (oligodendrocyte precursor cells, OPCs). In this chapter, we review the heterogeneity of oligodendrocytes and OPCs.

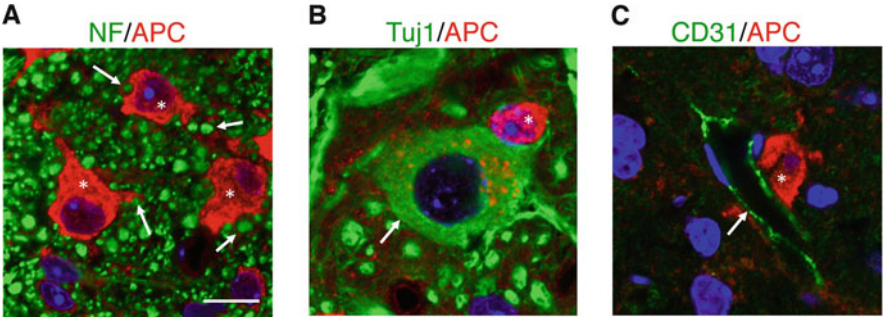

Fig. 5.1 Regionally different oligodendrocytes in the mouse spinal cord. (**a**) Myelinating oligodendrocytes among the axonal fiber tracts in the anterior funiculus of the white matter at P7. Asterisks: APC (Adenomatous Polyposis Coli) (red) positive interfascicular oligodendrocyte; allows: NF (neurofilament) (green) positive axon that is ensheathed or wrapped by an oligodendrocyte process. (**b**) Oligodendrocyte adhering to a neuronal cell body in the anterior horn of the gray matter in adult mice. Asterisks: APC (red) positive perineuronal oligodendrocyte; allow: Tuj1 (green) positive anterior horn neuron. (**c**) Oligodendrocyte attaching to a blood vessel in the gray matter at P7. Asterisks: APC (red) positive perivascular oligodendrocyte; allow: CD31 (green) positive endothelial cell. Nuclei are labeled by DAPI (blue). Scale bar, 10 μm

5.2 Heterogeneity of Oligodendrocytes

5.2.1 Regional Differences of Oligodendrocytes in the CNS

As mentioned previously, Del Río Hortega described three distinct localizations of oligodendroglial cells (Del Río Hortega 1928). First, the interfascicular oligodendrocytes localized in the axonal fiber tracts of the white matter are the most typical and well-characterized type among the three (Fig. 5.1a). These typical oligodendrocytes in the axonal fibers seem to be homogenous, however, several studies using gene-manipulated animals indicate that loss of gene expression exhibit regionally different phenotypes in the oligodendrocytes. Ablation of WAVE1 [WASP (Wkiskott-Aldrich syndrome proteins) family verprolin homologue-1] or B-Raf, an upstream kinase of MEK and ERK, causes hypomyelination of axonal tracts in the corpus callosum and the optic nerve, but not in the spinal cord (Kim et al. 2006; Galabova-Kovacs et al. 2008). On the other hand, knockout of mTOR (mammalian target of rapamycin) in oligodendrocytes results in myelination defect only in the white matter of the spinal cord (Wahl et al. 2014). In addition, more severe hypomyelination is observed in the spinal cord, compared with in the corpus callosum, of the transmembrane protein teneurin-4 (Ten-4) deficient mice (Suzuki et al. 2012). These observations represent region-specific signaling and mechanisms in CNS myelination. Further, analyzes of CNS tissues and using microfibers uncovered that oligodendrocytes in the spinal cord form longer internodes of myelin sheaths than those in the brain (Hildebrand et al. 1993; Bechler et al. 2015). Elongation of myelin internodes increases velocity of action potential (Stassart et al. 2018), therefore axonal tracts in the white matter of the spinal cord achieve more rapid conduction of action potential than those in the brain as "express way" of descending and ascending electrical signals between the brain and peripheral tissues. From these investigations, oligodendrocytes give rise to the region-specific features for each requirement in their locations.

The second perineuronal oligodendrocytes, called satellite oligodendrocytes, neighbor neuronal cell bodies in the gray matter and rarely form myelin (Fig. 5.1b). They are thought as reserve cells since they exert myelination ability after demyelination (Ludwin 1979). Furthermore, it has become clear that they regulate neuronal excitability by uptaking the extracellular potassium ion surrounding an active neuronal soma (Battefeld et al. 2016). Third, the perivascular oligodendrocytes are located around blood vessels in the CNS tissues (Fig. 5.1c). Their functions have not been characterized yet. However, perivascular oligodendrocytes may be a key player in metabolic support of axons, since there is a report demonstrating that oligodendrocytes transport pyruvate and lactate, which are probably derived from glucose secreted by blood vessels, to axons through MCT1, a monocarboxylate transporter localized in the most inner oligodendroglial cytoplasm inside myelin (Lee et al. 2012a). The latter two types of oligodendrocytes may possess more distinct functions from normal myelin formation. Further investigations are required for understanding them.

5.2.2 Morphological Differences of Oligodendrocytes in the CNS

Del Río Hortega categorized four different subclasses of oligodendrocytes with their morphologies and size/number of axons to form myelin (Del Río Hortega 1928). Type I oligodendrocytes have round and small cell body and form a lot of cell processes to myelinate many axons with small caliber sizes. The directions of these axons myelinated by type I cells are diverse. Type I oligodendrocytes exist in both white and gray matters. Type II oligodendrocytes are similar to type I cells, but their cell bodies are more cuboidal and axons they myelinate direct in parallel. These type II cells are found in the white matter, but not in the gray matter. Type III oligodendrocytes are located in both white and gray matters and the number of type III cells is less than type I and II cells. Type III oligodendrocytes have fewer cell processes forming myelin on larger diameter axons. Type IV oligodendrocytes are called Schwannoid oligodendrocytes alternatively. Their cell bodies are long and lie along large-diameter axons. Type IV cells myelinate only one large axon, like Schwann cells, and are usually observed in the white matter. After Del Río Hortega's report, several groups have demonstrated more defined characteristics of the four subtypes of oligodendrocytes. Butt et al. identified a couple of expression markers for the subtypes [CAII (carbonic anhydrase II) and long- and short-isoforms of MAG (myelin associated glycoprotein)] and a difference in internodal length among type I–IV oligodendrocytes in rat anterior medullary velum (Butt et al. 1995, 1998a, b).

5.2.3 Selectivity of Axonal Sizes in Myelination by Oligodendrocytes

One of the interesting aspects in the features of type I–IV oligodendrocytes is the difference in axonal diameters that they myelinate. A study using engineered nanofibers revealed that oligodendrocytes form myelin-like structures on the nanofibers and suggested that oligodendrocytes do not require axonal adhesion molecules, which is different from Schwann cells (Lee et al. 2012b). However, nanofibers whose diameter is less than 0.4 μm are not myelinated by oligodendrocytes, indicating that myelination of the smaller diameter fibers requires additional signaling, as it from axonal surface molecules.

There are several studies that indicate key molecules in myelination of small-diameter axons. Inhibiting integrin β1 signaling and conditional knockout of FAK (focal adhesion kinase), which is a downstream effector of integrin β1, in oligodendrocytes result in temporal hypomyelination during myelinating stage, while inhibition of integrin β3 signaling does not (Câmara et al. 2009). Further, the spontaneous null mutation of the laminin α2 chain, a component of laminin-211 that is a ligand of integrin β1, reduces myelination of small-diameter axon (Chun et al. 2003). These results suggest that the signaling of laminin α2-integrin β1-FAK may play an important role in myelination by type I/II oligodendrocytes. Further, the transmembrane protein Ten-4 is required for myelination of small-diameter axons through FAK activation (Suzuki et al. 2012) (Figs. 5.2 and 5.3). The

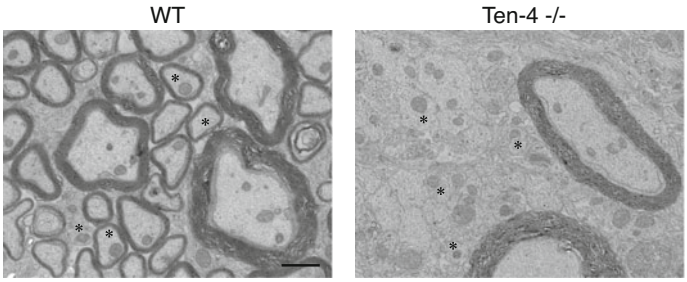

Fig. 5.2 Hypomyelination of small-diameter axons in the spinal cord of Ten-4 (teneurin-4)-deficient (−/−) mice. EM images in the lateral funiculus of 7-week-old WT and Ten-4 −/− mice are represented. Asterisks: Small-diameter axon. Scale bar, 1 μm

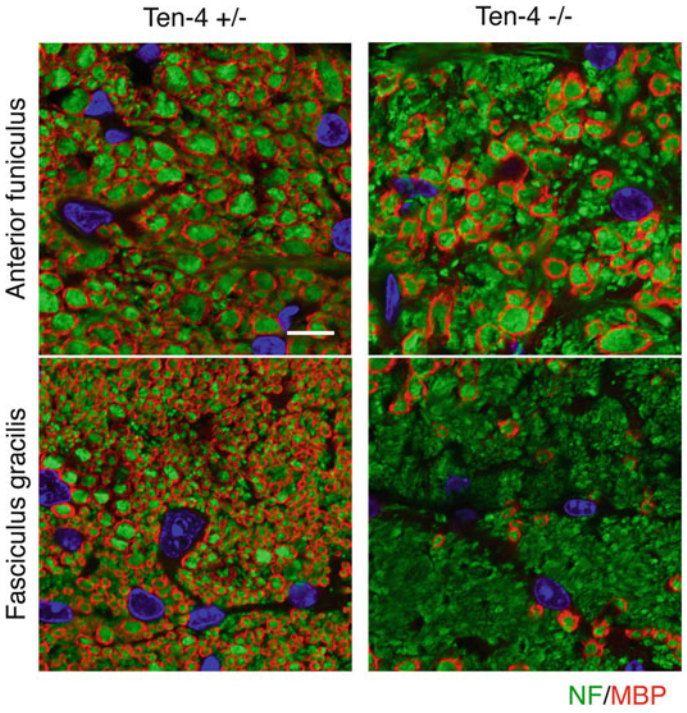

Fig. 5.3 Reduced myelination on small-diameter axons in the spinal cord of Ten-4 (teneurin-4)-deficient (−/−) mice. Immunohistochemical images of MBP (red) and neurofilament (NF) (green) in the anterior funiculus and fasciculus gracilis of 5-week-old Ten-4 heterozygous (+/−) and −/− mice are shown. Nuclei are labeled by DAPI (blue). Scale bar, 10 μm

hypomyelination of small axons and the neurological phenotype in Ten-4-deficient mice are more severe than those in the mutant mice of integrin β1, FAK, and laminin α2. Ten-4 may be a critical upstream molecule to regulate the signaling in type I/II oligodendrocyte myelination. Another regulator of myelination on small-diameter

axon is phosphatidylinositol 3,5-bisphosphate phosphatase Fig4. Interestingly, ectopic expression of Fig4 in neurons is sufficient to rescue the phenotype with hypomyelination of small-diameter axons in Fig4 null mice, whereas Fig4 is an intracellular protein (Winters et al. 2011). Neuronal Fig4 signaling presumably regulates the myelination through the expression of neuronal surface and/or secreted proteins for axon–oligodendrocyte interaction. These studies tell us that myelination of small-diameter axons by oligodendrocytes (type I and II) requires specific signaling, which is orchestrated by these molecules.

5.2.4 Oligodendrocyte Heterogeneity from Comprehensive Single-Cell RNA Sequencing

Recently, a comprehensive single-cell RNA sequencing analysis of oligodendrocyte lineage cells was reported and revealed the heterogeneity of these cells, particularly mature oligodendrocytes (Marques et al. 2016). In this report, a total of 5072 cells from 10 different regions of juvenile and adult CNS in mice were analyzed, and as a result, one population of OPCs, one population of differentiation-committed OPCs, two populations of newly formed oligodendrocytes, two populations of myelin-forming oligodendrocytes, and six populations of mature oligodendrocytes were identified. It is interesting to note that OPCs consist of the homogenous population according to the comprehensive analysis of single-cell gene expression, while OPCs are developmentally originated from a couple of distinct regions and timings, as reviewed below. Their data show that oligodendrocyte lineage cells are more homogenous during the sequential differentiation steps anywhere in the CNS, however, terminally differentiated oligodendrocytes consist of the heterogenous populations in a CNS region- and age-specific manner. Four of the six populations of mature oligodendrocytes are more abundant at the juvenile stage and express genes related to lipid synthesis and myelination at higher levels, compared with the other two populations. In contrast, one population of the six is specifically found in adult CNS and more highly express synapse-related genes, such as Grm3, a metabotropic glutamate receptor. The remaining population in the six is the most common throughout all the regions and ages. Functions of the oligodendrocytes in each population should be elucidated in near future.

5.3 Heterogeneity of OPCs

5.3.1 OPCs from Distinct Regions in the CNS

The single-cell RNA sequencing analysis shows that OPCs are composed of the homogenous population at the comprehensive gene expression level (Marques et al. 2016). However, there are a number of evidence that indicate OPCs are not uniformed. During development in the murine spinal cord, Shh (sonic hedgehog) and BMPs (bone morphogenetic proteins) are expressed in the ventricular zone of

the neural tube and function as morphogens in the regulation of specification and generation of OPCs. Shh plays a key role in determining cell fate toward OPCs, while BMPs suppress the production of OPCs. Regional differences in the expression of Shh and BMPs give rise to distinct OPC populations during development (Rowitch and Kriegstein 2010). Around embryonic day (E) 12, the first wave of OPC production occurs in the motor neuron progenitor domain of the ventral spinal cord. The second OPCs emerge from the dorsal region in the spinal cord around E16 (Kessaris et al. 2006; Rowitch and Kriegstein 2010). The OPCs derived from the dorsal region specifically express transcription factors, Pax7 and Mash1 (Cai et al. 2005). The expression of Olig1 is induced by the stimulation of Shh signaling in the ventrally derived OPCs, however, OPCs from the dorsal region develop normally without the Shh signaling (Cai et al. 2005). Instead, the development of dorsally derived OPCs is dependent on signaling induced by FGF (fibroblast growth factor) (Fogarty et al. 2005). The functional difference between these two populations has not been clearly elucidated yet, except for the ability of remyelination is higher in the OPCs from the dorsal spinal cord (Crawford et al. 2016).

5.3.2 OPCs in the Gray and White Matters

OPCs migrate and spread throughout CNS tissues, including both the gray and white matters. The characteristics of OPCs in the gray and white matters are different. For instance, white matter OPCs proliferate more rapidly than those in the gray matter, since OPCs in the white matter are more susceptible to PDGF (platelet-derived growth factor), which is widely used for OPC proliferation in culture (Chen et al. 2007; Hill et al. 2013; Viganò et al. 2013). Further, direct synaptic inputs to OPCs are reported and OPCs react with neuronal activity. However, white and gray matters OPCs possess sets of synapses with distinct neurotransmitters, glutamate and glutamate/GABA, respectively (Kukley et al. 2007; Ziskin et al. 2007). These evidence tell us that expression patterns of these proteins and biological roles in OPCs are distinguishable between in the white and gray matters. Finally, transplantation experiments in adult mouse brain demonstrated that white matter OPCs can differentiate to mature oligodendrocytes after transplantation into the gray matter, whereas gray matter OPCs are unable (Viganò et al. 2013). This suggests an intrinsic difference between these OPCs, which is not altered by their environmental factors.

5.3.3 Region-Specific Differentiation of OPCs

OPCs can differentiate to astrocytes, which are specifically called type II astrocytes, in the presence of a high concentration of serum in culture (Raff et al. 1983). In vivo, the differentiation from OPCs to type II astrocytes is region restrictedly observed in the ventral cortex (Zhu et al. 2008; Suzuki et al. 2017). This observation indicates that the differentiation capacity of OPCs is also different, although a mechanism of the regional difference in OPC differentiation is still unknown. In addition, OPCs

can differentiate not only into oligodendrocytes and astrocytes but also Schwann cells during the process of tissue regeneration in the CNS (Zawadzka et al. 2010). From these evidence, the fate of OPCs is altered by environmental factors in each region or condition, which may be important to control OPCs' behavior for the therapy of demyelinating diseases.

5.4 Conclusion

While sets of subtypes in neurons have been defined well, those in glial cells are still under discussion. One of the reasons is that glia cells are more flexible with their multipotency, compared with neurons. OPCs are able to differentiate into not only oligodendrocytes but also astrocytes as mentioned, furthermore, there is a report that demonstrates OPC differentiation to neurons (Rivers et al. 2008). In addition, differentiated oligodendrocytes can transdifferentiate into astrocytes under a pathological condition (Kohyama et al. 2008), and vice versa with an ectopic Sox10 expression (Mokhtarzadeh Khanghahi et al. 2018). However, many phenotypic differences of glia cells are clearly characterized and there are the evidence that indicate the heterogeneity of oligodendrocytes and OPCs in morphology, localization, and gene expression as described in this chapter. The specialized functions in each population of oligodendrocytes and OPCs need to be disclosed, and these discoveries will facilitate a better understanding of their biology, as well as the development of diagnostic and therapeutic reagents for dysmyelinating diseases and other related disorders.

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References

Battefeld A, Klooster F, Kole MHP (2016) Myelinating satellite oligodendrocytes are integrated in a glial syncytium constraining neuronal high frequency activity. Nat Commun 10(7):11298

Bechler ME, Byrne L, Ffrench-Constant C (2015) CNS myelin sheath lengths are an intrinsic property of oligodendrocytes. Curr Biol 25(18):2411–2416. https://doi.org/10.1016/j.cub.2015.07.056

Butt AM, Ibrahim M, Ruge FM, Berry M (1995) Biochemical subtypes of oligodendrocyte in the anterior medullary velum of the rat as revealed by the monoclonal antibody rip. Glia 14 (3):185–197

Butt AM, Ibrahim M, Berry M (1998a) Axon-myelin sheath relations of oligodendrocyte unit phenotypes in the adult rat anterior medullary velum. J Neurocytol 27(4):259–269

Butt AM, Ibrahim M, Gregson N, Berry M (1998b) Differential expression of the L- and S-isoforms of myelin associated glycoprotein (MAG) in oligodendrocyte unit phenotypes in the adult rat anterior medullary velum. J Neurocytol 27(4):271–280

Cai J, Qi Y, Hu X, Tan M, Liu Z, Zhang J, Li Q, Sander M, Qiu M (2005) Generation of oligodendrocyte precursor cells from mouse dorsal spinal cord independent of Nkx6 regulation and Shh signaling. Neuron 45(1):41–53

Câmara J, Wang Z, Nunes-Fonseca C, Friedman HC, Grove M, Sherman DL, Komiyama NH, Grant SG, Brophy PJ, Peterson A, ffrench-Constant C (2009) Integrin-mediated axoglial interactions initiate myelination in the central nervous system. J Cell Biol 185(4):699–712. https://doi.org/10.1083/jcb.200807010

Chen Y, Balasubramaniyan V, Peng J, Hurlock EC, Tallquist M, Li J, Lu QR (2007) Isolation and culture of rat and mouse oligodendrocyte precursor cells. Nat Protoc 2(5):1044–1051

Chun SJ, Rasband MN, Sidman RL, Habib AA, Vartanian T (2003) Integrin-linked kinase is required for laminin-2-induced oligodendrocyte cell spreading and CNS myelination. J Cell Biol 163(2):397–408

Crawford AH, Tripathi RB, Richardson WD, Franklin RJM (2016) Developmental origin of oligodendrocyte lineage cells determines response to demyelination and susceptibility to age-associated functional decline. Cell Rep 15(4):761–773. https://doi.org/10.1016/j.celrep.2016.03.069

Del Río Hortega P (1922) Son homologables la glia de escasas radiaciones y las células deSchwann. Bol Soc Esp Biol 10:25–28

Del Río Hortega P (1928) Tercera aportación al conocimiento morfológico e interpretación functional de la oligodendroglía. Mem Real Soc Esp Hist Nat 14:5–122

Fogarty M, Richardson WD, Kessaris N (2005) A subset of oligodendrocytes generated from radial glia in the dorsal spinal cord. Development 132(8):1951–1959

Galabova-Kovacs G, Catalanotti F, Matzen D, Reyes GX, Zezula J, Herbst R, Silva A, Walter I, Baccarini M (2008) Essential role of B-Raf in oligodendrocyte maturation and myelination during postnatal central nervous system development. J Cell Biol 180(5):947–955. https://doi.org/10.1083/jcb.200709069

Hildebrand C, Remahl S, Persson H, Bjartmar C (1993) Myelinated nerve fibres in the CNS. Prog Neurobiol 40(3):319–384

Hill RA, Patel KD, Medved J, Reiss AM, Nishiyama A (2013) NG2 cells in white matter but not gray matter proliferate in response to PDGF. J Neurosci 33(36):14558–14566. https://doi.org/10.1523/JNEUROSCI.2001-12.2013

Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD (2006) Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci 9(2):173–179

Kim HJ, DiBernardo AB, Sloane JA, Rasband MN, Solomon D, Kosaras B, Kwak SP, Vartanian TK (2006) WAVE1 is required for oligodendrocyte morphogenesis and normal CNS myelination. J Neurosci 26(21):5849–5859

Kohyama J, Kojima T, Takatsuka E, Yamashita T, Namiki J, Hsieh J, Gage FH, Namihira M, Okano H, Sawamoto K, Nakashima K (2008) Epigenetic regulation of neural cell differentiation plasticity in the adult mammalian brain. Proc Natl Acad Sci U S A 105(46):18012–18017. https://doi.org/10.1073/pnas.0808417105

Kukley M, Capetillo-Zarate E, Dietrich D (2007) Vesicular glutamate release from axons in white matter. Nat Neurosci 10(3):311–320

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ, Rothstein JD (2012a) Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature 487(7408):443–448. https://doi.org/10.1038/nature11314

Lee S, Leach MK, Redmond SA, Chong SY, Mellon SH, Tuck SJ, Feng ZQ, Corey JM, Chan JR (2012b) A culture system to study oligodendrocyte myelination processes using engineered nanofibers. Nat Methods 9(9):917–922. https://doi.org/10.1038/nmeth.2105

Ludwin SK (1979) The perineuronal satellite oligodendrocyte. A role in remyelination. Acta Neuropathol 47(1):49–53

Marques S, Zeisel A, Codeluppi S, van Bruggen D, Mendanha Falcão A, Xiao L, Li H, Häring M, Hochgerner H, Romanov RA, Gyllborg D, Muñoz Manchado A, La Manno G, Lönnerberg P, Floriddia EM, Rezayee F, Ernfors P, Arenas E, Hjerling-Leffler J, Harkany T, Richardson WD, Linnarsson S, Castelo-Branco G (2016) Oligodendrocyte heterogeneity in the mouse juvenile

and adult central nervous system. Science 352(6291):1326–1329. https://doi.org/10.1126/science.aaf6463

Mokhtarzadeh Khanghahi A, Satarian L, Deng W, Baharvand H, Javan M (2018) In vivo conversion of astrocytes into oligodendrocyte lineage cells with transcription factor Sox10; promise for myelin repair in multiple sclerosis. PLoS One 13(9):e0203785. https://doi.org/10.1371/journal.pone.0203785

Raff MC, Miller RH, Noble M (1983) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. Nature 303(5916):390–396

Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD (2008) PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci 11(12):1392–1401. https://doi.org/10.1038/nn.2220

Rowitch DH, Kriegstein AR (2010) Developmental genetics of vertebrate glial-cell specification. Nature 468(7321):214–222. https://doi.org/10.1038/nature09611

Stassart RM, Möbius W, Nave KA, Edgar JM (2018) The axon-myelin unit in development and degenerative disease. Front Neurosci 12:467. https://doi.org/10.3389/fnins.2018.00467

Suzuki N, Fukushi M, Kosaki K, Doyle AD, de Vega S, Yoshizaki K, Akazawa C, Arikawa-Hirasawa E, Yamada Y (2012) Teneurin-4 is a novel regulator of oligodendrocyte differentiation and myelination of small-diameter axons in the CNS. J Neurosci 32(34):11586–11599. https://doi.org/10.1523/JNEUROSCI.2045-11.2012

Suzuki N, Sekimoto K, Hayashi C, Mabuchi Y, Nakamura T, Akazawa C (2017) Differentiation of oligodendrocyte precursor cells from Sox10-venus mice to oligodendrocytes and astrocytes. Sci Rep 7(1):14133. https://doi.org/10.1038/s41598-017-14207-0

Viganò F, Möbius W, Götz M, Dimou L (2013) Transplantation reveals regional differences in oligodendrocyte differentiation in the adult brain. Nat Neurosci 16(10):1370–1372. https://doi.org/10.1038/nn.3503

Wahl SE, McLane LE, Bercury KK, Macklin WB, Wood TL (2014) Mammalian target of rapamycin promotes oligodendrocyte differentiation, initiation and extent of CNS myelination. J Neurosci 34(13):4453–4465. https://doi.org/10.1523/JNEUROSCI.4311-13.2014

Winters JJ, Ferguson CJ, Lenk GM, Giger-Mateeva VI, Shrager P, Meisler MH, Giger RJ (2011) Congenital CNS hypomyelination in the Fig4 null mouse is rescued by neuronal expression of the PI(3,5)P(2) phosphatase Fig4. J Neurosci 31(48):17736–17751. https://doi.org/10.1523/JNEUROSCI.1482-11.2011

Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, Rowitch DH, Kessaris N, Suter U, Richardson WD, Franklin RJ (2010) CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. Cell Stem Cell 6(6):578–590. https://doi.org/10.1016/j.stem.2010.04.002

Zhu X, Bergles DE, Nishiyama A (2008) NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development 135(1):145–157

Ziskin JL, Nishiyama A, Rubio M, Fukaya M, Bergles DE (2007) Vesicular release of glutamate from unmyelinated axons in white matter. Nat Neurosci 10(3):321–330

Part II

Specialized Structures Along the Myelinated Nerve Fibers

Functional Domains in Myelinated Axons

6

Leonid M. Yermakov, Lulu A. Hong, Domenica E. Drouet, Ryan B. Griggs, and Keiichiro Susuki

Abstract

Propagation of action potentials along axons is optimized through interactions between neurons and myelinating glial cells. Myelination drives division of the axons into distinct molecular domains including nodes of Ranvier. The high density of voltage-gated sodium channels at nodes generates action potentials allowing for rapid and efficient saltatory nerve conduction. At paranodes flanking both sides of the nodes, myelinating glial cells interact with axons, forming junctions that are essential for node formation and maintenance. Recent studies indicate that the disruption of these specialized axonal domains is involved in the pathophysiology of various neurological diseases. Loss of paranodal axoglial junctions due to genetic mutations or autoimmune attack against the paranodal proteins leads to nerve conduction failure and neurological symptoms. Breakdown of nodal and paranodal proteins by calpains, the calcium-dependent cysteine proteases, may be a common mechanism involved in various nervous system diseases and injuries. This chapter reviews recent progress in neurobiology and pathophysiology of specialized axonal domains along myelinated nerve fibers.

Keywords

Node of Ranvier · Paranode · Axon initial segment · Calpain

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6.1 Introduction

The nervous system's complex network receives and delivers information to and from different areas of our body. Inputs into neurons via somatodendritic synapses are integrated and converge on an output signal that is transmitted along axons toward the targets. Individual glial cells, oligodendrocytes in the central nervous system (CNS) or Schwann cells in the peripheral nervous system (PNS), sheath neuronal axons with tightly wrapped myelin in a segmented pattern (Sherman and Brophy 2005). Myelin has key roles and benefits for proper nervous system function. Myelin sheath electrically insulates the axon and helps establish rapid and efficient action potential propagation along myelinated axons in both CNS and PNS. In addition, myelination consequently drives further division of the axon into distinct molecular regions—unmyelinated spaces called nodes of Ranvier, paranodes and juxtaparanodes, and myelinated regions called the internode (Sherman and Brophy 2005; Rasband and Peles 2015). In addition, just proximal to the soma lies an axonal area called the axon initial segment (AIS). These excitable axonal domains, AIS and node of Ranvier, are particularly important because they function to create high-density clusters of ion channels, including voltage-gated sodium (Nav) channels.

In myelinated axons, action potentials are generated at the AIS (Bender and Trussell 2012; Kole and Stuart 2012), then regenerated at the nodes of Ranvier (Franssen and Straver 2013) allowing for rapid and efficient relay of information (Fig. 6.1). At paranodes flanking the distal and proximal sides of a node, myelinating glial cells interact with axons forming an axoglial junction that is essential for node formation and maintenance. In addition, paranodes are required for maintaining nerve conduction in myelinated axons: Even subtle disruption of paranodal junctions can result in conduction slowing (Babbs and Shi 2013).

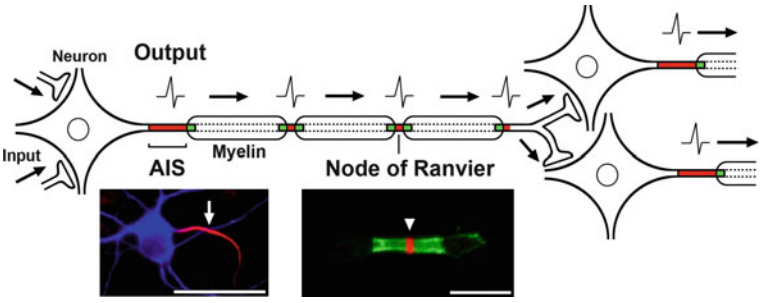

Fig. 6.1 Functional domains in myelinated axons. Axon initial segment (AIS) and nodes of Ranvier are the excitable axonal domains required for neuronal output and action potential propagation. Left panel: Mouse cortical neuron culture stained for AIS (arrow, ankyrinG in red) and somatodendritic domain (MAP2 in blue). Scale bar = 50 μm. Right panel: Mouse sciatic nerve section stained for node (arrowhead, ankyrinG in red) and paranode (Caspr in green). Scale bar = 10 μm

Recent advances in experimental and clinical pathology reveal that disruption of axonal domains is a key component in the pathogenesis of various neurological disorders, stressing the importance of understanding the basic neurobiology behind neuron–glia interactions and how their disruption accompanies nervous system injuries. This chapter describes recent progress in molecular mechanisms involved in the formation and maintenance of functional domains in myelinated axons. We also briefly describe diseases associated with disruption of AIS, nodes, and axon–glia interaction at paranodes, and discuss how these basic neurobiology discoveries facilitate our understanding of the pathophysiology of neurological disorders.

6.2 Structures and Protein Complexes at Specialized Axonal Domains

6.2.1 Axon Initial Segment and Nodes of Ranvier

Myelinated axons are divided into functionally and molecularly distinct domains including the AIS and nodes of Ranvier, which are the excitable domains that support neuronal outputs (Fig. 6.1). The AIS is a region approximately 10–60 μm long, located at the interface between the neuronal soma and the axon (Rasband 2010a). Nodes of Ranvier (nodes) are the 1 μm short gaps between two adjacent myelin sheaths (Rasband and Peles 2015). AIS and nodes share a specialized protein complex that is nearly identical (Fig. 6.2). A high molecular level of voltage-gated ion channel clusters, such as sodium (Nav), potassium (Kv), and calcium (Cav) channels are present at the nodes. Various forms of Nav channels exist including Nav1.1, Nav1.2, Nav1.6, Nav1.7, Nav1.8, and Nav1.9 [for a recent review, see Rasband and Peles 2015; Freeman et al. 2016]. A variety of Kv channels such as KCNQ2 (Kv7.2), KCNQ3 (Kv7.3), and Kv3.1b are also present at these sites (Battefeld et al. 2014). These ion channels regulate membrane properties of the AIS and nodal axolemma to ensure efficient generation and propagation of action potentials.

PNS nodal axolemma makes contact with Schwann cell microvilli whereas a specialized extracellular matrix (ECM) and perinodal astrocytes surround CNS nodes [reviewed in Rasband and Peles 2015]. In PNS, gliomedin and NrCAM are secreted from Schwann cells and are incorporated into perinodal ECM and mediate positioning of microvilli for proper contact with nodal axolemma (Eshed et al. 2005, 2007; Feinberg et al. 2010). In the CNS, specialized perinodal ECM components include brevican, versican, phosphacan, Bral1, neurocan, and tenascin-R [reviewed in Susuki et al. 2013]. These ECM molecules interact with cell adhesion molecule neurofascin (NF) 186 at the nodal axolemma, forming a structural and functional connection between the axolemma and myelinating glial cells and their secretions within the ECM.

AnkyrinG is a major scaffolding protein found at both AIS and nodes. AnkyrinG has multiple binding partners that include the cytoskeletal proteins βIV spectrin and αII spectrin, cell adhesion molecule NF186, and voltage-gated ion channels such as

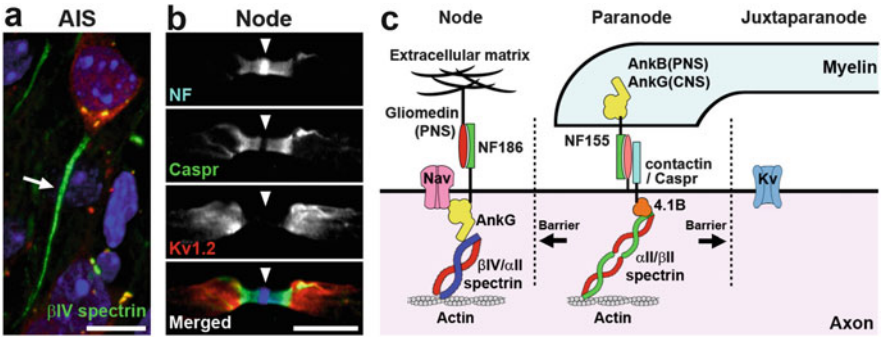

Fig. 6.2 Molecular organization of functional axonal domains. (**a**) Representative image of axon initial segment (AIS) in mouse cerebral cortex. AIS (arrow) is labeled in green (βIV spectrin), neuronal soma in red (NeuN), and cell nuclei in blue (Hoechst). Scale bar = 10 μm. (**b**) Representative image of node of Ranvier in mouse sciatic nerve section immunostained for neurofascin (NF) in blue, Caspr (paranodal axon) in green, and juxtaparanodal voltage-gated potassium channel (Kv1.2) in red. The antibody to NF stains NF186 at nodal axolemma strongly and NF155 at paranodal glia weakly. Arrowheads indicate node. Scale bar = 10 μm. (**c**) Molecular organization of node of Ranvier. Paranodal axoglial junctions are formed by cell adhesion molecules, axonal contactin and contactin-associated protein (Caspr), and glial NF155. These junctions act as a diffusion barrier to restrict the mobility of nodal and juxtaparanodal molecules. Nodal protein complex including voltage-gated sodium (Nav) channels is secured by the interaction between the axonal cell adhesion molecule NF186 and extracellular matrix molecules such as gliomedin in the peripheral nervous system (PNS). An ankyrinG (AnkG)-βIV/αII spectrin complex links Nav channels to the actin cytoskeleton for further stabilization. This panel is modified and reproduced from Susuki et al. (2016) with permission

Nav (Fig. 6.2) (Pan et al. 2006; Susuki et al. 2016; Huang et al. 2017; Nelson and Jenkins 2017).

6.2.2 Paranodes

The paranodes are located immediately adjacent to either side of the node. Paranodal junctions contain the enlarged, cytoplasm-filled ends of lateral loops formed by myelinating glia, and are the site of attachment between the axon and the myelin. The signature structural characteristics of paranodes are the transverse bands formed by the paranodal axoglial junctions—visualized at the transmission electron microscope (TEM) level by segmented dense lines (Mierzwa et al. 2010). The primary functions of paranodes are to facilitate saltatory conduction, prevent shunting of current, and stop lateral diffusion of ion channels (Rosenbluth 2009). Paranodal axoglial junctions are formed by cell adhesion complexes consisting of both axonal and glial-derived molecules. On the axonal side of the paranodal junction, the glycosylphosphatidylinositol protein contactin (Boyle et al. 2001) interacts in *cis* with contactin-associated protein (Caspr) (Peles et al. 1997; Bhat et al. 2001; Boyle et al. 2001). Caspr is involved in intracellular/intercellular signaling pathways and

aids in cellular communication between neurons and glia in the developing nervous system (Faivre-Sarrailh et al. 2000). The cytoplasmic domain of Caspr interacts with axonal adapter protein 4.1B, which in turn links it to the cytoskeletal αII and βII spectrin complex (Fig. 6.2) [reviewed in Susuki et al. 2016]. On the glial side, NF155 is critical for paranodal junction assembly (Tait et al. 2000; Sherman et al. 2005; Pillai et al. 2009) and was the first glial cell adhesion molecule to be identified. Contactin is also expressed in oligodendrocytes and regulates myelination and nodal and paranodal formation in the CNS (Çolakoğlu et al. 2014). Glial cells also contain ankyrinG (CNS) or ankyrinB (PNS), which through interaction with NF155 help assemble paranodal junctions (Chang et al. 2014). βII spectrin expressed in Schwann cells is enriched at paranodes, and contributes to the formation and maintenance of paranodal axoglial junctions (Susuki et al. 2018). Finally, in addition to the proteins, gangliosides (sialylated glycosphingolipids) are involved in the formation and maintenance of paranodal axoglial junctions (Susuki et al. 2007a; Silajdzić et al. 2009; Yao et al. 2014; Yoo et al. 2015). For other glycolipids, galactocerebroside and sulfatide, see Chap. 12 by Baba and Ishibashi.

6.2.3 Juxtaparanodes

Juxtaparanodes are located adjacent to paranodes and are segregated from nodes of Ranvier by a barrier formed by the paranodal axoglial junction (Fig. 6.2). Juxtaparanodes are characterized by high-density clusters of Kv channel, mostly Kv1 subtypes. In both PNS and CNS, Kv channels are situated underneath the glial-derived myelin sheath. For proper clustering of Kv1 channels, presence of Caspr2 and TAG-1 molecules at the juxtaparanodes is crucial (Rasband 2010b). Similar to the paranodal region, juxtaparanodal Caspr2 is linked to the axonal αII and βII spectrin complex through adapter protein 4.1B [reviewed in Susuki et al. 2016].

For more details on molecular complexes at the AIS and nodes, see other review articles (Chang and Rasband 2013; Rasband and Peles 2015; Susuki et al. 2016; Griggs et al. 2017; Nelson and Jenkins 2017; Huang and Rasband 2018).

6.3 Formation and Maintenance of Ion Channel Clustering at the Excitable Axonal Domains

6.3.1 Assembly of AIS

Despite the similarity of protein complexes at nodes of Ranvier and the AIS, the mechanisms for assembly of these excitable domains are significantly different. AIS assembly is a neuron-intrinsic process without glial cells, whereas node formation requires interaction between neurons and myelinating glia [reviewed in Rasband and Peles 2015; Ghosh et al. 2017; Nelson and Jenkins 2017]. During AIS assembly, ankyrinG is a master organizer for the AIS protein complex that includes Nav channels, NF186, and βIV spectrin. In developing axons that are unmyelinated,

ankyrinG is the first component that appears at the AIS and is excluded from the distal axon. A submembranous cytoskeletal complex in distal axons consisting of ankyrinB, αII spectrin, and βII spectrin defines an intra-axonal boundary, limiting ankyrinG and βIV spectrin to the AIS (Galiano et al. 2012). In addition, control of AIS assembly is proposed to be mediated by palmitoylation of ankyrinG, or phosphorylation of ankyrinG and its binding partners [reviewed in Nelson and Jenkins 2017].

6.3.2 Formation and Maintenance of Nodes of Ranvier

In contrast to the AIS, myelinating glial cells are required for the assembly of Nav channel clusters at the nodal axolemma. Two distinct glia-mediated mechanisms are involved in node formation. One mechanism is the interaction between ECM proteins secreted by myelinating glial cells and axonal cell adhesion molecule NF186. During peripheral nerve development, gliomedin and NrCAM are secreted by Schwann cells and incorporated into the ECM at the edge of forming myelin segments and promote NF186 clustering on the axolemma (Eshed et al. 2005, 2007; Feinberg et al. 2010). CNS nodes are associated with specialized ECM proteins including the chondroitin sulfate proteoglycans such as brevican that bind directly to NF186 (Susuki et al. 2013). Clustering of NF186 functions as an attachment site for the axonal scaffolding protein ankyrinG (Sherman et al. 2005), which recruits Nav channels at nodes (Gasser et al. 2012). Finally, the submembranous cytoskeletal protein βIV spectrin connects the nodal NF186–ankyrinG–Nav channel complex to the actin cytoskeleton for further stabilization. After the nodes are formed, glial contact with axonal NF186 mediated by ECM proteins contributes to long-term maintenance of the Nav channel complex (Amor et al. 2014; Desmazieres et al. 2014; Taylor et al. 2017).

A second, parallel mechanism for node formation mediated by myelinating glia is the paranodal axoglial junctions formed by glial NF155 and axonal contactin and Caspr. These paranodal junctions have been proposed to act as a diffusion barrier to restrict the mobility of nodal proteins such as Nav channels (Rasband et al. 1999; Feinberg et al. 2010; Susuki et al. 2013). Cytoskeletal components at paranodal axons are composed of actin-binding proteins αII spectrin, βII spectrin (Ogawa et al. 2006), and protein 4.1B (Ohara et al. 2000). Axonal βII spectrin is required for paranode-dependent clustering of Nav channels at nodes of Ranvier (Amor et al. 2017). Interaction between Caspr and protein 4.1B has a role in oligodendrocyte process extension and in the formation of Nav channel clustering at the tips of oligodendrocyte processes (Brivio et al. 2017). The paranodal axoglial junctions also function as a diffusion barrier for juxtaparanodal proteins such as Kv channels (Bhat et al. 2001; Boyle et al. 2001; Pillai et al. 2009). In mutant mice lacking the paranodal cell adhesion complex, juxtaparanodal proteins are mislocalized into the paranodal region (Fig. 6.3). The axonal cytoskeleton at paranodes also facilitates the stabilization of the diffusional barrier. Loss of βII spectrin in PNS sensory axons leads to mislocalization of juxtaparanodal Kv channels to paranodes, even when the

cell adhesion complex containing Caspr is intact within the paranodal axoglial junction (Zhang et al. 2013).

Paranodal axoglial junctions also contribute to long-term maintenance of ion channel clustering along myelinated axons. Ablation of glial NF155 induced after completion of myelination caused reduction of paranodal Caspr, loss of paranodal transverse bands, and mislocalization of juxtaparanodal Kv channels to paranodal area (Pillai et al. 2009). No nodal destabilization was observed after ablation of glial NF155 in mature nerves. However, ablation of both axonal NF186 and glial NF155 simultaneously in nerves with mature myelin caused accelerated destabilization of the node when compared to ablation of NF186 alone, suggesting that the stability of nodal complexes are enhanced when the flanking paranodal domains are present (Taylor et al. 2017). Furthermore, inducible loss of NF186 did not lead to a total absence of nodal Nav channels, suggesting that paranodal structures play a role in nodal maintenance (Desmazieres et al. 2014). Taken together, these studies underscore the importance of myelinating glial cells for the initial assembly and long-term maintenance of ion channel clustering at distinct axonal domains.

6.4 Disruption of Paranodal Axoglial Junctions in Neurological Diseases

Given the importance of the AIS and nodes of Ranvier for nervous system function, it is not surprising that changes in these domains play key roles in the pathophysiology of various neurological and psychiatric disorders (Buffington and Rasband 2011; Susuki 2013; Nelson and Jenkins 2017). Here we focus on the disruption of paranodal axoglial junctions, as the paranodal cell adhesion complex contributes to the stability and organization of nodes of Ranvier along axons. Recent progress of molecular neurobiology regarding paranodal axoglial junctions has provided the basic science foundation needed to elucidate mechanisms of how nerve function is damaged in neurological diseases.

6.4.1 Mutant Mouse Models for Loss of the Paranodal Cell Adhesion Complex

Perturbing the paranodal cell adhesion complex in Caspr knockout mice has been shown to cause mislocalization of juxtaparanodal proteins such as Kv1 channels to the paranodal region and elongation of nodal clusters (Fig. 6.3) (Bhat et al. 2001). This consequently prompts functional deficits such as nerve conduction slowing and behavioral signs of neurological dysfunction (Bhat et al. 2001). These neurological, physiological, and morphological deficits correspond to *shambling* mice with spontaneous mutations in the gene encoding Caspr (Sun et al. 2009). Most notably, transverse bands were absent in TEM analyses in both Caspr knockout (Bhat et al. 2001) and *shambling* mice (Sun et al. 2009). Similarly, mice with ablated contactin gene expression develop deficiencies in hind limb movement, decreased conduction

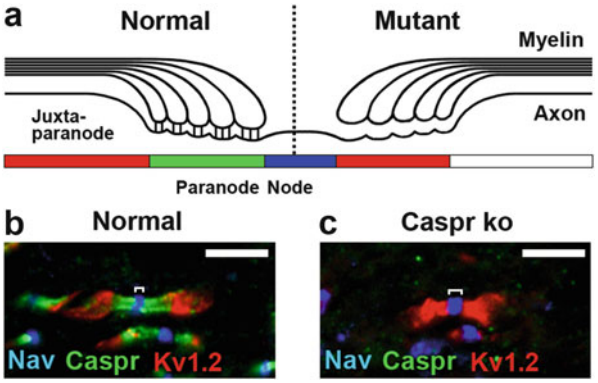

Fig. 6.3 Loss of paranodal axoglial junctions. (**a**) A schematic presentation of node (blue), paranodes (green), and juxtaparanodes (red). Normal (left side) and paranodal mutant (right side) are depicted. Note the loss of paranodal junctions, increased distance between paranodal lateral loops and axolemma, mild nodal elongation, and mislocalization of juxtaparanodal components to paranodal region in the mutant. (**b**, **c**) Representative images of nodes in wild type (**b**) or Caspr knockout (**c**) mouse optic nerves. Sections are immunostained for Caspr (paranodal axon) in green, Nav channel in blue, and juxtaparanodal voltage-gated potassium channel (Kv1.2) in red. Note the loss of Caspr immunostaining, elongation of nodal gap (brackets), and Kv1.2 clusters next to the node in Caspr knockout (**c**). Scale bars = 5 μm

velocity, absence of paranodal transverse bands, and altered Kv1 channel localization (Boyle et al. 2001). Furthermore, conditional knockout mice lacking NF155, specifically in myelinating glial cells (Pillai et al. 2009), show similar phenotypes and morphology to knockout mice lacking axonal Caspr (Bhat et al. 2001) or contactin (Boyle et al. 2001). These glial NF155 mutant mice exhibit muscle weakness, significant conduction slowing, loss of transverse bands, and mislocalized Kv1 channels. Nav channel clusters at nodes of Ranvier were elongated but still present in these paranodal mutant mice (Bhat et al. 2001; Boyle et al. 2001; Pillai et al. 2009), as other mechanisms such as the ECM and axonal cytoskeletal and scaffolding proteins can compensate for the absence of paranodal axoglial junctions in PNS (Feinberg et al. 2010) and CNS (Susuki et al. 2013). These findings strongly suggest that the paranodal cell adhesion complex is integral for paranodal organization and proper axoglial interactions between myelin loops and axon membranes.

Disruption to the paranodal Caspr/contactin/NF155 complex can lead to dysfunction of normal axonal circuitry and transport, accelerating axonal pathology. Paranodal disruptions also causally triggered axonal degeneration and cell death in *shambling* mice (Takagishi et al. 2016). Another Caspr mutant study linked cytoplasmic swellings of organelles to fragmented cytoskeletal features and degeneration in cerebellar Purkinje axons (Garcia-Fresco et al. 2006). Similar pronounced axonal swellings were observed in mice with mutated contactin (Boyle et al. 2001). Additionally, axonal degeneration was prominent in myelinated cerebellar fibers of NF155 mutant mice, with pathological accumulation of mitochondria and smooth endoplasmic reticulum within the degenerated axons (Pillai et al. 2009).

These observations in experimental models establish a vital link between the paranodal cell adhesion complex and normal axonal function, raising the possibility that neurodegenerative disorders may stem from disruption to this molecular structure. Clinical cases of paranodal disruption have likewise been shown to exhibit loss of transverse bands and elongated nodes of Ranvier, as described below.

6.4.2 Human Diseases Associated with Genetic Mutations of Paranodal Cell Adhesion Complex

A recent study described a patient with severe congenital hypotonia, contractures of fingers and toes, and no reaction to touch or pain (Smigiel et al. 2018). Whole exome sequencing revealed a homozygous mutation in *NFASC*, the gene encoding neurofascin, which is predicted to specifically eliminate glial NF155 leaving neuronal NF186 and NF140 intact. The absence of NF155 and disrupted paranodal junctions were confirmed by an immunofluorescent study of skin biopsies from the patient.

Homozygous frameshift mutations in *CNTNAP1*, the gene encoding Caspr, were found in patients with arthrogryposis multiplex congenita, characterized by multiple congenital contractures resulting from reduced fetal mobility (Laquérriere et al. 2014). Postpartum, infants with these mutations exhibited slowed peripheral nerve conduction velocity, and notable demyelination and elongated nodes of Ranvier in peripheral nerve biopsy specimens (Laquérriere et al. 2014). A recent study describing *CNTNAP1* mutations in humans provided further evidence of paranodal disruption: TEM images obtained from sural nerve biopsies of three patients showed disorganized Caspr-containing paranodes, the disappearance of transverse bands at paranodal axoglial junctions, and widened nodes (Vallat et al. 2016). The absence of paranodal axoglial junctions was also reported in another patient with *CNTNAP1* mutations (Conant et al. 2018). Furthermore, axonal degeneration has been associated with *CNTNAP1* mutations in patients with arthrogryposis multiplex congenita (Lakhani et al. 2017; Conant et al. 2018). These findings in human patients mirror the phenotype of genetically engineered Caspr knockout mice (Bhat et al. 2001) or spontaneous Caspr mutant mice (Sun et al. 2009). However, hypomyelination was observed in patients with *CNTNAP1* mutations (Laquérriere et al. 2014; Vallat et al. 2016; Conant et al. 2018). This is inconsistent with findings from Caspr mutant mice displaying normal myelin thickness (Bhat et al. 2001; Sun et al. 2009). The reason for the difference of myelination between humans and mice with Caspr mutations is not known.

Recently, mutations in *GLDN*, the gene encoding perinodal ECM molecule gliomedin, have been found to be associated with arthrogryposis multiplex congenita (Maluenda et al. 2016). TEM analysis of the sciatic nerve from a patient with *GLDN* mutation showed a reduced number of myelinated fibers and marked lengthening of the nodes of Ranvier compared to an age-matched control individual (Maluenda et al. 2016). In contrast, mutant mice lacking gliomedin had normal PNS myelin and display no overt neurological abnormalities, although these mutant mice

displayed altered Nav channel clustering during early development and impaired attachment of Schwann cell microvilli to the nodal axolemma (Feinberg et al. 2010). Nevertheless, these observations in patients with *NFASC*, *CNTNAP1*, or *GLDN* mutations further underscore the important consequences of paranodal and nodal disruption in human disease.

6.4.3 Autoimmune Reactions Targeting the Paranodal Cell Adhesion Complex

Cell adhesion molecules at paranodes can be targeted by immune reactions, leading to neurological diseases, such as chronic inflammatory demyelinating polyneuropathy (CIDP) [for a recent review, see Querol et al. 2017]. Serum autoantibodies against NF155 and contactin are present in certain subpopulations of CIDP with characteristic clinical features (Koike et al. 2017; Vallat et al. 2017). The detachment of paranodal lateral loops from axolemma was frequently noted in CIDP patients with circulating anti-NF155 antibodies, but not in patients without these antibodies (Koike et al. 2017). Anti-NF155 antibodies have also been implicated in destabilization of the paranode and retardation of conduction velocity (Vallat et al. 2017). Similarly, pathology in a CIDP patient with anti-contactin antibodies showed detachment of paranodal lateral loops from the axolemma (Koike et al. 2017). As mentioned above, these findings are consistent with loss of paranodal transverse bands in the mutant mice lacking NF155 (Pillai et al. 2009) or contactin (Boyle et al. 2001). Deposition of IgG4 was found at paranodes in these patients, suggesting that anti-NF155 and anti-contactin antibodies bound to the target cell adhesion molecules at paranodes (Koike et al. 2017). In another study, the passive transfer of anti-contactin-1 IgG4 from CIDP patients into rats with a mild neuropathy and blood–nerve barrier leakage (induced by immunization with low doses of P2 peptide) disrupted paranodal cell adhesion complex and exacerbated the severity of the clinical signs, further suggesting the pathogenic roles of these autoantibodies (Manso et al. 2016). Similarly, antibodies to Caspr or NF155 are elevated in sera from patients with a demyelinating form of Guillain-Barré syndrome, an acute immune-mediated polyneuropathy (Ng et al. 2012; Doppler et al. 2016). These autoantibodies against nodal and paranodal proteins are detected in only a portion of patients with these neuropathies, and the direct pathophysiological consequences of these autoantibodies remain unclear.

Autoantibodies against both glial NF155 and axonal NF186 were detected in the sera from patients with multiple sclerosis (MS), an immune-mediated disease of the CNS (Mathey et al. 2007; Kawamura et al. 2013). Transfer of anti-NF antibodies to mice/rats after induction of experimental autoimmune encephalomyelitis, a model of inflammatory demyelination, exacerbated immune-mediated axonal injury by selective targeting of complement to nodes of Ranvier (Mathey et al. 2007). Although lesions to CNS myelin in MS are proposed to be the primary pathology, emerging research in patients has shown that early lesions in MS involve paranodal disruption (Coman et al. 2006; Howell et al. 2006). However, the pathogenic roles of these

autoantibodies to NF155 and NF186 in human MS patients require further study. Nevertheless, these observations support the notion that autoimmune reactions against the paranodal cell adhesion complex contribute to the pathophysiology of immune-mediated neurological diseases.

6.4.4 Other Diseases Involving Paranodal Axoglial Junctions

Besides gene mutation and autoimmune mechanisms, paranodal disruption has been described in a variety of nervous system injuries and diseases. For instance, compression neuropathy has revealed disorganized paranodal myelin structures in human pathology (Neary et al. 1975). Experiments in a mouse model confirmed that chronic sciatic nerve compression leads to disorganized Caspr and NF155 clusters and partial loss of paranodal transverse bands (Otani et al. 2017) (Fig. 6.4). A pathological study performed on patients with type 1 diabetes revealed distinct loss of transverse bands at the axoglial junction, paranode swelling and de/remyelination of axons, along with reduced conduction velocity in sural nerves (Sima et al. 1988). A recent study of skin biopsy samples from patients with diabetes mellitus found dispersion of both NF and Caspr staining at the nodes of Ranvier (Doppler et al. 2017). A glucose metabolite methylglyoxal, which is known to be elevated in patients with diabetes, disrupts paranodal axoglial junctions in both PNS and CNS (Griggs et al. 2018). The molecular and cellular mechanisms of how paranodal axoglial junctions are disrupted in these conditions still remain unclear. Nevertheless, these studies further demonstrate that the disruption of paranodal axoglial junctions is involved in a wide variety of neurological diseases.

6.5 Disruption of Axonal Domains Due to Breakdown of Nodal/Paranodal Proteins

In addition to the loss of paranodal axoglial junctions as described above, disruption of excitable axonal domains along myelinated nerve fibers also may play a role in disease (Buffington and Rasband 2011; Susuki 2013; Nelson and Jenkins 2017). Recent studies using animal models suggest that the variable etiology and structural characteristics of axonal domain disruption in human disease may be due to a common mechanism. Calpains are a class of intracellular calcium-dependent proteases (Ma 2013). The mechanism of calpain activation varies depending on the disease condition, but it is typically associated with dysregulated calcium homeostasis (Vosler et al. 2008). Upon pathological over-activation, calpain can cleave many of the proteins that comprise myelin and excitable axonal domains (Fig. 6.5) (Ma 2013). Some of the known targets of calpain-mediated proteolysis are ankyrins, spectrins, Nav channels, and myelin basic protein (Banik 1992; Czogalla and Sikorski 2005; Schafer et al. 2009; von Reyn et al. 2009).

Pathological activation of calpains mediates nodal and paranodal disruption in conditions of nerve injury. For example, glutamate-evoked excitotoxicity results in

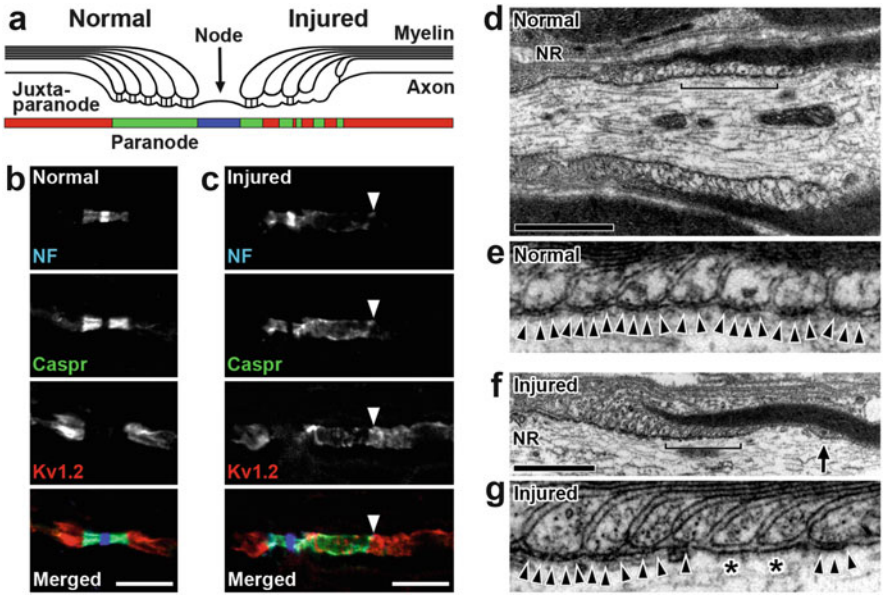

Fig. 6.4 Disruption of paranodal axoglial junctions in sciatic nerve injury. (**a**) A schematic presentation of nodes (blue), paranodes (green), and juxtaparanodes (red). Normal (left side) and disrupted paranode in a compression neuropathy model (Otani et al. 2017) (right side) are depicted. Note the partial loss of paranodal junctions, the ectopic innermost paranodal lateral loop, and disorganized paranodal and juxtaparanodal components in the injured nerve. (**b**, **c**) Representative images of nodes from control mouse sciatic nerve (**b**) or injured mouse nerve by compression (**c**). Sections are immunostained for Caspr (paranodal axon) in green, neurofascin (NF155 at paranodal glia and NF186 at nodal axon) in blue, and juxtaparanodal voltage-gated potassium channel (Kv1.2) in red. Note the disrupted paranodal Caspr cluster (right side of the node) with overlapping Kv1.2 (**c**). Arrowheads indicate the edge of paranodal clusters. Scale bars = 10 μm. (**d**) Transmission electron microscopy image of normal paranode in longitudinal section of control mouse sciatic nerve. NR indicates the location of node of Ranvier. Scale bar = 1 μm. (**e**) Enlarged image of the designated area in panel D (bracket) showing transverse bands (arrowheads). (**f**) Transmission electron microscopy image of disrupted paranode in longitudinal section of compressed mouse sciatic nerve. NR indicates the location of node of Ranvier. Arrow indicates remote lateral loop. Scale bar = 1 μm. (**g**) Enlarged image of the designated area in panel F (bracket). Transverse bands (arrowheads) are absent in two lateral loops (asterisks). This figure is modified and reproduced from Otani et al. (2017) with permission

calpain-mediated paranodal disruption in ex vivo white matter from the guinea pig spinal ventral column (Fu et al. 2009). Exposure to glutamate in the presence of calcium (but not in the absence of calcium) caused significant paranodal myelin splitting and retraction, as well as increased nodal length. Pharmacological inhibition of calpain prevented nodal elongation almost entirely, suggesting that calpain mediates glutamate-evoked damage to nodal/paranodal structures. Traumatic brain injury in rats resulted in a calpain-mediated breakdown of ankyrinG and αII spectrin and disruption of nodal and paranodal fine structures as early as 3 h post-injury

(Reeves et al. 2010). Calpain over-activation may also mediate nodal and paranodal disruption in the axonal form of Guillain-Barré syndrome. The hallmark of early human pathology in axonal Guillain-Barré syndrome is the abnormal elongation of nodes of Ranvier in spinal ventral roots (Griffin et al. 1996). A study in a rabbit model of Guillain-Barré syndrome mediated by anti-ganglioside antibodies suggests that autoimmune attack and selective complement deposition at the nodes of Ranvier leads to disruption of the molecular complex at nodes and paranodes (Susuki et al. 2007b). Importantly, this complement deposition forms pores in the affected nodal axolemma, presumably allowing uncontrolled calcium influx and calpain activation (McGonigal et al. 2010). Supporting this hypothesis, pharmacological inhibition of calpains resulted in preserved clusters of nodal ankyrinG/Nav1.6 and paranodal Caspr in an ex vivo model of Guillain-Barré syndrome (McGonigal et al. 2010). Similarly, methylglyoxal-induced paranodal disruption is prevented by pharmacological calpain inhibition (Griggs et al. 2018). These findings suggest that calpain over-activation is involved in the disruption of nodes and paranodes in various conditions.

Calpains are also implicated in the disruption of the AIS. In a rodent model of stroke induced by middle cerebral artery occlusion, AIS disruption was evident by decreased ankyrinG, βIV spectrin, and Nav channel immunoreactivity (Schafer et al. 2009). Pharmacological inhibition of calpain almost completely prevented AIS disruption. Interestingly, calpain-specific cleavage products of ankyrinG and βIV spectrin were found prior to Nav channel breakdown, suggesting that calpain-mediated disruption of intracellular proteins initiates functional AIS disruption by reduced clustering of Nav channels at the AIS (Fig. 6.5). Although similar molecular complexes are present in nodes of Ranvier, the authors found no evidence of nodal disruption (Schafer et al. 2009), suggesting that ischemia-induced dysfunction at axonal excitable domains preferentially affects the AIS. Calpain is also implicated in AIS disruption in a mouse model of acute neuroinflammation induced by intraperitoneal lipopolysaccharide injection (Benusa et al. 2017). Reduced ankyrinG

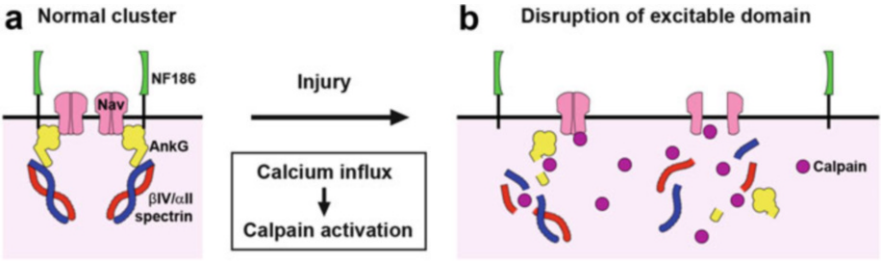

Fig. 6.5 Calpain-mediated disruption of excitable domains. (**a**) Nav channels, ankyrinG (AnkG), NF186, and βIV spectrin form clusters at the AIS and nodes of Ranvier. (**b**) Upon injury, calcium enters the axons and activates calpains. Calpains cleave the cytoskeletal and scaffolding proteins, ankyrinG and βIV/αII spectrin, resulting in the disruption of the clusters of ion channels and cell adhesion molecules. Calpains also cleave Nav channels, leading to dysfunction of excitable axonal domains. This figure is modified and reproduced from Griggs et al. (2017) with permission

immunostaining at the AIS occurred within 24 h of lipopolysaccharide administration. Administration of a calpain inhibitor prior to lipopolysaccharide injection completely prevented AIS disruption, demonstrating that calpains are required for the neuroinflammatory breakdown of the AIS in the CNS. Additional experiments in knockout mice suggest that NOX2-mediated calcium dysregulation leads to calpain over-activation in this neuroinflammatory model (Benusa et al. 2017). Recent studies suggest that structural changes in AIS play key roles in pathophysiology of a wide variety of conditions including traumatic brain injury (Baalman et al. 2013), epilepsy (Harty et al. 2013), Alzheimer's disease (Marin et al. 2016), multiple sclerosis (Clark et al. 2016), or diabetic brain complications (Yermakov et al. 2018), although it remains unknown if calpain mediates AIS alterations in these disease conditions.

These studies support the hypothesis that calpain-mediated disruption of excitable domains along myelinated axons is a pathological mechanism common to various neurological diseases (Fig. 6.5). Modulation of calpains or calpastatin, an endogenous inhibitor of calpains present in both neurons and myelinating glial cells (Takano et al. 2005), maybe a potential strategy to stop damage to myelinated nerve structures in pathological conditions or injuries to the nervous system.

6.6 Conclusion

As reviewed in this chapter, myelinating glial cells interact with neurons to form specialized axoglial domains such as nodes of Ranvier and paranodal junctions that are required for proper nervous system function. Recent progress in basic myelin biology has facilitated the understanding of the pathophysiology of neurological diseases. Despite this progress, the exact mechanism(s) that lead to the disruption of axonal domains that is associated with many neurological diseases remains elusive. Further research is needed to elucidate these unknown mechanisms of axonal domain disruption, as this could lead to the discovery of new therapeutic targets to counteract the progression of neurological disease.

References

Amor V, Feinberg K, Eshed-Eisenbach Y, Vainshtein A, Frechter S, Grumet M, Rosenbluth J, Peles E (2014) Long-term maintenance of Na^+ channels at nodes of Ranvier depends on glial contact mediated by gliomedin and NrCAM. J Neurosci 34:5089–5098

Amor V, Zhang C, Vainshtein A, Zhang A, Zollinger DR, Eshed-Eisenbach Y, Brophy PJ, Rasband MN, Peles E (2017) The paranodal cytoskeleton clusters Na^+ channels at nodes of Ranvier. elife 6:e21392

Baalman KL, Cotton RJ, Rasband SN, Rasband MN (2013) Blast wave exposure impairs memory and decreases axon initial segment length. J Neurotrauma 30:741–751

Babbs CF, Shi R (2013) Subtle paranodal injury slows impulse conduction in a mathematical model of myelinated axons. PLoS One 8:e67767

Banik NL (1992) Pathogenesis of myelin breakdown in demyelinating diseases: role of proteolytic enzymes. Crit Rev Neurobiol 6:257–271

Battefeld A, Tran BT, Gavrilis J, Cooper EC, Kole MHP (2014) Heteromeric Kv7.2/7.3 channels differentially regulate action potential initiation and conduction in neocortical myelinated axons. J Neurosci 34:3719–3732

Bender KJ, Trussell LO (2012) The physiology of the axon initial segment. Annu Rev Neurosci 35:249–265

Benusa SD, George NM, Sword BA, DeVries GH, Dupree JL (2017) Acute neuroinflammation induces AIS structural plasticity in a NOX2-dependent manner. J Neuroinflammation 14:116

Bhat MA, Rios JC, Lu Y, Garcia-Fresco GP, Ching W, St Martin M, Li J, Einheber S, Chesler M, Rosenbluth J, Salzer JL, Bellen HJ (2001) Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. Neuron 30:369–383

Boyle ME, Berglund EO, Murai KK, Weber L, Peles E, Ranscht B (2001) Contactin orchestrates assembly of the septate-like junctions at the paranode in myelinated peripheral nerve. Neuron 30:385–397

Brivio V, Faivre-Sarrailh C, Peles E, Sherman DL, Brophy PJ (2017) Assembly of CNS nodes of Ranvier in myelinated nerves is promoted by the axon cytoskeleton. Curr Biol 27:1068–1073

Buffington SA, Rasband MN (2011) The axon initial segment in nervous system disease and injury. Eur J Neurosci 34:1609–1619

Chang K-J, Rasband MN (2013) Excitable domains of myelinated nerves: axon initial segments and nodes of Ranvier. Curr Top Membr 72:159–192

Chang K-J, Zollinger DR, Susuki K, Sherman DL, Makara MA, Brophy PJ, Cooper EC, Bennett V, Mohler PJ, Rasband MN (2014) Glial ankyrins facilitate paranodal axoglial junction assembly. Nat Neurosci 17:1673–1681

Clark KC, Josephson A, Benusa SD, Hartley RK, Baer M, Thummala S, Joslyn M, Sword BA, Elford H, Oh U, Dilsizoglu-Senol A, Lubetzki C, Davenne M, DeVries GH, Dupree JL (2016) Compromised axon initial segment integrity in EAE is preceded by microglial reactivity and contact. Glia 64:1190–1209

Çolakoğlu G, Bergstrom-Tyrberg U, Berglund EO, Ranscht B (2014) Contactin-1 regulates myelination and nodal/paranodal domain organization in the central nervous system. Proc Natl Acad Sci U S A 111:E394–E403

Coman I, Aigrot MS, Seilhean D, Reynolds R, Girault JA, Zalc B, Lubetzki C (2006) Nodal, paranodal and juxtaparanodal axonal proteins during demyelination and remyelination in multiple sclerosis. Brain 129:3186–3195

Conant A, Curiel J, Pizzino A, Sabetrasekh P, Murphy J, Bloom M, Evans SH, Helman G, Taft RJ, Simons C, Whitehead MT, Moore SA, Vanderver A (2018) Absence of axoglial paranodal junctions in a child with *CNTNAP1* mutations, hypomyelination, and arthrogryposis. J Child Neurol 33:642–650

Czogalla A, Sikorski AF (2005) Spectrin and calpain: a "target" and a "sniper" in the pathology of neuronal cells. Cell Mol Life Sci 62:1913–1924

Desmazieres A, Zonta B, Zhang A, Wu L-MN, Sherman DL, Brophy PJ (2014) Differential stability of PNS and CNS nodal complexes when neuronal neurofascin is lost. J Neurosci 34:5083–5088

Doppler K, Appeltshauser L, Villmann C, Martin C, Peles E, Krämer HH, Haarmann A, Buttmann M, Sommer C (2016) Auto-antibodies to contactin-associated protein 1 (Caspr) in two patients with painful inflammatory neuropathy. Brain 139:2617–2630

Doppler K, Frank F, Koschker A-C, Reiners K, Sommer C (2017) Nodes of Ranvier in skin biopsies of patients with diabetes mellitus. J Peripher Nerv Syst 22:182–190

Eshed Y, Feinberg K, Poliak S, Sabanay H, Sarig-Nadir O, Spiegel I, Bermingham JR, Peles E (2005) Gliomedin mediates Schwann cell-axon interaction and the molecular assembly of the nodes of Ranvier. Neuron 47:215–229

Eshed Y, Feinberg K, Carey DJ, Peles E (2007) Secreted gliomedin is a perinodal matrix component of peripheral nerves. J Cell Biol 177:551–562

Faivre-Sarrailh C, Gauthier F, Denisenko-Nehrbass N, Le Bivic A, Rougon G, Girault JA (2000) The glycosylphosphatidyl inositol-anchored adhesion molecule F3/contactin is required for surface transport of paranodin/contactin-associated protein (caspr). J Cell Biol 149:491–502

Feinberg K, Eshed-Eisenbach Y, Frechter S, Amor V, Salomon D, Sabanay H, Dupree JL, Grumet M, Brophy PJ, Shrager P, Peles E (2010) A glial signal consisting of gliomedin and NrCAM clusters axonal Na^+ channels during the formation of nodes of Ranvier. Neuron 65:490–502

Franssen H, Straver DCG (2013) Pathophysiology of immune-mediated demyelinating neuropathies - Part I: Neuroscience. Muscle Nerve 48:851–864

Freeman SA, Desmazières A, Fricker D, Lubetzki C, Sol-Foulon N (2016) Mechanisms of sodium channel clustering and its influence on axonal impulse conduction. Cell Mol Life Sci 73:723–735

Fu Y, Sun W, Shi Y, Shi R, Cheng JX (2009) Glutamate excitotoxicity inflicts paranodal myelin splitting and retraction. PLoS One 4:e6705

Galiano MR, Jha S, Ho TS-Y, Zhang C, Ogawa Y, Chang K-J, Stankewich MC, Mohler PJ, Rasband MN (2012) A distal axonal cytoskeleton forms an intra-axonal boundary that controls axon initial segment assembly. Cell 149:1125–1139

Garcia-Fresco GP, Sousa AD, Pillai AM, Moy SS, Crawley JN, Tessarollo L, Dupree JL, Bhat M a (2006) Disruption of axo-glial junctions causes cytoskeletal disorganization and degeneration of Purkinje neuron axons. Proc Natl Acad Sci U S A 103:5137–5142

Gasser A, Ho TS-Y, Cheng X, Chang K-J, Waxman SG, Rasband MN, Dib-Hajj SD (2012) An ankyrinG-binding motif is necessary and sufficient for targeting Nav1.6 sodium channels to axon initial segments and nodes of Ranvier. J Neurosci 32:7232–7243

Ghosh A, Sherman DL, Brophy PJ (2017) The axonal cytoskeleton and the assembly of nodes of Ranvier. Neuroscientist. https://doi.org/10.1177/1073858417710897

Griffin JW, Li CY, Macko C, Ho TW, Hsieh ST, Xue P, Wang FA, Cornblath DR, McKhann GM, Asbury AK (1996) Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain-Barré syndrome. J Neurocytol 25:33–51

Griggs RB, Yermakov LM, Susuki K (2017) Formation and disruption of functional domains in myelinated CNS axons. Neurosci Res 116:77–87

Griggs RB, Yermakov LM, Drouet DE, Nguyen DVM, Susuki K (2018) Methylglyoxal disrupts paranodal axoglial junctions via calpain activation. ASN Neuro 10. https://doi.org/10.1177/1759091418766175

Harty RC, Kim TH, Thomas EA, Cardamone L, Jones NC, Petrou S, Wimmer VC (2013) Axon initial segment structural plasticity in animal models of genetic and acquired epilepsy. Epilepsy Res 105:272–279

Howell OW, Palser A, Polito A, Melrose S, Zonta B, Scheiermann C, Vora AJ, Brophy PJ, Reynolds R (2006) Disruption of neurofascin localization reveals early changes preceding demyelination and remyelination in multiple sclerosis. Brain 129:3173–3185

Huang CY-M, Rasband MN (2018) Axon initial segments: structure, function, and disease. Ann N Y Acad Sci 1420:46–61

Huang CY-M, Zhang C, Zollinger DR, Leterrier C, Rasband MN (2017) An αII spectrin-based cytoskeleton protects large-diameter myelinated axons from degeneration. J Neurosci 37:11323–11334

Kawamura N, Yamasaki R, Yonekawa T, Matsushita T, Kusunoki S, Nagayama S, Fukuda Y, Ogata H, Matsuse D, Murai H, Kira J-i (2013) Anti-neurofascin antibody in patients with combined central and peripheral demyelination. Neurology 81:714–722

Koike H, Kadoya M, Kaida K-I, Ikeda S, Kawagashira Y, Iijima M, Kato D, Ogata H, Yamasaki R, Matsukawa N, Kira J-I, Katsuno M, Sobue G (2017) Paranodal dissection in chronic inflammatory demyelinating polyneuropathy with anti-neurofascin-155 and anti-contactin-1 antibodies. J Neurol Neurosurg Psychiatry. https://doi.org/10.1136/jnnp-2016-314895

Kole MHP, Stuart GJ (2012) Signal processing in the axon initial segment. Neuron 73:235–247

Lakhani S, Doan R, Almureikhi M, Partlow JN, Al Saffar M, Elsaid MF, Alaaraj N, James Barkovich A, Walsh CA, Ben-Omran T (2017) Identification of a novel *CNTNAP1* mutation causing arthrogryposis multiplex congenita with cerebral and cerebellar atrophy. Eur J Med Genet. https://doi.org/10.1016/j.ejmg.2017.02.006

Laquérriere A et al (2014) Mutations in *CNTNAP1* and *ADCY6* are responsible for severe arthrogryposis multiplex congenita with axoglial defects. Hum Mol Genet 23:2279–2289

Ma M (2013) Role of calpains in the injury-induced dysfunction and degeneration of the mammalian axon. Neurobiol Dis 60:61–79

Maluenda J, Manso C, Quevarec L, Vivanti A, Marguet F, Gonzales M, Guimiot F, Petit F, Toutain A, Whalen S, Grigorescu R, Coeslier AD, Gut M, Gut I, Laquerrière A, Devaux J, Melki J (2016) Mutations in *GLDN*, encoding gliomedin, a critical component of the nodes of Ranvier, are responsible for lethal arthrogryposis. Am J Hum Genet 99:928–933

Manso C, Querol L, Mekaouche M, Illa I, Devaux JJ (2016) Contactin-1 IgG4 antibodies cause paranode dismantling and conduction defects. Brain 139:1700–1712

Marin MA, Ziburkus J, Jankowsky J, Rasband MN (2016) Amyloid-β plaques disrupt axon initial segments. Exp Neurol 281:93–98

Mathey EK, Derfuss T, Storch MK, Williams KR, Hales K, Woolley DR, Al-Hayani A, Davies SN, Rasband MN, Olsson T, Moldenhauer A, Velhin S, Hohlfeld R, Meinl E, Linington C (2007) Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 204:2363–2372

McGonigal R, Rowan EG, Greenshields KN, Halstead SK, Humphreys PD, Rother RP, Furukawa K, Willison HJ (2010) Anti-GD1a antibodies activate complement and calpain to injure distal motor nodes of Ranvier in mice. Brain 133:1944–1960

Mierzwa AJ, Arevalo J-C, Schiff R, Chao MV, Rosenbluth J (2010) Role of transverse bands in maintaining paranodal structure and axolemmal domain organization in myelinated nerve fibers: Effect on longevity in dysmyelinated mutant mice. J Comp Neurol 518:2841–2853

Neary D, Ochoa J, Gilliatt RW (1975) Sub-clinical entrapment neuropathy in man. J Neurol Sci 24:283–298

Nelson AD, Jenkins PM (2017) Axonal membranes and their domains: assembly and function of the axon initial segment and node of Ranvier. Front Cell Neurosci 11:136

Ng JKM et al (2012) Neurofascin as a target for autoantibodies in peripheral neuropathies. Neurology 79:2241–2248

Ogawa Y, Schafer DP, Horresh I, Bar V, Hales K, Yang Y, Susuki K, Peles E, Stankewich MC, Rasband MN (2006) Spectrins and ankyrinB constitute a specialized paranodal cytoskeleton. J Neurosci 26:5230–5239

Ohara R, Yamakawa H, Nakayama M, Ohara O (2000) Type II brain 4.1 (4.1B/KIAA0987), a member of the protein 4.1 family, is localized to neuronal paranodes. Mol Brain Res 85:41–52

Otani Y, Yermakov LM, Dupree JL, Susuki K (2017) Chronic peripheral nerve compression disrupts paranodal axoglial junctions. Muscle Nerve 55:544–554

Pan Z, Kao T, Horvath Z, Lemos J, Sul J-Y, Cranstoun SD, Bennett V, Scherer SS, Cooper EC (2006) A common ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. J Neurosci 26:2599–2613

Peles E, Nativ M, Lustig M, Grumet M, Schilling J, Martinez R, Plowman GD, Schlessinger J (1997) Identification of a novel contactin-associated transmembrane receptor with multiple domains implicated in protein-protein interactions. EMBO J 16:978–988

Pillai AM, Thaxton C, Pribisko AL, Cheng G, Dupree JL, Bhat MA (2009) Spatiotemporal ablation of myelinating glia-specific *neurofascin* ($Nfasc^{NF155}$) in mice reveals gradual loss of paranodal axoglial junctions and concomitant disorganization of axonal domains. J Neurosci Res 87:1773–1793

Querol L, Devaux J, Rojas-Garcia R, Illa I (2017) Autoantibodies in chronic inflammatory neuropathies: diagnostic and therapeutic implications. Nat Rev Neurol 13:533–547

Rasband MN (2010a) The axon initial segment and the maintenance of neuronal polarity. Nat Rev Neurosci 11:552–562

Rasband MN (2010b) Clustered K^+ channel complexes in axons. Neurosci Lett 486:101–106
Rasband MN, Peles E (2015) The nodes of Ranvier: molecular assembly and maintenance. Cold Spring Harb Perspect Biol 8:a020495
Rasband MN, Peles E, Trimmer JS, Levinson SR, Lux SE, Shrager P (1999) Dependence of nodal sodium channel clustering on paranodal axoglial contact in the developing CNS. J Neurosci 19:7516–7528
Reeves TM, Greer JE, Vanderveer AS, Phillips LL (2010) Proteolysis of submembrane cytoskeletal proteins ankyrin-G and αII-spectrin following diffuse brain injury: a role in white matter vulnerability at nodes of Ranvier. Brain Pathol 20:1055–1068
Rosenbluth J (2009) Multiple functions of the paranodal junction of myelinated nerve fibers. J Neurosci Res 87:3250–3258
Schafer DP, Jha S, Liu F, Akella T, McCullough LD, Rasband MN (2009) Disruption of the axon initial segment cytoskeleton is a new mechanism for neuronal injury. J Neurosci 29:13242–13254
Sherman DL, Brophy PJ (2005) Mechanisms of axon ensheathment and myelin growth. Nat Rev Neurosci 6:683–690
Sherman DL, Tait S, Melrose S, Johnson R, Zonta B, Court FA, Macklin WB, Meek S, Smith AJH, Cottrell DF, Brophy PJ (2005) Neurofascins are required to establish axonal domains for saltatory conduction. Neuron 48:737–742
Silajdzić E, Willison HJ, Furukawa K, Barnett SC (2009) In vitro analysis of glial cell function in ganglioside-deficient mice. J Neurosci Res 87:2467–2483
Sima AA, Nathaniel V, Bril V, McEwen TA, Greene DA (1988) Histopathological heterogeneity of neuropathy in insulin-dependent and non-insulin-dependent diabetes, and demonstration of axo-glial dysjunction in human diabetic neuropathy. J Clin Invest 81:349–364
Smigiel R, Sherman DL, Rydzanicz M, Walczak A, Mikolajkow D, Krolak-Olejnik B, Kosinska J, Gasperowicz P, Biernacka A, Stawinski P, Marciniak M, Andrzejewski W, Boczar M, Krajewski P, Sasiadek MM, Brophy PJ, Ploski R (2018) Homozygous mutation in the Neurofascin gene affecting the glial isoform of Neurofascin causes severe neurodevelopment disorder with hypotonia, amimia and areflexia. Hum Mol Genet. https://doi.org/10.1093/hmg/ddy277
Sun X, Takagishi Y, Okabe E, Chishima Y, Kanou Y, Murase S, Mizumura K, Inaba M, Komatsu Y, Hayashi Y, Peles E, Oda S, Murata Y (2009) A novel Caspr mutation causes the shambling mouse phenotype by disrupting axoglial interactions of myelinated nerves. J Neuropathol Exp Neurol 68:1207–1218
Susuki K (2013) Node of Ranvier disruption as a cause of neurological diseases. ASN Neuro 5: AN20130025
Susuki K, Baba H, Tohyama K, Kanai K, Kuwabara S, Hirata K, Furukawa K, Furukawa K, Rasband MN, Yuki N (2007a) Gangliosides contribute to stability of paranodal junctions and ion channel clusters in myelinated nerve fibers. Glia 55:746–757
Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, Funakoshi K, Hirata K, Baba H, Yuki N (2007b) Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. J Neurosci 27:3956–3967
Susuki K, Chang K-J, Zollinger DR, Liu Y, Ogawa Y, Eshed-Eisenbach Y, Dours-Zimmermann MT, Oses-Prieto JA, Burlingame AL, Seidenbecher CI, Zimmermann DR, Oohashi T, Peles E, Rasband MN (2013) Three mechanisms assemble central nervous system nodes of Ranvier. Neuron 78:469–482
Susuki K, Otani Y, Rasband MN (2016) Submembranous cytoskeletons stabilize nodes of Ranvier. Exp Neurol 283:446–451
Susuki K, Zollinger DR, Chang K-J, Zhang C, Huang CY-M, Tsai C-R, Galiano MR, Liu Y, Benusa SD, Yermakov LM, Griggs RB, Dupree JL, Rasband MN (2018) Glial βII spectrin contributes to paranode formation and maintenance. J Neurosci 38:3647–3617

Tait S, Gunn-Moore F, Collinson JM, Huang J, Lubetzki C, Pedraza L, Sherman DL, Colman DR, Brophy PJ (2000) An oligodendrocyte cell adhesion molecule at the site of assembly of the paranodal axo-glial junction. J Cell Biol 150:657–666

Takagishi Y, Katanosaka K, Mizoguchi H, Murata Y (2016) Disrupted axon-glia interactions at the paranode in myelinated nerves cause axonal degeneration and neuronal cell death in the aged Caspr mutant mouse shambling. Neurobiol Aging 43:34–46

Takano J, Tomioka M, Tsubuki S, Higuchi M, Iwata N, Itohara S, Maki M, Saido TC (2005) Calpain mediates excitotoxic DNA fragmentation via mitochondrial pathways in adult brains. J Biol Chem 280:16175–16184

Taylor AM, Saifetiarova J, Bhat MA (2017) Postnatal loss of neuronal and glial neurofascins differentially affects node of Ranvier maintenance and myelinated axon function. Front Cell Neurosci 11:11

Vallat J-M, Nizon M, Magee A, Isidor B, Magy L, Péréon Y, Richard L, Ouvrier R, Cogné B, Devaux J, Zuchner S, Mathis S (2016) Contactin-associated protein 1 (*CNTNAP1*) mutations induce characteristic lesions of the paranodal region. J Neuropathol Exp Neurol 75:1155–1159

Vallat J-M, Yuki N, Sekiguchi K, Kokubun N, Oka N, Mathis S, Magy L, Sherman DL, Brophy PJ, Devaux JJ (2017) Paranodal lesions in chronic inflammatory demyelinating polyneuropathy associated with anti-Neurofascin 155 antibodies. Neuromuscul Disord 27:290–293

von Reyn CR, Spaethling JM, Mesfin MN, Ma M, Neumar RW, Smith DH, Siman R, Meaney DF (2009) Calpain mediates proteolysis of the voltage-gated sodium channel -α-subunit. J Neurosci 29:10350–10356

Vosler PS, Brennan CS, Chen J (2008) Calpain-mediated signaling mechanisms in neuronal injury and neurodegeneration. Mol Neurobiol 38:78–100

Yao D, McGonigal R, Barrie JA, Cappell J, Cunningham ME, Meehan GR, Fewou SN, Edgar JM, Rowan E, Ohmi Y, Furukawa K, Furukawa K, Brophy PJ, Willison HJ (2014) Neuronal expression of GalNAc transferase is sufficient to prevent the age-related neurodegenerative phenotype of complex ganglioside-deficient mice. J Neurosci 34:880–891

Yermakov LM, Drouet DE, Griggs RB, Elased KM, Susuki K (2018) Type 2 diabetes leads to axon initial segment shortening in db/db mice. Front Cell Neurosci 12:146

Yoo S-W, Motari MG, Susuki K, Prendergast J, Mountney A, Hurtado A, Schnaar RL (2015) Sialylation regulates brain structure and function. FASEB J 29:3040–3053

Zhang C, Susuki K, Zollinger DR, Dupree JL, Rasband MN (2013) Membrane domain organization of myelinated axons requires βII spectrin. J Cell Biol 203:437–443

Physiology of Myelinated Nerve Conduction and Pathophysiology of Demyelination

7

Hessel Franssen

Abstract

Nerve conduction in myelinated axons is a fascinating subject due to the intricate structure and complex properties of the axon and its relation to the equally complex Schwann cells surrounding it. This chapter first deals with normal functional aspects of voltage-gated ion channels in the axon and Schwann cell membranes as well as their related proteins. Next, the pathophysiological alterations that are induced by experimental studies to mimic and study neuropathic disorders in humans are discussed. Finally, a link is made with human neuropathies associated with antibodies against gangliosides, and the putative mechanisms of axonal degeneration in demyelinating neuropathies are discussed. Although this chapter is relevant to understand symptoms in human neuropathies, the reader is referred to Franssen and Straver (Muscle Nerve 49:4–20, 2014) for a review of translational and clinical studies in human patients.

Keywords

Axon · Compound action potential · Conduction · Demyelination · Experimental allergic neuritis · Ion channel · Myelin · Nerve · Neuropathy · Schwann cell

Abbreviations

AMAN	Acute motor axonal neuropathy
AMP	Adenine monophosphate
ATP	Adenine triphosphate
cAMP	Cyclic adenine triphosphate

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CAP	Compound action potential
Caspr	Contactin-associated glycoprotein
CMAP	Compound muscle action potential
Cx29	Connexin-29
EAN	Experimental allergic neuritis
ECF	Extracellular fluid
ERM	Ezrin-radixin-moesin
GD1a	Ganglioside GD1a
GM1	Ganglioside GM1
GT1b	Ganglioside GT1b
HCN	Hyperpolarization-activated cyclic-nucleotide-gated
HSPG	Heparin-sulfate proteoglycan
Kv	Voltage-gated potassium channel nomenclature
L-type	Long-duration and large current generated by calcium channels
MAC	Membrane attack complex
MAG	Myelin-associated glycoprotein
MMN	Multifocal motor neuropathy
Nav	Voltage-gated sodium channel nomenclature
NF	Neurofascin
NO	Nitric oxide
Nr-CAM	Neuronal cell adhesion molecule
P0	Protein zero
P2	Protein two
P2X	Purinergic receptor nomenclature
PMP22	Peripheral myelin protein twenty-two
T-type	Transient and tiny current generated by calcium channels

7.1 Micro-anatomy of Myelinated Nerve Fibers

A myelinated nerve fiber consists of a succession of node–internode configurations. At internodes, the axon is surrounded by a myelinating Schwann cell with a narrow space being left between the internodal axolemma and the adaxonal Schwann cell membrane. Although the nodes are devoid of myelin they are covered by Schwann cell microvilli and a nodal gap matrix substance consisting of proteoglycans and mucopolysaccharides, which form a moderate resistance between the nodal membrane and the extracellular fluid (ECF). There is a close mutual molecular signaling relationship between Schwann cell and axon, which is important for development, maintenance, and restoration after demyelination (Martini 2001; Clark et al. 2017).

The axon is not a uniform tube because the node and the different sections of the internode have specific geometrical properties (Fig. 7.1). The node has the smallest diameter with its surface area occupying only 0.1% of the total surface of node and internode. An internode from one node to the next consists of paranode, juxtaparanode, stereotype internode and, again, a juxtaparanode and a paranode.

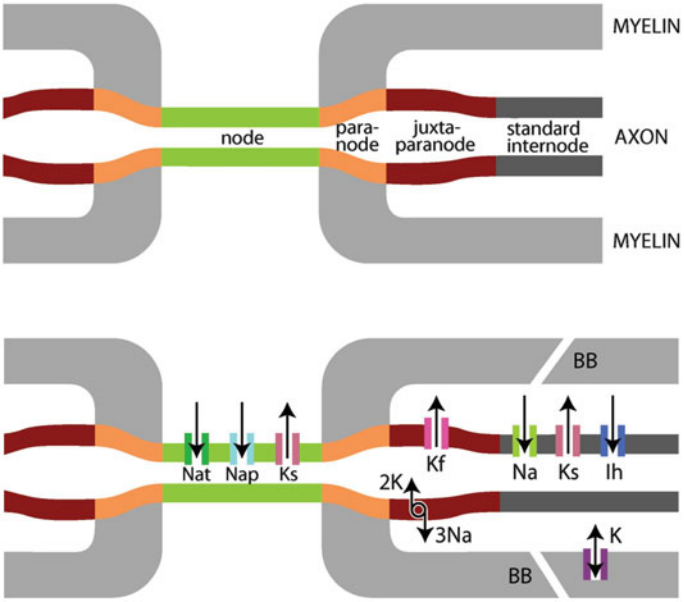

Fig. 7.1 Schematic diagrams of a myelinated axon. Upper: Subdivision of the axon into sections with different diameter. Lower: Distribution of axolemmal voltage-gated ion channels; the arrows indicate the current through each channel type under normal physiological conditions. Nat, transient Na^+ current; Nap, persistent Na^+ current; Ks, slow K^+ current; Kf, fast K^+ current; Ih, hyperpolarization-activated cation current; 2K/3Na, Na^+/K^+ pump; BB, the low Barrett and Barrett resistance through the myelin sheath possibly formed by Kv1/Cx29 complexes (Reproduced with permission from Franssen and Straver (2013) Pathophysiology of immune-mediated demyelinating neuropathies—part I: neuroscience. Muscle Nerve 48:851–864, © 2013 Wiley Periodicals, Inc.)

The juxtaparanode has the widest diameter and its surface is fluted so that its surface area is large (Berthold and Rydmark 1995). The diameter of mammalian myelinated axons ranges from 1 to 20 μm with the axon forming about 90% of the total cell volume of axon and soma. The distance between two adjacent nodes is up to 1–2 mm for the thickest fibers. Thicker nerve fibers have more myelin lamellae, wider axon diameter, longer internodal distance, and faster internodal impulse conduction until a maximum is reached after which conduction remains constant despite longer internodes (Wu et al. 2012).

The myelin sheath between two adjacent nodes is formed by a Schwann cell that, during development, has rotated 10–100 times around the axon. If a myelinating Schwann cell could be unrolled, it would form a flat trapezoid cell with an apical edge (attached to the axon) of 1–2 mm and a somewhat longer basal edge (Scherer and Arroyo 2002). Perpendicularly to the axon run broad strands of compact myelin, separated by small strands of non-compact myelin. In compact myelin, the Schwann cell cytoplasm has been squeezed out during development so that, there, the Schwann cell membranes are opposed. In non-compact myelin, the Schwann cell membranes are separated by a thin layer of cytoplasm. When rolled back around the

axon, the lateral edges of the more basal (outer) parts of the Schwann cell are longer than the internode and bend over the paranodal axolemma as paranodal loops and over the node as microvilli. The three-dimensional picture of the nodal gap is a circular groove with the nodal axolemmal membrane at the bottom, rather high walls formed by paranodal myelin loops of the two adjacent Schwann cells and a narrowed top, largely covered by up to 1000 Schwann cell microvilli (reviewed by Wilson and Chiu 1990). Because the nodal gap substance, which fills the pit, has strong cation binding properties, it was suggested to store Na^+ ions for release into the axon during an impulse and to take-up K^+ ions released from the nodal axolemma after repetitive impulse firing.

The paranodal loops are fixed to the axon by Schwann cell neurofascin 155 (NF155), which forms complexes with contactin and contactin-associated protein-1 (Caspr1) of the axolemma (Salzer et al. 2008). Schwann cell microvilli express ezrin-radixin-moesin molecules (ERMs), gliomedin, laminin, heparin sulfate proteoglycans (HSPGs), and collagen-V, which form complexes with axolemmal neuronal cell adhesion molecules (Nr-CAMs) and neurofascin 186 (NF186). These complexes, as well as the axolemmal ion channel clusters, are anchored by ankyrins to spectrin of the axonal cytoskeleton. The paranodal complexes form septate-like junctions that allow only restricted current flow between the ECF at the node and the narrow peri-axonal space between myelin sheath and internode; furthermore, they separate the nodal sodium (Na^+) channels from the juxtaparanodal potassium (K^+) channels.

The anatomical distribution of voltage-gated ion channels over the axolemma is also non-uniform (Fig. 7.1). The node expresses Nav1.6 channels in high density (1000–2000 μm^{-2}), Kv7.2 channels and T-type Ca^{2+} channels (Ritchie 1995; IUPHAR 2005; Zhang and David 2016). The juxtaparanode expresses Kv1.1 and Kv1.2 channels in such high numbers (up to 0.9 million for the two juxtaparanodes of one internode) that there is probably no space left for other ion channels (IUPHAR 2005; Rash et al. 2016). In mammalian axons, but not in amphibian axons, the Kv1.1 and Kv1.2 channels in the juxtaparanodal axolemma are aligned with and coupled to connexin-29 (Cx29) channels in the overlying Schwann cell membrane, thus forming an ionic current pathway between axonal and Schwann cell cytoplasm (Rash et al. 2016). The stereotype internode expresses K^+ channels and Na^+ channels in lower densities as well as Cl^- channels, L-type Ca^{2+} channels, and hyperpolarization-activated cyclic-nucleotide-gated cation (HCN) channels. The internodal Na^+ channel density (<25 μm^{-2}) is insufficient for impulse propagation. The location of Na^+/K^+ pumps was suggested to be nodal in an early study but internodal in subsequent advanced electrophysiological and staining experiments (reviewed by Franssen and Straver 2013).

7.2 Resting Membrane Potential

The resting membrane potential is the sum of all ionic equilibrium potentials across the axolemma plus the membrane potential difference generated by the electrogenic Na^+/K^+ pumps. The most important equilibrium potentials are those for potassium (E_K) and sodium (E_{Na}). E_K arises because part of the K^+ channels are still open at rest and the intra-axonal K^+ concentration $[K]_i$ is higher than the extracellular K^+ concentration $[K]_o$. The resulting chemical driving force moves K^+ ions outward thereby creating a thin, positively-charged layer on the outside of the axolemma. This layer, however, also produces an opposite electrostatic force that repels further outward movement of the positive K^+ ions. E_K is the membrane potential at which these opposite forces have equal absolute value and the net K^+ current is zero; its value is slightly more negative (−84 mV) than resting membrane potential (−75 mV). E_{Na} is positive (58 mV) because $[Na]_o$ exceeds $[Na]_i$ and because the positive extracellular Na^+ ions are attracted electrostatically by the negative resting membrane potential; thus both forces are directed inwards. Na^+/K^+ pumps contribute to resting membrane potential because, per pump cycle, three Na^+ ions are expelled from the axon and only two K^+ ions are moved into the axon, thereby generating a net outward current. The contribution of Cl^- channels is minimal as E_{Cl} approximates resting membrane potential.

7.3 Physiology of Axonal Ion Channels

Like in physics, current in electrophysiology is defined as a net flow of positive charges. This is literally true for currents through Na^+, K^+, and HCN channels because these are indeed carried by positively charged ions. Chloride channels, however, pass negatively charged Cl^- ions so that an inward flow of Cl^- ions is defined as an outward current. Within Na^+, K^+, and Cl^- channels, the current is mainly carried by the relevant ions although specificity does not reach 100%. HCN channels carry a mixture of Na^+ and K^+ ions (Nygren and Halter 1999). The intra- and extra-axonal longitudinal currents are carried by a mixture of positive ions, negative ions, and electrons. When an axolemmal ion channel opens, the induced current forms a loop consisting of the *ionic current* through the channel, an oppositely directed *capacitative current* to and from a more distant part of the axon membrane, and longitudinal currents through the inside and along the outside of the axon (Fig. 7.2). The direction of the capacitative current determines the direction of the induced membrane potential change.

The Nav1.6 channel consists of four subunits forming a pore with a *m-gate* halfway and a *h-gate* at its axoplasmic end. At resting membrane potential, the channel is the *closed state*, characterized by m-gate closure and h-gate opening. Depolarization of the membrane in which the channel is embedded induces a small outward shift of a positively charged residue within the Na^+ channel protein because the positive residue is attracted by the more negative extracellular and repelled by the more positive intracellular potential. As the residue is connected with the m-gate, the

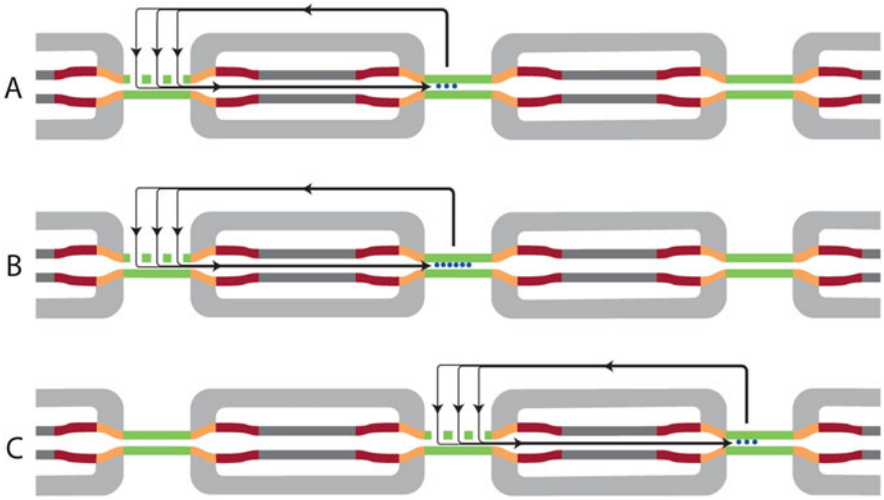

Fig. 7.2 Saltatory impulse propagation in a myelinated axon. (**a**) At an active node (left) open Na^+ channels sustain an inward ionic (action) current which induces an outward capacitative (driving) current that starts to charge the middle node. (**b**) The driving current at the middle node has led to the accumulation of positive charges at its inside and loss of positive charges at its outside, inducing nodal depolarization. (**c**) The depolarization at the middle node was sufficient to induce Na^+ channel opening so that the outward driving current has changed into an inward action current, the latter inducing an outward driving current at the right node. (Reproduced with permission from Franssen and Straver (2013) Pathophysiology of immune-mediated demyelinating neuropathies—part I: neuroscience. Muscle Nerve 48:851–864, © 2013 Wiley Periodicals, Inc.)

outward residue shift opens the m-gate and the channel is in a short-lasting *open state*. This is because m-gate opening has activated another, spring-like, residue, which closes the h-gate over the next 1–2 ms. The channel is now in its *inactivated state*. When membrane potential returns to its resting value, the m-gate closes and the h-gate opens.

Na^+ channel opening induces an inward ionic current of Na^+ ions and an outward capacitative current at an adjacent part of the axolemma or, more distant, at the node that has to be activated (Fig. 7.2). The outward capacitative current generates an accumulation of positive charges on the inside and loss of positive charges on the outside of the axolemma and leads, therefore, to a more positive resting membrane potential. Virtually no ionic current passes through the axon membrane since its bilipid structure is hydrophobic. Macroscopic Na^+ currents are divided into *transient currents*, which sustain action potentials and *persistent Na^+ currents*, which contribute to setting action potential threshold. Each of these currents is sustained by the Nav1.6 channel subtype (Bostock and Rothwell 1997; Caldwell et al. 2000). In motor axons, 99% of the total Na^+ current is transient and 1% persistent; in sensory axons, 98% is transient and 2% persistent. The persistent Na^+ current decreases impulse threshold, which is usually defined as the stimulus-current eliciting an impulse in 50% of trials. Because the small persistent Na^+ current is active at rest,

it slightly depolarizes resting membrane potential so that the voltage-step to reach the threshold for an impulse is slightly smaller. Due to the persistent Na^+ leak, however, more current will be needed to achieve a voltage step over the axolemma.

K^+ channel opening results in a net outward flow of K^+ ions because the outward chemical driving force (high $[K]_i$, low $[K]_o$) exceeds the inward electrical driving force (the positive K^+ ions are electrostatically attracted inwards by the negative resting membrane potential). The outward ionic K^+ current generates an inward capacitative current resulting in accumulation of positive charges on the outside of the axolemma and loss of positive charges on its inside and, therefore, a more negative resting membrane potential. In myelinated mammalian axons, three macroscopic K^+ currents can be recorded, including two fast types (I_{Kf1} and I_{Kf2}) and one slow type (I_{Ks}), each being activated by depolarization. In classic membrane physiology, fast K^+ channels are also known as *delayed outward rectifiers* because, contrary to the immediate activation of Na^+ channels, they are activated by depolarization after a delay of 1–2 ms and pass an outward current. Patch clamping of human axon membranes showed that K^+ currents are generated by at least five different K^+ channel types with overlapping properties for single-channel conductance, membrane potential for activation, deactivation time, and inactivation time (Reid et al. 1999). Inactivation of these channels is either slow, ranging from milliseconds to seconds, or absent. In mammalian axons, I_{Kf1} and I_{Kf2} prevent spreading of excitation beyond the node after a single impulse whereas in amphibian axons they are essential for repolarization after an impulse. I_{Ks} activates over tens of milliseconds after an impulse; during short-lasting repetitive firing, the summation of its effects repolarizes membrane potential so that extreme depolarization is prevented. Patch clamping of amphibian axolemma revealed K_{Ca} channels, activated by depolarization and by increased axoplasmic Ca^{2+}, as well as K_{ATP} channels, activated by axoplasmic ATP depletion; these channels might modify axonal excitability under metabolic demands (Jonas et al. 1991). The different types of nodal and internodal K^+ channels together are of major importance to the setting of resting membrane potential.

HCN channel opening gives rise to an inward ionic current of cations (mainly K^+ ions and Na^+ ions) and an outward capacitative current that shifts resting membrane potential to a more positive value. HCN channels are slowly activated over hundreds of milliseconds by strong hyperpolarization. Their function is to prevent extreme hyperpolarization of resting membrane potential which may arise from electrogenic Na^+/K^+ pump activation following sustained firing (see section on rate-dependent block).

7.4 Schwann Cell Ion Channels

Schwann cells express several types of ion channels on their membrane, nucleus, and cytoplasm. Some of these ion channels are only expressed in pure Schwann cell cultures during development, and during remyelination. This section deals mainly

with ion channels in mature Schwann cells that were shown to be electrically active in the presence of axons.

Schwann cells of adult rat sciatic nerve express different types of K^+ channels in spatially distinct distributions. Perinuclear Schwann cell cytoplasm expresses Kv1.1, the basal Schwann cell surface expresses Kv1.5 in discrete bands, and Schwann cell microvilli Kv1.5 and Kir2.1 (IUPHAR 2005; Mi et al. 1995; Baker 2002). Patch clamping of Schwann cell membranes from paranodal regions showed active delayed rectifying K^+ channels (Kv1.5), which start opening when membrane potential is depolarized above −55 to −75 mV. These channels, which normally pass an outward current, may be activated when repetitive firing has led to a local increase in $[K]_o$ and a decrease in E_K beyond the K^+ reversal potential. The normally outward current through these channels will then reverse to inward so that the excess extracellular K^+ can be taken up by the Schwann cell (Wilson and Chiu 1990). Kir2 inward rectifiers are open at resting membrane potential so that these channels probably form the first line of defense against an increase in $[K]_o$. They pass a voltage-independent K^+ current into the Schwann cell and their expression is controlled by second messengers such as cyclic adenine monophosphate (cAMP) (Baker 2002). Finally, Na^+/K^+ pumps in axonal and Schwann cell membranes will slowly return extracellular K^+ ions to intracellular spaces. Possibly, extracellular K^+ ions that have been taken up into perinodal Schwann cell microvilli by delayed and inward rectifiers are subsequently released into the extracellular space by the delayed and outward rectifying Kv1.5 channels located at the outer Schwann cell membrane (Baker 2002).

Other Schwann cell ion channels include Na^+ channels, Ca^{2+} channels, and ligand-gated purinergic (P2X) channels. The extracellular β-subunit of Na^+ channels in the Schwann cell membrane may contribute to adhesion between Schwann cell and axolemma and adhesion between myelin lamellae (Baker 2002). Interestingly, Schwann cells transfer ribosomes for assembly of Na^+ channel subunits to the underlying axon, suggesting that part of the channel proteins may be manufactured outside neuronal cell bodies (Court et al. 2008). The soma of cultured mouse Schwann cells expresses Ca^{2+} channels in the presence of cAMP. The recorded Ca^{2+} currents are of the T-type with a tiny conductance and generating a transient inactivating current, or of the L-type that is characterized by a large single-channel conductance and long duration current; the T-type was shown to be dependent on the presence of neurons or neuronal activity (reviewed by Baker 2002). P2X channels are expressed in mouse and rat Schwann cell somata and perinodal loop membranes. They are activated by high extracellular levels of ATP and generate an specific inward cation current which shifts membrane potential towards depolarization (Grafe et al. 1999).

Early computational models of conduction in myelinated axons incorporated the high electrical resistance and low capacitance that were measured in compact myelin by Tasaki (1955). The extrapolation of these features to the entire myelin sheath implied an inability of myelin to pass current or store charges, an inactive internodal axolemma and the setting of the entire nodal and internodal resting membrane potentials by the tiny node; the latter would also require a considerable fast K^+

efflux from the node in order to compensate for the Na^+ influx during an impulse. Subsequently, however, the nodal density of fast K^+ channels was shown to be very low and voltage-clamp studies on isolated nodes recorded an outward current with a reversal potential of zero, which is remote from E_K (Ritchie 1995). Investigations into the mechanisms of afterpotentials (the slow and small potential changes that normally follow an action potential) suggested that current passed through low resistance cytoplasmic pathways in non-compact myelin (Barrett and Barrett 1982). Revised mathematical models, therefore, included a low resistance-low capacitance myelin sheath and an internodal axolemma with active properties (setting of resting membrane potential by the great number of internodal K^+ channels) and passive properties (large capacitance enabling it to slowly store electric charges and generate afterpotentials) (Barrett and Barrett 1982; Blight 1985). Subsequent studies provided support for these models. Intracellular dye injection and video microscopy revealed that small molecules can indeed pass between adaxonal and perinuclear Schwann cell cytoplasm (Balice-Gordon et al. 1998). The substrates of these pathways are likely formed by the recently discovered Kv1/Cx29 channel complexes, which connect the axoplasm with Schwann cell cytoplasm (Rash et al. 2016). These channel complexes may sustain rapid repolarization after an impulse and may circumvent accumulation of K^+ ions in the narrow space between internodal myelin and axon after repetitive firing. Furthermore, recordings with ultrasharp microelectrodes revealed depolarizations in deeper myelin layers coinciding with axonal action potentials but with a longer duration (David et al. 1993). As these myelin depolarizations disappeared after administration of K^+ channel blockers, they may be sustained by Kv1/Cx29 channels.

7.5 Action Potential Propagation

For the description of action potential propagation in pathophysiology, the terms action current and driving current are convenient (Fig. 7.2). *Action current* is the inward ionic current at the entire node through the opened Na^+ channels during an action potential. As with all ionic currents, the action current induces a capacitative outward *driving current* which depolarizes the node that has to be activated next. At that node, the depolarization opens transient Na^+ channels so that the outward driving current changes into an inward ionic current. The Na^+ ions flowing into the axon give rise to further depolarization, which induces more Na^+ channel openings; this regenerative process is known as *action potential* or *impulse*. In mammalian and human myelinated fibers, the action potential is mainly terminated by the above described Na^+ channel inactivation, whereas in amphibian myelinated and unmyelinated fibers, it ends by fast K^+ channel-opening induced by the depolarization of the nodal and juxtaparanodal membrane. This fast K^+ channel opening leads to K^+ efflux and repolarization.

The inward Na^+ current of the impulse returns outward via three pathways, namely (1) as capacitative driving current to and from the membrane of the next node that has to be activated, (2) as ionic current through the low-resistance

pathways of the myelin sheath, and (3) as capacitative current to and from the internodal axon membrane, thereby inducing a slight depolarization due to accumulation of positive charges on the large internodal axon membrane. When the inward Na^+ current stops due to Na^+ channel inactivation, a part of these positive charges flows back to the inside of the nodal membrane, giving rise to the *depolarizing afterpotential*. This slow nodal depolarization subsequently activates nodal slow nodal K^+ channels thereby inducing the *hyperpolarizing afterpotential*.

High-frequency action potentials likely activate nodal T-type Ca^{2+} channels as action potentials evoked by 500 electrical stimuli at 50 Hz in mouse ventral roots were shown to induce a temporary nodal axoplasmic Ca^{2+} increase on calcium imaging (Zhang and David 2016).

7.6 Recording Methods

In whole roots or nerves, impulse propagation can be studied by delivering a brief electrical shock to a mixed nerve and recording a compound potential (CAP) arising from summated impulses in motor and sensory axons at distant sites. With this method, conduction block is revealed by a lower CAP on more distant than on nearby stimulation and slowing by a decreased maximal conduction velocity, calculated by dividing conduction distance by latency. A decreased CAP after stimulation at the distant and the nearby site, either indicates loss of axons or conduction block between the nearby stimulation site and the recording site. Pure motor conduction can be studied by recording compound muscle action potentials (CMAP) from a muscle innervated by the nerve and pure sensory conduction by recording a CAP from a distal sensory branch of the nerve.

Single myelinated fibers can be studied by using intra- or extracellular glass pipettes for recording and stimulation in ex-vivo preparations. *Current clamping* involves the passing of constant current through the axon membrane and recording the induced change in membrane potential. In *voltage clamping*, membrane potential is held (by an electronic feedback circuit) at values set by the investigator so that transmembrane currents (e.g., through the node) can be recorded at each of the induced membrane potentials. In this setup, the current through a group of specific ion channels can be assessed by selective pharmacological blockade of the other ion channel types. In *external longitudinal current recording* a pair of electrodes, 80 μm apart, is placed alongside a single myelinated axon at the outside of an intact nerve (Bostock and Sears 1978). These electrodes record the current flowing between them during the passage of an impulse elicited at a distant site. A micromanipulator allows recording from different adjacent sites along the fiber. Any difference in current between two adjacent recording sites reflects current entering or leaving the axon. Comparing external longitudinal current at a node with that just before the node first reveals a strong outward current (reflecting the capacitative driving current) that is followed by a strong inward current (reflecting inward ionic current through open Na^+ channels). The current characteristics allow assessment of internodal distance and internodal conduction time, both in normal and in demyelinated axons. These

macroscopic recording techniques have successfully characterized events occurring in experimental demyelination of amphibian and lower mammalian axons. Due to their fragility, however, recording from higher mammalian and human myelinated nerve fibers is technically extremely challenging and, so far, only one researcher was able to investigate human myelinated fibers (Schwarz et al. 1995). *Patch clamping* employs extremely fine micropipettes with a tip diameter of about 1 μm for current or voltage clamping on an intact cell body, or an excised membrane patch which contains a single to several ion channels. By this method, normal and genetically mutated human ion channels have been characterized electrophysiologically.

7.7 Experimental Demyelination

Experimental generalized demyelination can be induced by chemical substances such as lysolecithin, immunization with diphtheria toxin, injection with specific myelin peptides, or injection with peripheral nerve myelin homogenate. The latter two methods result in experimental allergic neuritis (EAN), a disorder characterized by a T-cell-mediated response to the myelin proteins P2, P0, or PMP22 with activated macrophages invading the myelin sheath and inducing demyelination. EAN shares important features with the human disorder acute inflammatory demyelinating polyneuropathy, a subtype of the Guillain-Barré syndrome.

The physiological consequences of demyelination of a single axon are slowing of internodal conduction time, persistent conduction block, warm block, cold block, increased refractory period, and rate-dependent block. These phenomena can be explained by a persistent or temporary decrease in the *safety factor*, defined as the ratio: (available driving current)/(required driving current). In normal axons it has a value of 5–7, but in demyelinated axons it can be reduced due to a decrease in available driving current, an increase in required driving current, or both (reviewed by Franssen and Straver 2013). If the safety factor is reduced, but above one, conduction is possible, albeit with slowed internodal conduction time. If it falls below one, internodal conduction is blocked. At critically demyelinated internodes with a safety factor just above one, conduction slowing may change into conduction block (defined as the failure of impulse propagation at a given site of a viable axon) if unfavorable physiological circumstances, such as temperature change or sustained impulse firing, cause an additional reduction in safety factor to less than one. This mechanism underlies rate-dependent block, heat block, and cold block.

Segmental demyelination leads to loss of driving current because part of the intra-axonal current toward nodes that have to be depolarized by the driving current flows outward across the damaged myelin sheath, which has a reduced thickness (Fig. 7.3). Comparison of whole nerve conduction and histopathology in an EAN model with predictable time course revealed that progressive loss of myelin layers was associated with progressive conduction slowing until conduction became blocked (Saida et al. 1980; Sumner et al. 1982). In the acute phase of complete segmental demyelination, conduction is usually blocked because the low internodal Na^+ channel density cannot sustain continuous conduction over the denuded axolemma.

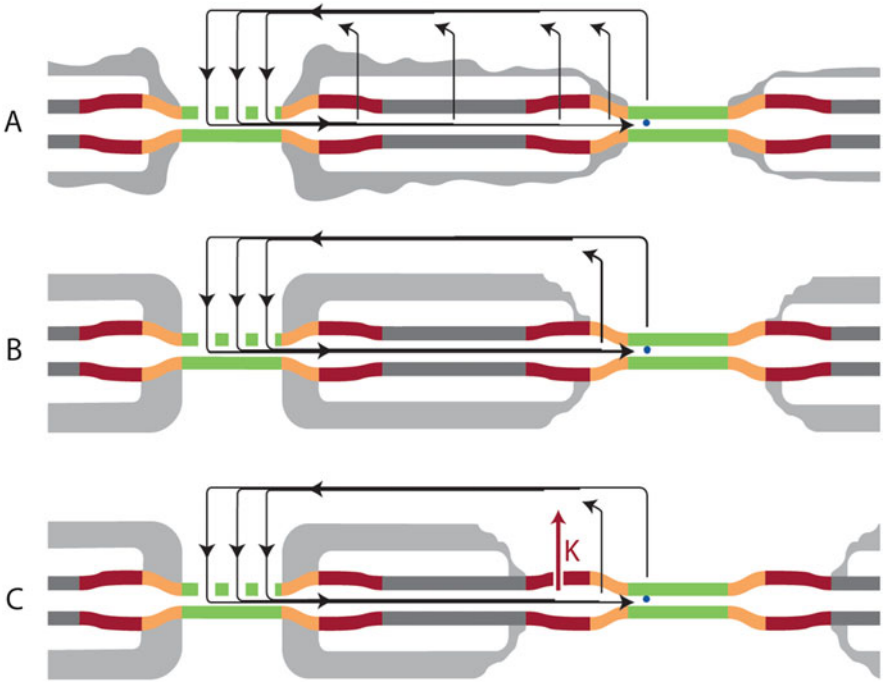

Fig. 7.3 Different types of demyelination leading to a reduced safety factor. (**a**) Segmental demyelination with leakage of driving current through the damaged myelin and loss of driving current at the right node. (**b**) Paranodal demyelination with spreading of driving current over an area consisting of the node and the denuded paranode; the large capacitance of this area impairs its depolarization. (**c**) Juxtaparanodal demyelination with spreading of driving current over the nodal, paranodal, and juxtaparanodal areas of which the capacitance is even larger than that in (**b**); impulse generation is further impaired because the depolarizing driving current also activates exposed juxtaparanodal fast K^+ channels. (Reproduced with permission from Franssen and Straver (2013) Pathophysiology of immune-mediated demyelinating neuropathies—part I: neuroscience. Muscle Nerve 48:851–864, © 2013 Wiley Periodicals, Inc.)

Computer simulations of segmental demyelination showed that conduction is still possible if myelin thickness is 2.7% of normal at one internode or 4% of normal at two adjacent internodes (Koles and Rasminsky 1972).

Paranodal demyelination results in the above described driving current leakage as well as spreading of the remaining driving current over an area comprising the former node and the denuded paranodal axolemma (Fig. 7.3). Because this area is larger and has a greater electrical capacity than a normal node, it takes more time and more electric charging to depolarize it to impulse threshold and internodal conduction time may increase from a normal value of 20–600 μs, or internodal conduction may become blocked; this is known as impedance mismatch (Rasminsky and Sears 1972; Waxman et al. 1995).

Juxtaparanodal demyelination may lead to exposure, or dispersion to the node, of juxtaparanodal fast K^+ channels (Schwarz et al. 1991) (Fig. 7.3). Activation of these

channels by the depolarizing driving current will then shorten action potential duration and induce block. Consistent with this, pharmacological inhibition of these channels by application of 4-aminopyridine on single demyelinated mammalian motor axons was shown to resolve conduction block or increase impulse duration in intra- or extra-axonal recordings (Bostock et al. 1981).

Several studies showed that disruption of nodal and juxtaparanodal ion channel clusters contributes to the conduction deficit and clinical signs. Slight demyelination in EAN was associated with decreased peak Na^+ currents and increased membrane capacitance, suggesting dispersion of nodal Na^+ channels to the paranode due to paranodal myelin detachment (Schwarz et al. 1991). A study of spinal roots in EAN showed loss of nodal Na^+ channel staining or diffuse Na^+ channel immunostaining from the node to the paranode; at that stage whole nerve recording showed conduction block, heat block, and slowing (Novakovic et al. 1998). Thereafter Na^+ channel staining was lost, indicating that Na^+ channel density was less than 50 channels/μm^2; this was associated with severe clinical signs and paranodal or total segmental demyelination. Mathematical models could explain the electrophysiological findings by diffusion of nodal Na^+ channel clusters and loss of paranodal seals (Novakovic et al. 1998). In EAN, induced by immunization against peripheral myelin, the density of nodal adhesion molecules gliomedin and neurofascin became diminished and autoantibodies to these molecules were found in the earliest stage (Lonigro and Devaux 2009). This was followed by paranodal retraction resulting in nodal widening, demyelination, disruption of nodal Na1.6 and Kv7.2 channel clusters, and mislocalization of juxtaparanodal Kv1.2 channels to the paranode and node. Nodal complement depositions were not detected. Clinical signs started when the adhesion molecules disappeared and their severity correlated with the number of disrupted nodes. CAP recordings showed slowing, conduction block, and increased refractory period. Signs of conduction block diminished after application of the Kv1 channel blocker 4-aminopyridine but not after a blocker of nodal Kv7.2 channels, suggesting that exposure of juxtaparanodal Kv1-channels contributed to the block. In rats, immunized with P2 peptide, passive transfer of anti-contactin-1 IgG4 induced selective loss of paranodal Caspr1/contactin-1/NF155 complexes and moderate nodal lengthening in motor, not sensory, fibers (Manso et al. 2016). Because electrophysiology showed decreased CAPs without slowing and pathology showed no axonal degeneration or demyelination, it is likely that the prominent clinical signs resulted from conduction block caused by the destruction of paranodal adhesion molecules.

7.8 Repetitive Impulse Firing

Repetitive firing of action potentials puts extra stress on demyelinated axons even if only two closely spaced impulses are evoked. External longitudinal current recording along single demyelinated axons showed that progressively decreasing the interval between two closely spaced electrical stimuli progressively increased internodal conduction time for the second stimulus until this stimulus could not

elicit an impulse anymore (Rasminsky and Sears 1972). This inter-stimulus interval, defined as the refractory period, ranged from 1.8 to 4.3 ms in demyelinated single axons whereas it was about 1.0 ms in normal axons. In normal myelinated axons, refractoriness is caused by temporary inactivation of transient nodal Na^+ channels after an impulse and, to a lesser extent, by fast K^+ channel activation. The prolonged refractory period in demyelinated axons reflects the longer time needed to depolarize a node due to the combined effects of increased electrical membrane capacity, leakage of driving current, and dispersion of nodal and juxtaparanodal ion channels.

Rate-dependent block was demonstrated when single demyelinated axons were stimulated for several minutes at frequencies of 80–200 Hz or 0–50 Hz. This gave rise to a gradually increasing internodal conduction time that was followed by intermittent internodal conduction and, finally, block (Rasminsky and Sears 1972; Bostock and Grafe 1985; Kaji and Sumner 1989). Because conduction failure occurred at inter-stimulus intervals that were longer than the refractory period, other mechanisms than refractoriness must have been involved. Essentially, each of these mechanisms may cause an extra reduction in safety factor which is already reduced by demyelination. Short trains of 10–20 impulses cause brief hyperpolarization due to the summation of the hyperpolarizing afterpotentials that follow each impulse (Baker et al. 1987). More prolonged repetitive firing increases $[K]_o$ which, in turn, leads to decreased E_K and depolarization; the latter first decreases threshold but later increases it due to Na^+ channel inactivation (Fig. 7.4) (Brismar 1991; Bostock et al. 1991). Sustained firing also increases intra-axonal Na^+ concentration which decreases the Na^+ concentration gradient; the resulting decreased Na^+ influx during an impulse induces a decrease in driving current (Rasminsky and Sears 1972). The most important mechanism for rate-dependent block, however, is hyperpolarization induced by increased activity of the electrogenic Na^+/K^+ pump, which is driven by the increased $[Na]_i$. Pump involvement is likely because blocking did neither arise after replacement of Na^+ ions by Li^+ ions in the medium nor after topical application of the pump blocker ouabain (Bostock and Grafe 1985; Kaji and Sumner 1989). To attain sufficient depolarization of hyperpolarized axons, a larger-than-normal potential step has to be made in order to reach threshold for impulse generation. This requires extra driving current and, when this is not available due to demyelination-induced leakage, rate-dependent block ensues (Fig. 7.4). A contributing factor is that most Na^+ channels are in the closed state (m-gate closed, h-gate opened) during hyperpolarization (Fig. 7.4).

7.9 Temperature-Dependent Block

Heat block was shown to arise in single demyelinated axons when the temperature was raised from 36.0 °C to 37.0 °C and to resolve after restoring temperature (Rasminsky 1973). Heat block may occur at critically demyelinated internodes with a safety factor of just above one. Raising temperature at such internodes further decreases the safety factor as it diminishes action potential amplitude. This is because the Q10 of the rate constant for Na^+ permeability activation is smaller

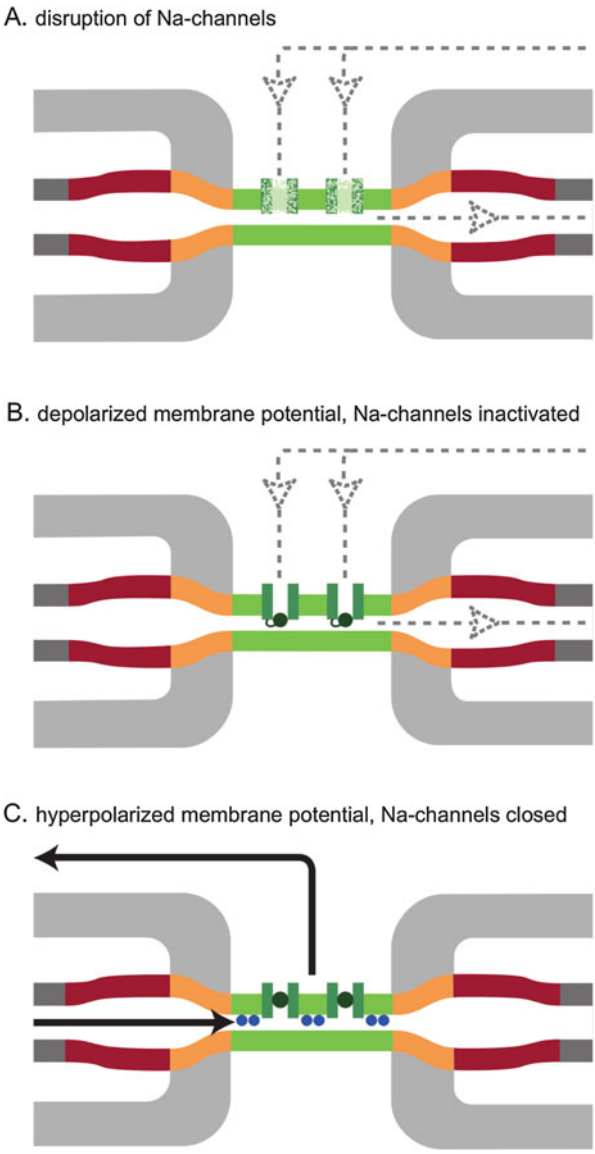

Fig. 7.4 Nodal causes of reduced safety factor. (**a**) Damage to Na^+ channels or clusters results in loss of action current. (**b**) Inactivation of Na^+ channels in a permanently depolarized axon impairs action current. (**c**) In a hyperpolarized axon, a larger-than-normal voltage-step is needed to reach the threshold for an impulse; this requires more driving current. (Reproduced with permission from Franssen and Straver (2013) Pathophysiology of immune-mediated demyelinating neuropathies—part I: neuroscience. Muscle Nerve 48:851–864, © 2013 Wiley Periodicals, Inc.)

than that for inactivation, leading to a shorter open-time of nodal Na^+ channels (Schwarz and Eikhof 1987). Furthermore, the increase with temperature of the rate constant α_n for fast K^+ current activation is higher than that of the rate constant α_m for transient Na^+ current activation, implying that warming induces an increase in fast K^+ current which is more prominent than the increase in Na^+ current (Frankenhaeuser and Moore 1963). When action current is opposed by exposure of juxtaparanodal fast K^+ channels due to demyelination, this relatively prominent

fast K^+ current increase may lead to further reduction in action current and conduction block.

Cold block occurs if normal axons are cooled to 5–8 °C and demyelinated axons to 16 °C, the latter because both cold and demyelination reduce safety factor (Low and McLeod 1997). Cold block results from slowing of Na^+ channel activation, yielding reduced action, and driving currents (Schwarz and Eikhof 1987). Thermal reduction of Na^+/K^+ pump activity probably contributes to cold block as it leads to depolarization of resting membrane potential and, consequently, Na^+ channel inactivation (Franssen et al. 2010). The common cause of these mechanisms is that chemical reactions, including those sustaining changes in protein conformation, slow down with decreasing temperature.

7.10 Restoration of Conduction After Demyelination

External longitudinal current recordings in total demyelination, induced by diphtheria toxin, showed very slow (1–2 ms^{-1}) continuous conduction 4–6 days after onset, suggesting increased diffuse internodal Na^+ channel expression (Bostock and Sears 1978). This, and another study of total chemical demyelination, also revealed internodal foci of inward current, known as phi-nodes (Bostock and Sears 1978; Smith et al. 1982). As the distance between phi-nodes equals the shortened internodal distance after remyelination, phi-nodes may represent small Na^+ channel clusters out of which new nodes may develop. During remyelination, Na^+ channel clusters appear on both edges of remyelinating Schwann cells (Novakovic et al. 1998). At a later stage, characterized by clinical recovery, the clusters of neighboring Schwann cells fuse and form a new node; the distance between these new nodes is then shorter than normal.

Correlation between findings on pathology and whole nerve conduction showed that restoration of previously absent CMAPs was associated with the appearance of small numbers of axons encircled by 2–8 turns of myelin lamellae, representing 8–20% of the normal number of turns (Saida et al. 1980). Remarkably, maximal conduction velocity had returned to normal when myelin thickness was still only one-third of normal, indicating that even moderate demyelination may go undetected by conduction velocity measurements.

7.11 Antibodies Against Gangliosides

Gangliosides are glycolipids consisting of an extracellular sugar portion to which sialic acid residues may be attached and a lipid ceramide portion located in the bilipid membrane. High-resolution light microscopy and electron microscopy of teased fibers from rat roots and rat sciatic nerves have identified ganglioside GM1 on the nodal axolemma, paranodal axolemma, abaxonal Schwann cell membrane, and Schwann cell microvilli. The ceramide portion of ganglioside GM1 was found to differ slightly between motor and sensory fibers, a finding which might explain the

occurrence of pure motor neuropathies associated with anti-GM1 antibodies in man (Ogawa-Goto et al. 1990). Ganglioside GT1b was found on the nodal axolemma, internodal axolemma, and abaxonal Schwann cell membrane and, finally, ganglioside GD1a on nodes and abaxonal Schwann cell membrane (Gong et al. 2002). Some neuropathies in human are associated with serum antibodies against gangliosides. These include acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy, both variants of the Guillain-Barré syndrome, and multifocal motor neuropathy (MMN), a chronic neuropathy affecting motor fibers only. The ganglioside GM1-sugar portion Gal(β1–3)GalNAc was found to be present in human axolemma and Schwann cells but was not obligatory colocalized with Na^+ channel or K^+ channel proteins, suggesting that anti-ganglioside induced damage to ion channel clusters arises from disruption of the axon membrane in which these channels are embedded (Sheikh et al. 1999).

An important animal model of AMAN was developed by immunization against subcutaneously injected GM1-ganglioside in rabbits (Susuki et al. 2007). The acute phase showed complement-dependent damage to nodal Na^+ channel clusters, axonal cytoskeleton (βIV-spectrin), and adhesion molecules that separate the nodal from the juxtaparanodal ion channel clusters (moesin in Schwann cell microvilli and axolemmal Caspr in the paranode). The severity of damage was correlated with the extent of membrane attack complex (MAC) formation and paranodal complement deposits. During recovery, Na^+ channel clusters first appeared at each side of the former nodal area and fused later to form new nodes. Axonal degeneration was mild, suggesting that the clinical signs of weakness were caused by damage to nodal Na^+ channel clusters and the resulting conduction block (Fig. 7.4).

Injection of anti-GM1 serum from a patient with MMN in rat sciatic nerve induced conduction block and increased temporal dispersion on whole nerve motor NCS (Santoro et al. 1992). Pathology showed nodal immunoglobulin deposits but demyelination in only 6.5% of axons, suggesting that the block was not due to demyelination but to interference with impulse generation at the nodes of Ranvier. Injection of ganglioside antibodies into rat sciatic nerve also induced the above-described abnormalities, with mainly motor fibers being affected by injection of anti-GD1a or anti-GT1b ganglioside and mainly sensory fibers by injection of anti-GD1b ganglioside (Susuki et al. 2012). Serial motor conduction studies of the sciatic nerve showed a temporary segmental CMAP drop without features of slowing or temporal dispersion, consistent with nodal dysfunction without demyelination.

Application of high-titer anti-GM1 ganglioside sera without complement increased depolarization-induced fast outward K^+ currents on voltage-clamping of rat myelinated single axons (Takigawa et al. 1995). This suggested that juxtaparanodal demyelination exposed fast K^+ channels. Decreased Na^+ currents were only observed after addition of complement. External longitudinal current recording along rat ventral root fibers incubated with high-titer anti-GM1 sera without complement from patients with acute motor axonal neuropathy or multifocal motor neuropathy showed blocking in only 2% of fibers (Hirota et al. 1997). The current patterns in these blocked fibers revealed intensive passive outward currents, which did not resemble those induced by the Na^+ channel blocker tetrodotoxin,

suggesting that the block arose due to demyelination only. Incubation, for up to 6 h, of desheathed sciatic nerve with anti-GM1 (IgM or IgG) or anti-GQ1b (IgG) ganglioside plus complement resulted in nodal antibody depositions but not in conduction block on whole nerve recordings (Paparounas et al. 1999).

7.12 Axonal Degeneration in Demyelinating Neuropathies

In human demyelinating neuropathies, irreversible loss of axons is possibly the main cause of clinically observed deficits (reviewed by Franssen and Straver 2014). Demyelination or loss of normal Schwann cell function may lead to rapid or slow axonal degeneration by several mechanisms. The terms primary and secondary axonal degeneration should, therefore, either be carefully defined or avoided.

Pathology in AMAN showed nodal IgG and complement depositions in man and rabbits and membrane attack complex (MAC) depositions in rabbits (Susuki et al. 2007). MAC causes 5-nanometer-wide pores in the axolemma allowing a massive influx of ions and water. The resulting high intra-axonal Ca^{2+} concentration activates the calpain pathway, causing proteolytic neurofilament cleavage and damage to the intra-axonal parts of ion channels, including the inactivation gates of Na^+ channels (Von Reyn et al. 2009). The resulting failure to inactivate contributes to the persistent Na^+ influx in these disorders. In a model where desheathed terminal motor myelinated axons were incubated with anti-GD1a antibodies, loss of nodal Na^+ channel staining started 15–30 min after adding complement (McGonigal et al. 2010). External longitudinal current recording indicated loss of inward Na^+ currents and outward K^+ currents. The pathological changes were prevented by adding complement inhibitors or calpain inhibitors whereas electrophysiological changes were prevented only by complement inhibitors. This suggests that, although MAC formation results in loss of ionic concentration gradients and inability to generate impulses, MAC-formation alone will not induce axonal degeneration unless the calpain pathway is activated.

Nitric oxide (NO) production in experimental demyelinating nerve lesions may inhibit mitochondria and, therefore, Na^+/K^+ pump function (Kapoor et al. 2003). Exposure of rat dorsal roots to NO during 100 Hz electrical stimulation gave rise to irreversible CAP loss on whole nerve recording. CAP loss could, however, be prevented by adding Na^+ channel or Na^+/Ca^{2+} exchanger blockers to the medium before the start of electrical stimulation. These results suggest that axonal degeneration in this model may arise from a combination of Na^+ influx induced by repetitive firing, Na^+ influx through persistent Na^+ channels due to the depolarization induced Na^+/K^+ pump failure, reversal of the Na^+/Ca^{2+} exchanger driven by the high $[Na_i]$, intra-axonal Ca^{2+} accumulation, and calpain-mediated axonal degeneration.

Increased expression of Na^+ channels along denuded parts of formerly myelinated axons may result in continuous, albeit slowed, conduction (Saida et al. 1980; Bostock and Sears 1978). The high number of Na^+ channels along long stretches of denuded axons may, however, result in excessive Na^+ influx when impulses are propagated continuously. The resulting intra-axonal Na^+ accumulation may result in

calpain-mediated axonal degeneration. Animal studies and pathology studies of multiple sclerosis revealed that upregulation of the Nav1.6 subtype was associated with axonal degeneration whereas that of Nav1.2 was not (Kapoor et al. 2003; Waxman 2006). The explanation was that Nav1.6 produces a larger persistent Na^+ current than Nav1.2, giving rise to Ca^{2+}-mediated axonal degeneration by the above-described mechanism. Finally, depolarization by the driving current may activate L-type Ca^{2+} channels in denuded paranodal and juxtaparanodal axolemma and contribute to Ca^{2+} influx and axonal degeneration (Zhang and David 2016).

Neurofilament accumulation may impair axonal transport which, in turn, may lead to slow axonal degeneration (reviewed by Franssen and Straver 2014). Myelin-associated glycoprotein (MAG), a glycoprotein expressed in non-compact myelin, normally ensures adhesion and spacing of non-compact myelin lamellae and maintains axon diameter by promoting attachment of negatively charged phosphate groups to axonal neurofilament side-arms; the resulting repelling forces between these phosphate groups induce spacing so that axon diameter can be maintained. Loss of normal MAG function, as in MAG knockout mice and in human neuropathy with anti-MAG antibodies, induces loosening of myelin lamellae, loss of neurofilament spacing, and axonal stenosis.

References

Baker MD (2002) Electrophysiology of mammalian Schwann cells. Prog Biophys Mol Biol 78:83–103

Baker M, Bostock H, Grafe P, Martius P (1987) Function and distribution of three types of rectifying channel in rat spinal root myelinated axons. J Physiol 383:45–67

Balice-Gordon RJ, Bone LJ, Scherer SS (1998) Functional gap junctions in the Schwann cell myelin sheath. J Cell Biol 142:1095–1104

Barrett EF, Barrett JN (1982) Intracellular recording from vertebrate myelinated axons: mechanism of the depolarizing afterpotential. J Physiol 323:117–144

Berthold CH, Rydmark M (1995) Morphology of normal peripheral axons. In: Waxman SG, Kocsis JD, Stys PK (eds) The Axon. Oxford University Press, New York, pp 13–48

Blight AR (1985) Computer simulation of action potentials and afterpotentials in mammalian myelinated axons: the case for a lower resistance myelin sheath. Neurosci 15:13–31

Bostock H, Grafe P (1985) Activity-dependent excitability changes in normal and demyelinated rat spinal root axons. J Physiol 365:239–257

Bostock H, Rothwell JC (1997) Latent addition in motor and sensory fibres of human peripheral nerve. J Physiol 498(1):277–294

Bostock H, Sears TA (1978) The internodal axon membrane: electrical excitability and continuous conduction in segmental demyelination. J Physiol 280:273–301

Bostock H, Sears TA, Sherratt RM (1981) The effects of 4-aminopyridine and tetraethylammonium ions on normal and demyelinated mammalian nerve fibers. J Physiol 313:301–315

Bostock H, Baker M, Reid G (1991) Changes in excitability of human motor axons underlying post-ischaemic fasciculations: evidence for two stable states. J Physiol 441:537–557

Brismar T (1991) Electrical properties of isolated demyelinated rat nerve fibers. Acta Physiol Scand 113:161–166

Caldwell JH, Schaller KL, Lasher RS, Peles E, Levinson SR (2000) Sodium channel Nav1.6 is localized at nodes of Ranvier, dendrites, and synapses. PNAS 97:5616–5620

Clark AJ, Kaller MS, Galino J, Willison HJ, Rinaldi S, Bennett DLH (2017) Co-cultures with stem cell-derived human sensory neurons reveal regulators of peripheral myelination. Brain 140:898–913

Court FA, Hendriks WTJ, MacGillavry HD, Alvarez J, Van Minnen J (2008) Schwann cell to axon transfer of ribosomes: toward a novel understanding of the role of glia in the nervous system. J Neurosci 28:11024–11029

David G, Barrett JN, Barrett EF (1993) Activation of internodal potassium conductance in rat myelinated axons. J Physiol 472:177–202

Frankenhaeuser B, Moore LE (1963) The effect of temperature on the sodium and potassium permeability changes in myelinated nerve fibres of *Xenopus laevis*. J Physiol 169:431–437

Franssen H, Straver DCG (2013) Pathophysiology of immune-mediated demyelinating neuropathies – part I: neuroscience. Muscle Nerve 48:851–864

Franssen H, Straver DCG (2014) Pathophysiology of immune-mediated demyelinating neuropathies – part II: neurology. Muscle Nerve 49:4–20

Franssen H, Gebbink TA, Wokke JH, Van den Berg LH, Van Schelven LJ (2010) Is cold paresis related to axonal depolarization? J Periph Nerv Syst 15:227–237

Gong Y, Tagawa Y, Lunn MPT, Laroy W, Heffer-Lauc M, Li CY, Griffin JW, Schnaar RL, Sheikh KA (2002) Localization of major gangliosides in the PNS: implications for immune neuropathies. Brain 125:2491–2506

Grafe P, Mayer C, Takigawa T, Kamleiter M, Sanchez-Brandelik R (1999) Confocal calcium imaging reveals an inotropic P2 nucleotide receptor in the paranodal membrane of rat Schwann cells. J Physiol 515:377–383

Hirota N, Kaji R, Bostock H, Shindo K, Kawasaki T, Mizutani K, Oka N, Kohara N, Saida T, Kimura J (1997) The physiological effect of anti-GM1 antibodies on saltatory conduction and transmembrane currents in single motor axons. Brain 120:2159–2169

IUPHAR (2005) Compendium of voltage-gated ion channels. Pharmacol Rev 57:385–540

Jonas P, Koh DS, Kampe K, Hermsteiner M, Vogel W (1991) ATP-sensitive and Ca-activated K channels in vertebrate axons: novel links between metabolism and excitability. Pflügers Arch 418:68–73

Kaji R, Sumner AJ (1989) Ouabain reverses conduction disturbances in single demyelinated nerve fibers. Neurology 39:1364–1368

Kapoor R, Davies M, Blaker PA, Hall SM, Smith KJ (2003) Blockers of sodium and calcium entry protect axons from nitric oxide-mediated degeneration. Ann Neurol 53:174–180

Koles ZJ, Rasminsky M (1972) A computer simulation of conduction in demyelinated nerve fibres. J Physiol 227:351–364

Lonigro A, Devaux JJ (2009) Disruption of neurofascin and gliomedin at nodes of Ranvier precedes demyelination in experimental allergic neuritis. Brain 132:260–273

Low PA, McLeod JG (1997) Refractory period, conduction of trains of impulses, and effect of temperature on conduction in chronic hypertrophic neuropathy. J Neurol Neurosurg Psychiatry 40:434–447

Manso C, Querol L, Mekaouche M, Illa I, Devaux JJ (2016) Contactin-1 IgG4 antibodies cause paranode dismantling and conduction defects. Brain 139:1700–1712

Martini R (2001) The effect of myelinating Schwann cells on axons. Muscle Nerve 24:456–466

McGonigal R, Rowan EG, Greenshields KN, Halstead SK, Humphreys PD, Rother RP, Furukawa K, Willison HJ (2010) Anti-GD1a antibodies activate complement and calpain to injure distal motor nodes of Ranvier in mice. Brain 133:1944–1960

Mi H, Deerinck TJ, Ellisman MH, Schwarz TL (1995) Differential distribution of closely related potassium channels in rat Schwann cells. J Neurosci 15:3761–3774

Novakovic SD, Levinson SR, Schachner M, Shrager P (1998) Disruption and reorganization of sodium channels in experimental allergic neuritis. Muscle Nerve 21:1019–1032

Nygren A, Halter JA (1999) A general approach to modeling conduction and concentration dynamics in excitable cells of concentric cylindrical geometry. J Theor Biol 199:329–358

Ogawa-Goto K, Funamoto N, Abe T, Nagashima K (1990) Different ceramide compositions of gangliosides between human motor and sensory nerves. J Neurochem 55:1486–1493

Paparounas K, O'Hanlon GM, O'Leary CP, Rowan EG, Willison HJ (1999) Anti-ganglioside antibodies can bind peripheral nerve nodes of Ranvier and activate the complement cascade without inducing acute conduction block in vitro. Brain 122:807–816

Rash JE, Vanderpool KG, Yasumura T, Hickman J, Beatty JT, Nagy JI (2016) Kv1 channels identified in rodent myelinated axons, linked to Cx29 in innermost myelin: support for electrically active myelin in mammalian saltatory conduction. J Neurophysiol 115:1836–1859

Rasminsky M (1973) The effects of temperature on conduction in demyelinated single nerve fibers. Arch Neurol 28:287–292

Rasminsky M, Sears TA (1972) Internodal conduction in undissected demyelinated nerve fibers. J Physiol 227:323–350

Reid G, Scholz A, Bostock H, Vogel W (1999) Human axons contain at least five types of voltage-dependent potassium channel. J Physiol 518(3):681–696

Ritchie JM (1995) Physiology of axons. In: Waxman SG, Kocsis JD, Stys PK (eds) The Axon. Oxford University Press, New York, pp 68–96

Saida K, Sumner AJ, Saida T, Brown MJ, Silberberg DH (1980) Antiserum-mediated demyelination: relationship between remyelination and functional recovery. Ann Neurol 8:12–24

Salzer JL, Brophy PJ, Peles E (2008) Molecular domains of myelinated axons in the peripheral nervous system. Glia 56:1532–1540

Santoro M, Uncini A, Corbo M, Staugaitis SM, Thomas FP, Hays AP, Latov N (1992) Experimental conduction block induced by serum from a patient with anti-GM1 antibodies. Ann Neurol 31:385–390

Scherer SS, Arroyo EJ (2002) Recent progress on the molecular organization of myelinated axons. J Periph Nerv Syst 7:1–12

Schwarz JR, Eikhof G (1987) Na currents and action potentials in rat myelinated nerve fibers at 20 and 37 degrees C. Pflügers Arch 409:569–577

Schwarz JR, Corrette BJ, Mann K, Wiethölter H (1991) Changes of ionic channel distribution in myelinated nerve fibers from rats with experimental allergic neuritis. Neurosci Lett 122:205–209

Schwarz JR, Reid G, Bostock H (1995) Action potentials and membrane currents in the human node of Ranvier. Pflügers Arch 430:283–292

Sheikh KA, Deerinck TJ, Ellisman MH, Griffin JW (1999) The distribution of ganglioside-like moieties in peripheral nerves. Brain 122:449–460

Smith KJ, Bostock H, Hall SM (1982) Saltatory conduction precedes remyelination in axons demyelinated with lysophosphatidyl choline. J Neurol Sci 54:13–31

Sumner AJ, Saida K, Saida T, Silberberg DH, Asbury AK (1982) Acute conduction block associated with experimental antiserum-mediated demyelination of peripheral nerve. Ann Neurol 11:469–477

Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, Funakoshi K, Hirata K, Baba H, Yuki N (2007) Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. J Neurosci 27:3956–3967

Susuki K, Yuki N, Schafer DP, Hirata K, Zhang G, Funakoshi K, Rasband MN (2012) Dysfunction of nodes of Ranvier: a mechanism for anti-ganglioside antibody-mediated neuropathies. Exp Neurol 233:534–542

Takigawa T, Yasuda H, Kikkawa R, Shigata Y, Saida T, Kitsato H (1995) Antibodies against GM1 ganglioside affect K^+ and Na^+ currents in isolated rat myelinated nerve fibers. Ann Neurol 37:436–442

Tasaki I (1955) New measurements of the capacity and the resistance of the myelin sheath and the nodal membrane of the isolated frog nerve fiber. Am J Phys 181:639–650

Von Reyn CR, Spaethling JM, Mesfin MN, Ma M, Neumar RW, Smith DH, Siman R, Meaney DF (2009) Calpain mediates proteolysis of the voltage-gated sodium channel α-subunit. J Neurosci 29:10350–10356

Waxman SG (2006) Axonal conduction and injury in multiple sclerosis: the role of sodium channels. Nat Rev Neurosci 7:932–941

Waxman SG, Kocsis JD, Black JA (1995) Pathophysiology of demyelinated axons. In: Waxman SG, Kocsis JD, Stys PK (eds) The Axon. Oxford University Press, New York, pp 438–461

Wilson GF, Chiu SY (1990) Ion channels in axon and Schwann cell membranes at paranodes of mammalian myelinated fibers studied with patch clamp. J Neurosci 10:3263–3274

Wu LMN, Williams A, Delaney A, Sherman DL, Brophy PJ (2012) Increasing internodal distance in myelinated nerves accelerates nerve conduction to a flat maximum. Curr Biol 22:1957–1961

Zhang Z, David G (2016) Stimulation-induced Ca^{2+} influx at nodes of Ranvier in mouse peripheral motor axons. J Physiol 594(1):39–57

8 Under the ECM Dome: The Physiological Role of the Perinodal Extracellular Matrix as an Ion Diffusion Barrier

Yoko Bekku and Toshitaka Oohashi

Abstract

Enriched Na^+ channel clustering allows for rapid saltatory conduction at a specialized structure in myelinated axons, the node of Ranvier, where cations are exchanged across the axon membrane. In the extracellular matrix (ECM), highly negatively charged molecules accumulate and wrap around the nodal gaps creating an ECM dome, called the perinodal ECM. The perinodal ECM has different molecular compositions in the central nervous system (CNS) and peripheral nervous system (PNS). Chondroitin sulfate proteoglycans are abundant in the ECM at the CNS nodes, whereas heparan sulfate proteoglycans are abundant at the PNS nodes. The proteoglycans have glycosaminoglycan chains on their core proteins, which makes them electrostatically negative. They associate with other ECM molecules and form a huge stable ECM complex at the nodal gaps. The polyanionic molecular complexes have high affinity to cations and potentially contribute to preventing cation diffusion at the nodes.

In this chapter, we describe the molecular composition of the perinodal ECM in the CNS and PNS, and discuss their physiological role at the node of Ranvier.

Keywords

ECM · Proteoglycans · Perinodal ECM · Node of Ranvier · Microenvironment · Glia

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8.1 The ECM in the Nervous System

The extracellular matrix (ECM) proteins are ubiquitously expressed throughout the body including in the nervous system during the developmental stage and in adults. In the mature brain, the volume fraction of the extracellular space (ECS) is approximately 20% (Nicholson and Sykova 1998). The ECS is predominantly occupied by the hyaluronan, a large, linear, and non-sulfated glycosaminoglycan (GAG). Hyaluronan exists in either a diffuse or a condensed form in the ECS. Two forms of condensed hyaluronan-based ECM are found along the axon: the axon initial segment (AIS) domain of the perineuronal net (PNN) and the node of Ranvier (Fawcett et al. 2019). At the node of Ranvier, the myelin sheath is interrupted, and a few micrometers of the axonal membrane are directly exposed to the extracellular fluid. The nodes are highly enriched in voltage-gated Na^+ and K^+ channels, which allows action potentials to propagate via saltatory conduction. Astrocytic and NG2 immunoreactive glial fingers come into close apposition with the nodal membrane in the CNS, whereas Schwann cell microvilli cover the node of Ranvier in the PNS. The perinodal ECM is embedded in the nodal gaps between axons and glial cells. In addition to hyaluronan, chondroitin sulfate proteoglycans (CSPGs), especially the hyaluronan binding CSPG lectican family, are enriched in the perinodal space and the PNN of the CNS, whereas heparan sulfate proteoglycans (HSPGs) are abundant in the PNS nodes. The complex of hyaluronan, link proteins and lecticans, can be further interconnected with glycoprotein tenascins at the C-termini of lecticans (Yamaguchi 2000). These aggregations are strongly negatively charged due to the GAG chains of hyaluronan, CS, and HS chains; thus, the polyanionic polymers were visualized by cationic staining—such as staining of ferric ion at the node of Ranvier in the PNS and CNS (Quick and Waxman 1977; Waxman and Quick 1978), and cationic iron colloid in the PNN (Murakami et al. 1993; Bruckner et al. 1993) before their composition was revealed by immunostaining. Hyaluronectin, which has an affinity to hyaluronan, was the first extracellular glycoprotein identified by an antibody at the node of Ranvier and in the PNN in the CNS (Delpech et al. 1982). It turned out that hyaluronectin was an enzymatically cleaved fragment of versican (Courel et al. 1998). Since then, the components of the ECM in the CNS, which include tenascin (Pesheva et al. 1989), aggrecan (Fryer et al. 1992), neurocan (Rauch et al. 1992), versican (Bignami et al. 1993), brevican (Yamada et al. 1994), and link proteins (Hirakawa et al. 2000; Bekku et al. 2003), have been increasingly identified, along with the components of the ECM in the PNS, which include syndecan 3 and 4 (Goutebroze et al. 2003) and perlecan (Colombelli et al. 2015) (Fig. 8.1). Over the past decades, various studies have revealed the molecular composition, localization, and functions of the ECM in the nervous system.

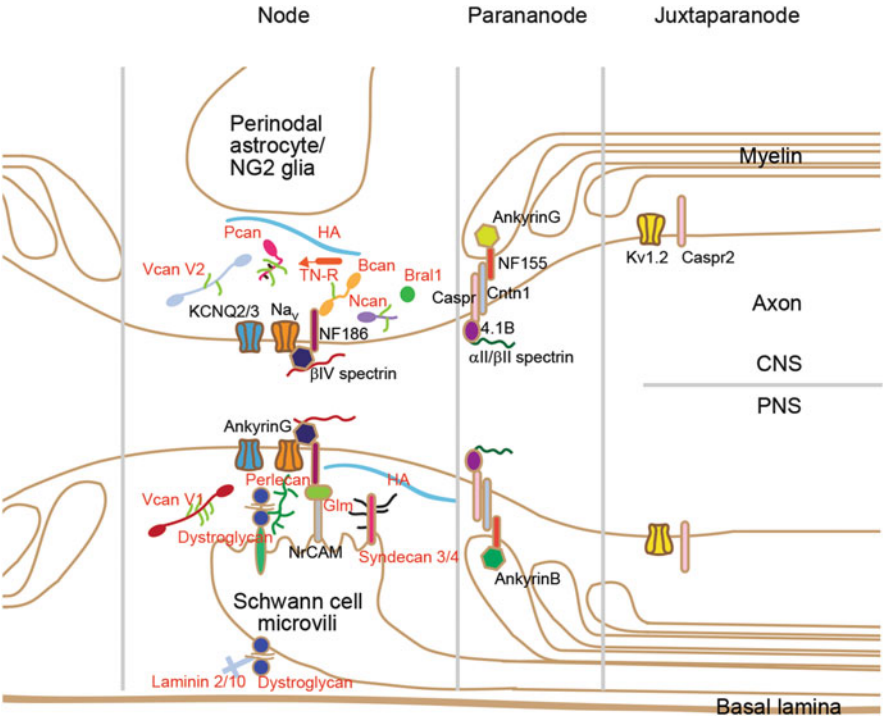

Fig. 8.1 The structural and molecular composition of the node of Ranvier. Oligodendrocytes (CNS) or Schwann cells (PNS) wrap around the axons, forming compact myelin. The nodes are flanked by paranodes and contacted by perinodal astrocytes/NG2 glia (CNS) or Schwann cell microvilli (PNS). Adhesion molecules (NF186, NF155, caspr, NrCAM, and cntn1) mediate axo–glial attachment, which forms multiprotein complexes with ion channels (Na^+ channel Na_v, and potassium channels KCNQ2/3 and $K_v1.2$) that are stabilized by their association with cytoskeletal molecules (ankyrins and spectrins). Perinodal matrix molecules (Vcan V2, Bcan, Ncan, Pcan, HA, TN-R, and Hapln2/Bral1 in CNS, Vcan V1, syndecan-3/4, glm, and perlecan in PNS) fill the nodal extracellular space. Abbreviations: *Bcan* brevican, *Bral1* Hapln2/Bral1, *cntn1* contactin 1, *Glm* gliomedin, *HA* hyaluronan, *Ncan* neurocan, *NF186* neurofascin 186, *NF155* neurofascin 155, *Pcan* phosohacan, *TN-R* tenascin-R, *Vcan V1* versican V1, *Vcan V2* versican V2

8.1.1 The Molecular Composition of the ECM at the CNS Nodes

8.1.1.1 Link Proteins

Link proteins belong to a link module family that contains a common structural domain of ~100 amino acids in length, termed a link module, which is involved in hyaluronan binding (Day and Prestwich 2002). They are also called hyaluronan and proteoglycan link proteins (HAPLNs) (Spicer et al. 2003). Link proteins consist of an immunoglobulin-like loop (also known as A-subdomain) and two contiguous link modules (also known as B-subdomain). The primary sequence and structural domain of link proteins are highly homologous to those of the G1 domain of lecticans, which

share the common property of binding to hyaluronan. Both link proteins and the G1 domain of lectican are necessary for the formation of a stable ternary complex with hyaluronan (Matsumoto et al. 2003; Seyfried et al. 2005; Shi et al. 2004; Rauch et al. 2004).

The first link protein was purified from cartilage (Oegema et al. 1975) and was characterized as cartilage link protein Crtl1 (Osborne-Lawrence et al. 1990). The link protein family is composed of four members, Crtl1/HAPLN1, Bral1/HAPLN2, Lp3/HAPLN3, and Bral2/HAPLN4. The molecular weights of all four link proteins are approximately 36–43 kDa. The four link proteins share between 45 and 58% amino acid identity; link modules have particularly high homology (Oohashi and Bekku 2007). Crtl1, Bral1, and Bral2 are expressed in the CNS, in particular, brain link proteins, Bral1 and Bral2, are exclusively expressed in the CNS (Oohashi et al. 2002; Bekku et al. 2003). In the CNS, Bral1 is localized at the node of Ranvier (Oohashi et al. 2002), and Crtl1 and Bral2 are expressed in the PNN (Ripellino et al. 1989; Bekku et al. 2003). On the other hand, Lp3 is broadly distributed in the pericellular matrix of vascular smooth muscle cells throughout the body, including the brain but is not expressed in either the PNN or the nodal gaps (Ogawa et al. 2004).

8.1.1.2 Lecticans

Lecticans (also called hyalectans) are a family of CSPGs that also belong to a link module family, encompassing aggrecan, versican, neurocan, and brevican. They display a high degree of homology in their N- and C-terminal globular domains. While the extended central region of lecticans is diverse in length and in the numbers of potential GAG attachment sites (Yamaguchi 2000). Lecticans are named from the presence of a well-conserved C-type lectin domain (CLD) in the C-terminal G3 domain. Hyalectans arose from the hyaluronan-binding ability of the G1 domain and the potential lectin-like ability of the G3 domain. A number of lectican isoforms are derived from the alternative splicing of exons encoding central regions or G3 domains, which leads to the functional diversity of lecticans. At least four isoforms exist in versican (V0, V1, V2, and V3 isoforms), and the alternative splicing of these isoforms occurs in the central GAG-attachment region (Ito et al. 1995). A new splicing isoform, V4, which was recently reported in breast cancer (Kischel et al. 2010), has not been well characterized in the brain. Brevican has a unique alternatively spliced glycosylphosphatidylinositol (GPI)-anchored isoform (Seidenbecher et al. 1995). In this isoform, the central region is followed by a GPI-anchor; thus, it lacks the G3 domain. However, this membrane-bound isoform constitutes less than 1% of the total brain brevican transcripts (John et al. 2006). In aggrecan, the G3 domain is subject to the alternative splicing of epidermal growth factor (EGF)-like repeats and a complement regulatory protein (CRP) repeat (Matthews et al. 2002; Morawski et al. 2012). Five splicing isoforms were assessed in the human cerebral cortex using some antibodies that were found to react with epitopes within defined aggrecan domains. The result suggests that selected aggrecan isoforms were linked to defined subsets of cortical interneurons and pyramidal cells (Virgintino et al. 2009). Several metalloproteinases (Mmp)—or a disintegrin and metalloproteases

with thrombospondin motifs (Adamts)—are reported to play a role in the activity-dependent decrease of PNN or the reshaping of PNN (Foscarin et al. 2011; Ganguly et al. 2013; Levy et al. 2015; Rossier et al. 2015). Not only the core protein part but also the CS moiety is important for the biological function of lecticans to be exerted in the PNN. The changes of CS sulfation patterns from the embryonic form (6-sulfated rich) to the postnatal form (4-sulfated rich) during development have been reported (Kitagawa et al. 1997; Miyata et al. 2012). The biological importance of CS chain-binding molecules, such as Semaphorin 3A and Otx2 have been highlighted (Dick et al. 2013; Sugiyama et al. 2008). For detailed information about the biological functions of sulfated-GAGs in the CNS, we refer the reader to recent reviews (Kadomatsu and Sakamoto 2014; Smith et al. 2015).

8.1.1.3 Other ECMs

Tenascins are a multimeric glycoprotein, and four distinct tenascins, TN-C, TN-R, TN-X, and TN-W (-N) have been identified in mammals (Chiquet-Ehrismann and Chiquet 2003). Among these, TN-C and TN-R are expressed in the nervous system: TN-R is exclusively expressed in the CNS, and TN-C is present in the CNS as well as in the PNS (Joester and Faissner 2001). In particular, TN-R is expressed in the PNN and the node of Ranvier in the CNS. A cysteine-rich N-terminal region allows tenascins to form trimers (TN-R) and hexamers (TN-C) (Chiquet-Ehrismann and Chiquet 2003). Both TN-C and TN-R bind to many types of ECM molecules, including lecticans and phosphacan, via the fibronectin type-III repeat. The multimeric tenascins function as molecular crosslinkers between lecticans and hyaluronan aggregation; consequently, a massive ECM network is generated. In fact, Lundell et al. demonstrated that TN-C and TN-R crosslink aggrecan–hyaluronan complexes by electron microscopy (Lundell et al. 2004). All four lecticans bind TN-R and TN-C via the G3 domains of lecticans (Aspberg et al. 1997; Day et al. 2004), in particular, brevican has at least ten-fold higher affinity to TN-R than other lecticans (Aspberg et al. 1997). Phosphacan is a secreted isoform of the receptor-type protein-tyrosine phosphatase β (RPTPβ) and a non-hyaluronan-binding type of CSPG. Developmentally regulated glycoforms of phosphacan containing keratan sulfate (phosphacan-KS) are also present in the brain (Rauch et al. 1991). Strong immunostaining of phosphacan-KS was observed in the prospective white matter in the rat cerebellum on postnatal day 7, whereas phosphacan without KS was not observed (Rauch et al. 1991; Grumet et al. 1994). Thus, phosphacan-KS might be the major form localizing at the node of Ranvier in adults. Both phosphacan with and without KS binds tenascin (Grumet et al. 1994), and phosphacan binds TN-C and TN-R in a calcium-dependent manner (Milev et al. 1997; Bekku et al. 2009). Phosphacan core protein showed colocalization with TN-R at the node of Ranvier in the optic nerve (Milev et al. 1997) and the facial nerve tract (Bekku et al. 2009), as well as in the PNN (Xiao et al. 1997; Haunso et al. 1999).

8.1.2 The Molecular Composition of ECM at the PNS Nodes

8.1.2.1 Gliomedin

Gliomedin is a transmembrane protein that belongs to subfamily VI of the olfactomedin protein family (Anholt 2014), and the group of type II transmembrane collagens that include collagens XIII and XVII (Maertens et al. 2007). Gliomedin contains two collagenous domains and olfactomedin-like domain, and the collagenous domain contains a putative α-helical coiled-coil sequence, which serves as an oligomerization motif (Maertens et al. 2007; Eshed et al. 2007). Gliomedin is expressed by myelinating Schwann cells and accumulates at the edges of each myelin segment (Eshed et al. 2005). Proteolytic cleavage of the protein by furin generates both the transmembrane and the secreted form; only the secreted form is detected at the node of Ranvier (Maertens et al. 2007; Eshed et al. 2007). The secreted form assembles with HSPG (Eshed et al. 2007). The olfactomedin domain mediates its interaction with cell adhesion molecules, neurofascin, and NrCAM (Eshed et al. 2005).

8.1.2.2 Heparan Sulfate Proteoglycans

Perlecan is a large HSPG that contains a multi-domain core protein of approximately 500 kDa (Murdoch et al. 1992). The core protein is divided into several unique structural regions, each imparting distinct aspects of biofunctional diversity on perlecan, such as cell adhesion, endocytosis, bone formation, and PNS node assembly (Gubbiotti et al. 2017). Dystroglycan (DG), a receptor of perlecan at the PNS node, is one of the components of dystrophin–glycoprotein complex. DG is composed of an α subunit and a transmembrane β subunit; the α subunit binds to perlecan, laminin 2, and another HSPG, agrin at the PNS nodes (Colombelli et al. 2015). DG and laminin 2 are required for Na^+ channel clustering and node formation (Saito et al. 2003; Occhi et al. 2005; Colombelli et al. 2015). Perlecan is recruited to the nodal gaps by DG and binds to gliomedin (Colombelli et al. 2015).

The syndecans are transmembrane HSPGs. The vertebrate syndecan family is composed of two subfamilies: syndecan 1 and 3, and syndecan 2 and 4 (Couchman 2003). Syndecan 1 and 3 sometimes carry CS-chains as well (Bernfield et al. 1999; Couchman 2003). Syndecan 3 and 4 are both localized at the perinodal gaps in the PNS (Goutebroze et al. 2003), and the nodal components are normally expressed in syndecan 3-deficient mice (Melendez-Vasquez et al. 2005).

8.2 The ECM Complex in the Nervous System

8.2.1 The ECM Complex at the CNS Nodes

To date, hyaluronan, Bral1, versican V2, brevican, neurocan, TN-R, and phosphacan have been reported as ECM molecules that are secreted at the node of Ranvier in the CNS (Delpech et al. 1982; Bartsch et al. 1993; Oohashi et al. 2002; Hedstrom et al. 2007; Bekku et al. 2009; Bekku and Oohashi 2010). NG2, a transmembrane CSPG,

and positive glia cells extend their processes to the nodes (Butt et al. 1999). Among these ECMs, Bral1, and versican V2 are localized at almost all nodes, whereas other ECM molecules are expressed in specific regions (Bekku et al. 2009; Bekku and Oohashi 2010). The expression of brevican has been observed in the facial nerve tract, the vestibulocochlear nerve tract, the ventral spinocerebellar tract, the pyramidal tract, the trapezoid body, the optic nerve tract, the white matter of the cerebellum and the spinal cord. Intriguingly, brevican, TN-R, and phosphacan are present at the nodes, in particular at large-diameter axons. In the brevican-deficient mice, the axon diameter-dependent expression of TN-R and phosohacan was altered and shifted to a diameter-independent manner. Immunohistochemistry and an immunoprecipitation experiment indicated that a ligand shift of TN-R from brevican to versican V2 occurred in brevican-deficient mice (Bekku et al. 2009). Taken together with the results of a surface plasmon resonance analysis (Aspberg et al. 1997), brevican is considered to assemble extracellular components at the large diameter node of Ranvier in the CNS. In addition, neurocan—particularly the N-terminal 130 kDa fragment (neurocan-N)—was predominantly detected in the optic nerve by Western blotting, indicating that neurocan-N existed at the node of Ranvier (Bekku and Oohashi 2010). In the rat cerebellum, 1D1 antibody, which recognizes the C-terminal of neurocan (full-length and a 150 kDa fragment, neurocan-C) showed immunoreactivity in the 7-day postnatal prospective white matter, whereas the immunoreaction could not be detected in the adult white matter (Rauch et al. 1991). During development, neurocan is cleaved to neurocan-C and -N, and neurocan-N associates with the perinodal ECM in the white matter. The nodal pattern of neurocan was evident, not only in the regions in which nodal brevican is expressed but also in the white matter of the corpus callosum and the internal capsule where brevican is not distributed. The number of neurocan-positive punctuate signals is greater than that of brevican signals; thus lecticans at the nodes are thought to be present in the following order: versican V2 > neurocan > brevican (Bekku and Oohashi 2010). Neurocan has the lowest ability to bind to TN-R among the lecticans (Aspberg et al. 1997), and neurocan-N lacks a C-type lectin domain that recognizes tenascins. Thus, it can be presumed that neurocan does not associate with TN-R and that brevican is the binding partner of TN-R at the nodes. More elaborate ECM associations surround the nodes that are larger in diameter (Fig. 8.2). Interestingly, the immunostaining patterns of TN-R and phosphacan at the nodes are abolished in the cerebellum and the spinal cord in versican V2-deficient mice (Dours-Zimmermann et al. 2009). Brevican, a binding partner of TN-R, is localized at the nodes in these regions (Bekku et al. 2009); thus, the disappearance of TN-R and phosphacan nodal patterns might be caused by the disruption of the entire perinodal ECM assembly in the region. Bral1 deficiency caused hyaluronan-based ECM no longer showed a nodal pattern in the CNS; thus, Bral1 is indispensable for stabilizing ECM assembly (including versican V2, brevican, neurocan, TN-R, phosphacan, and hyaluronan) at the node of Ranvier (Bekku and Oohashi 2010; Bekku et al. 2010).

Bral1 is produced by the neurons throughout the brain (Oohashi et al. 2002) and by oligodendrocytes in the cerebellum (Carulli et al. 2006). Versican V2 is synthesized by oligodendrocyte lineage cells (Schmalfeldt et al. 2000; Asher et al.

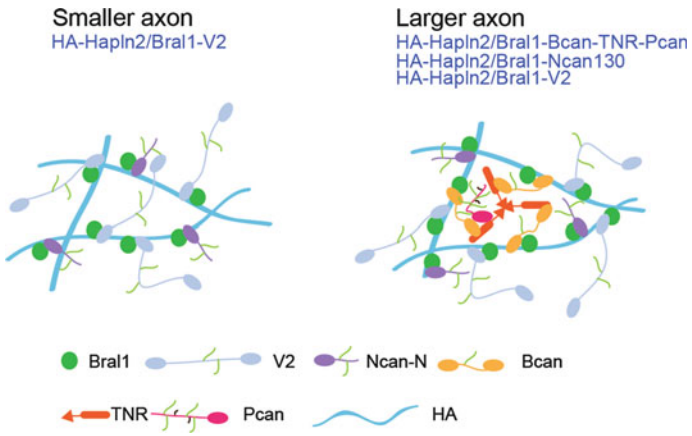

Fig. 8.2 A model of the axon diameter-dependent assembly of the hyaluronan-binding matrix at the CNS nodes. The minimum unit of the perinodal ECM consists of Vcan V2, HA, and Hapln2/Bral1 in smaller axons, and a more complex matrix surrounds axons that are larger in diameter

2002) and NG2 immunoreactive cells (Carulli et al. 2006). Brevican is made by glial cells, including oligodendrocytes and astrocyte, as well as neurons. Although the detailed localization of each brevican isoform in the CNS remains unclear (Yamada et al. 1994, 1997; Seidenbecher et al. 1998; Ogawa et al. 2001; Carulli et al. 2006), full-length brevican was reported to be localized at the node of Ranvier (Bekku et al. 2009). Neurocan is produced by the neurons in the cerebellum of juvenile and adult rodents (Engel et al. 1996; Carulli et al. 2006). Phosphacan is expressed by the Bergman glia in the juvenile cerebellum (Engel et al. 1996) and by NG2-positive cells in the adult cerebellum (Carulli et al. 2006). TN-R is highly synthesized by oligodendrocytes during the period of myelination, and is constantly produced by type-2 astrocytes and some neuronal cell types during postnatal development to adulthood (Wintergerst et al. 1993). Neurofascin 186 (NF186), which is required for the localization of brevican and versican V2 at the AIS (Hedstrom et al. 2007; Susuki et al. 2013), binds to Bral1, versican V2, and brevican in vitro (Susuki et al. 2013). CD44, a receptor for hyaluronan, is expressed in GFAP-positive astrocytes while there is no CD44 expression in NG2-positive cells in the optic nerves (Bekku et al. 2010). CD44-positive astrocyte processes project to some nodal gaps, but no changes were seen in the CD44 expression and their processes at the node of Ranvier in Bral1-deficient mice (Bekku et al. 2010). In the nodal region, more than 95% of nodes are projected by astrocytes, while 33–49% of nodal gaps are projected by NG2 glia (Serwanski et al. 2017). Interestingly, NG2 cells prefer to contact larger diameter axons (Serwanski et al. 2017). The role in the perinodal astrocytes/NG2 cells has not been elucidated, but there might be some roles on perinodal ECMs such as their diameter-dependent localization and maintenance. Further studies are needed to address the mechanisms of diameter-dependent ECM localization.

8.2.2 The ECM Complex at the PNS Nodes

None of the link proteins are localized in the PNS nodes (Bekku and Oohashi, unpublished data), irrespective of the existence of versican V1 (Melendez-Vasquez et al. 2005) and hyaluronan (Abood and Abul-Haj 1956). In addition to stabilizing the aggregation of lecticans, link proteins may also protect the lectican aggregates from degradation (Rodriguez and Roughley 2006). It is still unclear how these hyaluronan-based ECMs are stably associated at the perinodal gaps. TN-C is also localized at PNS nodes (Martini et al. 1990). TN-C has the ability to bind to proteoglycans, including phosphacan, neurocan, syndecan, perlecan, and the β2 subunit of the Na^+ channel (Joester and Faissner 2001; Midwood et al. 2016).

Type V collagen binds to syndecan 3 (Chernousov et al. 1996; Erdman et al. 2002) and is present in the abaxonal membrane of Schwann cells and nodal gaps (Melendez-Vasquez et al. 2005). In syndecan 3-deficient mice, immunostaining of type V collagen was more diffuse in the nodal region than in the abaxonal membrane (Melendez-Vasquez et al. 2005). Type XXVII collagen also showed a similar expression pattern to type V collagen (Grimal et al. 2010). Type XXVII collagen was partially colocalized with laminin γ1, which was present in the basal laminae above the node of Ranvier (Grimal et al. 2010; Occhi et al. 2005); thus, collagens may be associated with the DG/perlecan/laminin 2/agrin complex in addition to syndecans. As mentioned above, perlecan binds to gliomedin; thus, these extracellular molecules may create a huge ECM complex around the perinodal gaps in the PNS.

8.3 The Functional Roles of the Perinodal ECM

The perinodal ECM has been well studied in relation to node formation (Eshed et al. 2007; Saito et al. 2003; Occhi et al. 2005; Susuki et al. 2013; Colombelli et al. 2015); however, there has been little research on its physiological role. Since Susuki describes CNS node formation in Chap. 2-i, we focus on the physiological role of the perinodal ECM. The perinodal ECM has been investigated in several knockout mouse models, including TN-R-knockout (Weber et al. 1999), RPTPβ/phosphacan-knockout (Harroch et al. 2000), brevican-knockout (Bekku et al. 2009), versican V2-knockout (Dours-Zimmermann et al. 2009), and Bral1-knockout (Bekku et al. 2010) mice. With the exception of the brevican-knockout, the electrophysiological features of the nodes were analyzed in each of these mouse models; TN-R- and Bral1-deficient mice showed a significant decrease in conduction velocity, even though no difference was seen in the number of Na^+ channel clusters or in the morphology at the nodes of these two mutant mouse models (Weber et al. 1999; Bekku et al. 2010). TN-R and RPTPβ interact with Na^+ channels in vitro (Xiao et al. 1999; Ratcliffe et al. 2000). Both β1 and β2 subunits interact with fibronectin-type III repeats and the EGF-like domain of TN-R, and the application of EGF-like peptide to *Xenopus* oocytes expressing $Na_v1.2$ and β2 increased the Na^+ current amplitude; thus, TN-R might regulate the functional activity of the voltage-gated Na^+ channel via interaction with the β subunits at the node of Ranvier (Ratcliffe et al. 2000; Brackenbury and Isom 2008).

As mentioned above, almost all of the nodal gaps are surrounded by ECM; however, fibers that are particularly large in diameter are enclosed by TN-R and phosphacan at the node of Ranvier in the CNS. The important question with regard to the physiological role of ECM is why a more elaborate ECM association is required around the larger diameter nodes in the CNS. In agreement, a larger axon diameter increases the conduction velocity. Computational models have demonstrated the accumulation and depletion of ions in the local extracellular nodal region (Lopreore et al. 2008). Both GAGs, hyaluronan, and CS, are composed of an acidic repeating disaccharide unit that can provide a strong negatively charged environment. These macromolecules have a high affinity to cations such as Na^+ (Hunter et al. 1988; Scott 1989). It appears that the water-filled compartment could pool hydrated cations around the place in which the action potentials are generated and that it may function as a soluble cation exchanger. A more elaborate ECM association could create greater hydrated aggregation around larger caliber fibers. ECM molecules play a role in the tissue cytoarchitecture, not only through maintaining the optimal size of intercellular pores but also through the creation of diffusion barriers (Roitbak and Sykova 1999; Zamecnik et al. 2004; Sykova et al. 2005). Changes in the ECS diffusion parameters and anisotropy significantly affect the accumulation and diffusion of neuroactive substances (Vorisek and Sykova 1997). The digestion of GAG chains of CSPG by chondroitinase ABC enhances the diffusion of extracellular calcium in the brain (Hrabetová et al. 2009). Interestingly, chondroitin sulfates modulated the gating properties of voltage-operated ion channels by modulating the calcium concentration in the extracellular environment in *Xenopus laevis* (Vigetti et al. 2008). In Bral1-deficient mice, a reduction in the diffusion hindrance of the white matter was observed by measuring the ECS parameters while, in addition, a decrease in the conduction velocity also occurred (Bekku et al. 2010). Taken together, the Bral1-based extracellular environment is suggested to contribute to saltatory conduction in terms of the accumulation of cations due to an ion diffusion barrier around the CNS nodes.

The PNS nodal gaps seem to be a more closed environment with Schwann cell microvilli tightly covering the gaps. The CNS nodes—in contrast—are exposed to the extracellular fluid, while the perinodal glia project the processes to the CNS nodes. HSPGs and collagens are abundant at the PNS nodal gaps, which can also create a highly negatively charged environment around the nodal spaces. They may also contribute to generating an ion diffusion barrier in the PNS. Although their role in nodal formation has been well studied, their physiological role at the nodes remains unclear.

References

Abood LG, Abul-Haj SK (1956) Histochemistry and characterization of hyaluronic acid in axons of peripheral nerve. J Neurochem 1(2):119–125

Anholt RR (2014) Olfactomedin proteins: central players in development and disease. Front Cell Dev Biol 2:6. https://doi.org/10.3389/fcell.2014.00006

Asher RA, Morgenstern DA, Shearer MC, Adcock KH, Pesheva P, Fawcett JW (2002) Versican is upregulated in CNS injury and is a product of oligodendrocyte lineage cells. J Neurosci 22 (6):2225–2236

Aspberg A, Miura R, Bourdoulous S, Shimonaka M, Heinegard D, Schachner M et al (1997) The C-type lectin domains of lecticans, a family of aggregating chondroitin sulfate proteoglycans, bind tenascin-R by protein-protein interactions independent of carbohydrate moiety. Proc Natl Acad Sci U S A 94(19):10116–10121

Bartsch U, Pesheva P, Raff M, Schachner M (1993) Expression of janusin (J1-160/180) in the retina and optic nerve of the developing and adult mouse. Glia 9(1):57–69. https://doi.org/10.1002/glia.440090108

Bekku Y, Oohashi T (2010) Neurocan contributes to the molecular heterogeneity of the perinodal ECM. Arch Histol Cytol 73(2):95–102

Bekku Y, Su WD, Hirakawa S, Fassler R, Ohtsuka A, Kang JS et al (2003) Molecular cloning of Bral2, a novel brain-specific link protein, and immunohistochemical colocalization with brevican in perineuronal nets. Mol Cell Neurosci 24(1):148–159

Bekku Y, Rauch U, Ninomiya Y, Oohashi T (2009) Brevican distinctively assembles extracellular components at the large diameter nodes of Ranvier in the CNS. J Neurochem 108 (5):1266–1276. https://doi.org/10.1111/j.1471-4159.2009.05873.x

Bekku Y, Vargova L, Goto Y, Vorisek I, Dmytrenko L, Narasaki M et al (2010) Bral1: its role in diffusion barrier formation and conduction velocity in the CNS. J Neurosci 30(8):3113–3123. https://doi.org/10.1523/JNEUROSCI.5598-09.2010

Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M (1999) Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem 68(1):729–777. https://doi.org/10.1146/annurev.biochem.68.1.729

Bignami A, Perides G, Rahemtulla F (1993) Versican, a hyaluronate-binding proteoglycan of embryonal precartilaginous mesenchyma, is mainly expressed postnatally in rat brain. J Neurosci Res 34(1):97–106. https://doi.org/10.1002/jnr.490340110

Brackenbury WJ, Isom LL (2008) Voltage-gated Na+ channels: potential for beta subunits as therapeutic targets. Expert Opin Ther Targets 12(9):1191–1203. https://doi.org/10.1517/14728222.12.9.1191

Bruckner G, Brauer K, Hartig W, Wolff JR, Rickmann MJ, Derouiche A et al (1993) Perineuronal nets provide a polyanionic, glia-associated form of microenvironment around certain neurons in many parts of the rat brain. Glia 8(3):183–200. https://doi.org/10.1002/glia.440080306

Butt AM, Duncan A, Hornby MF, Kirvell SL, Hunter A, Levine JM, Berry M (1999) Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white matter. Glia 26 (1):84–91

Carulli D, Rhodes KE, Brown DJ, Bonnert TP, Pollack SJ, Oliver K et al (2006) Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. J Comp Neurol 494(4):559–577. https://doi.org/10.1002/cne.20822

Chernousov MA, Stahl RC, Carey DJ (1996) Schwann cells secrete a novel collagen-like adhesive protein that binds N-syndecan. J Biol Chem 271(23):13844–13853

Chiquet-Ehrismann R, Chiquet M (2003) Tenascins: regulation and putative functions during pathological stress. J Pathol 200(4):488–499. https://doi.org/10.1002/path.1415

Colombelli C, Palmisano M, Eshed-Eisenbach Y, Zambroni D, Pavoni E, Ferri C et al (2015) Perlecan is recruited by dystroglycan to nodes of Ranvier and binds the clustering molecule gliomedin. J Cell Biol 208(3):313–329. https://doi.org/10.1083/jcb.201403111

Couchman JR (2003) Syndecans: proteoglycan regulators of cell-surface microdomains? Nat Rev Mol Cell Biol 4(12):926–937. https://doi.org/10.1038/nrm1257

Courel MN, Marret S, Girard N, Chauzy C, Olivier A, Bertrand P et al (1998) Hyaluronectin is produced by oligodendrocytes and Schwann cells in vitro. J Neurocytol 27(1):27–32

Day AJ, Prestwich GD (2002) Hyaluronan-binding proteins: tying up the giant. J Biol Chem 277 (7):4585–4588

Day JM, Olin AI, Murdoch AD, Canfield A, Sasaki T, Timpl R et al (2004) Alternative splicing in the aggrecan G3 domain influences binding interactions with tenascin-C and other extracellular matrix proteins. J Biol Chem 279(13):12511–12518. https://doi.org/10.1074/jbc.M400242200

Delpech A, Girard N, Delpech B (1982) Localization of hyaluronectin in the nervous system. Brain Res 245(2):251–257

Dick G, Tan CL, Alves JN, Ehlert EM, Miller GM, Hsieh-Wilson LC, Sugahara K, Oosterhof A, van Kuppevelt TH, Verhaagen J, Fawcett JW, Kwok JC (2013) Semaphorin 3A binds to the perineuronal nets via chondroitin sulfate type E motifs in rodent brains. J Biol Chem 288 (38):27384–27395. https://doi.org/10.1074/jbc.M111.310029

Dours-Zimmermann MT, Maurer K, Rauch U, Stoffel W, Fassler R, Zimmermann DR (2009) Versican V2 assembles the extracellular matrix surrounding the nodes of ranvier in the CNS. J Neurosci 29(24):7731–7742. https://doi.org/10.1523/JNEUROSCI.4158-08.2009

Engel M, Maurel P, Margolis RU, Margolis RK (1996) Chondroitin sulfate proteoglycans in the developing central nervous system. I. Cellular sites of synthesis of neurocan and phosphacan. J Comp Neurol 366(1):34–43. https://doi.org/10.1002/(SICI)1096-9861(19960226)366:1<34::AID-CNE3>3.0.CO;2-L

Erdman R, Stahl RC, Rothblum K, Chernousov MA, Carey DJ (2002) Schwann cell adhesion to a novel heparan sulfate binding site in the N-terminal domain of alpha 4 type V collagen is mediated by syndecan-3. J Biol Chem 277(9):7619–7625. https://doi.org/10.1074/jbc.M111311200

Eshed Y, Feinberg K, Poliak S, Sabanay H, Sarig-Nadir O, Spiegel I et al (2005) Gliomedin mediates Schwann cell-axon interaction and the molecular assembly of the nodes of Ranvier. Neuron 47(2):215–229. https://doi.org/10.1016/j.neuron.2005.06.026

Eshed Y, Feinberg K, Carey DJ, Peles E (2007) Secreted gliomedin is a perinodal matrix component of peripheral nerves. J Cell Biol 177(3):551–562. https://doi.org/10.1083/jcb.200612139

Fawcett JW, Oohashi T, Pizzorusso T (2019) The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. Nat Rev Neurosci 20:451–465. https://doi.org/10.1038/s41583-019-0196-3

Foscarin S, Ponchione D, Pajaj E, Leto K, Gawlak M, Wilczynski GM, Rossi F, Carulli D (2011) Experience-dependent plasticity and modulation of growth regulatory molecules at central synapses. PLoS One 6(1):e16666. https://doi.org/10.1371/journal.pone.0016666

Fryer HJ, Kelly GM, Molinaro L, Hockfield S (1992) The high molecular weight Cat-301 chondroitin sulfate proteoglycan from brain is related to the large aggregating proteoglycan from cartilage, aggrecan. J Biol Chem 267(14):9874–9883

Ganguly K, Rejmak E, Mikosz M, Nikolaev E, Knapska E, Kaczmarek L (2013) Matrix metalloproteinase (MMP) 9 transcription in mouse brain induced by fear learning. J Biol Chem 288(29):20978–20991. https://doi.org/10.1074/jbc.M113.457903

Goutebroze L, Carnaud M, Denisenko N, Boutterin MC, Girault JA (2003) Syndecan-3 and syndecan-4 are enriched in Schwann cell perinodal processes. BMC Neurosci 4:29. https://doi.org/10.1186/1471-2202-4-29

Grimal S, Puech S, Wagener R, Venteo S, Carroll P, Fichard-Carroll A (2010) Collagen XXVIII is a distinctive component of the peripheral nervous system nodes of ranvier and surrounds nonmyelinating glial cells. Glia 58(16):1977–1987. https://doi.org/10.1002/glia.21066

Grumet M, Milev P, Sakurai T, Karthikeyan L, Bourdon M, Margolis RK, Margolis RU (1994) Interactions with tenascin and differential effects on cell adhesion of neurocan and phosphacan, two major chondroitin sulfate proteoglycans of nervous tissue. J Biol Chem 269 (16):12142–12146

Gubbiotti MA, Neill T, Iozzo RV (2017) A current view of perlecan in physiology and pathology: a mosaic of functions. Matrix Biol 57-58:285–298. https://doi.org/10.1016/j.matbio.2016.09.003

Harroch S, Palmeri M, Rosenbluth J, Custer A, Okigaki M, Shrager P et al (2000) No obvious abnormality in mice deficient in receptor protein tyrosine phosphatase beta. Mol Cell Biol 20 (20):7706–7715

Haunso A, Celio MR, Margolis RK, Menoud PA (1999) Phosphacan immunoreactivity is associated with perineuronal nets around parvalbumin-expressing neurones. Brain Res 834 (1–2):219–222

Hedstrom KL, Xu X, Ogawa Y, Frischknecht R, Seidenbecher CI, Shrager P, Rasband MN (2007) Neurofascin assembles a specialized extracellular matrix at the axon initial segment. J Cell Biol 178(5):875–886. https://doi.org/10.1083/jcb.200705119

Hirakawa S, Oohashi T, Su WD, Yoshioka H, Murakami T, Arata J, Ninomiya Y (2000) The brain link protein-1 (BRAL1): cDNA cloning, genomic structure, and characterization as a novel link protein expressed in adult brain. Biochem Biophys Res Commun 276(3):982–989. https://doi.org/10.1006/bbrc.2000.3583

Hrabetová S, Masri D, Tao L, Xiao F, Nicholson C (2009) Calcium diffusion enhanced after cleavage of negatively charged components of brain extracellular matrix by chondroitinase ABC. J Physiol 587(Pt 16):4029–4049. https://doi.org/10.1113/jphysiol.2009.170092

Hunter GK, Wong KS, Kim JJ (1988) Binding of calcium to glycosaminoglycans: an equilibrium dialysis study. Arch Biochem Biophys 260(1):161–167

Ito K, Shinomura T, Zako M, Ujita M, Kimata K (1995) Multiple forms of mouse PG-M, a large chondroitin sulfate proteoglycan generated by alternative splicing. J Biol Chem 270(2):958–965

Joester A, Faissner A (2001) The structure and function of tenascins in the nervous system. Matrix Biol 20(1):13–22

John N, Krügel H, Frischknecht R, Smalla KH, Schultz C, Kreutz MR, Gundelfinger ED, Seidenbecher CI (2006) Brevican-containing perineuronal nets of extracellular matrix in dissociated hippocampal primary cultures. Mol Cell Neurosci 31(4):774–784

Kadomatsu K, Sakamoto K (2014) Mechanisms of axon regeneration and its inhibition: roles of sulfated glycans. Arch Biochem Biophys 558:36–41. https://doi.org/10.1016/j.abb.2014.06.009

Kischel P, Waltregny D, Dumont B, Turtoi A, Greffe Y, Kirsch S, De Pauw E, Castronovo V (2010) Versican overexpression in human breast cancer lesions: known and new isoforms for stromal tumor targeting. Int J Cancer 126(3):640–650

Kitagawa H, Tsutsumi K, Tone Y, Sugahara K (1997) Developmental regulation of the sulfation profile of chondroitin sulfate chains in the chicken embryo brain. J Biol Chem 272 (50):31377–31381

Levy C, Brooks JM, Chen J, Su J, Fox MA (2015) Cell-specific and developmental expression of lectican-cleaving proteases in mouse hippocampus and neocortex. J Comp Neurol 523 (4):629–648. https://doi.org/10.1002/cne.23701

Lopreore CL, Bartol TM, Coggan JS, Keller DX, Sosinsky GE, Ellisman MH, Sejnowski TJ (2008) Computational modeling of three-dimensional electrodiffusion in biological systems: application to the node of Ranvier. Biophys J 95(6):2624–2635. https://doi.org/10.1529/biophysj.108.132167

Lundell A, Olin AI, Morgelin M, al-Karadaghi S, Aspberg A, Logan DT (2004) Structural basis for interactions between tenascins and lectican C-type lectin domains: evidence for a crosslinking role for tenascins. Structure 12(8):1495–1506. https://doi.org/10.1016/j.str.2004.05.021

Maertens B, Hopkins D, Franzke CW, Keene DR, Bruckner-Tuderman L, Greenspan DS, Koch M (2007) Cleavage and oligomerization of gliomedin, a transmembrane collagen required for node of ranvier formation. J Biol Chem 282(14):10647–10659. https://doi.org/10.1074/jbc.M611339200

Martini R, Schachner M, Faissner A (1990) Enhanced expression of the extracellular matrix molecule J1/tenascin in the regenerating adult mouse sciatic nerve. J Neurocytol 19(4):601–616

Matsumoto K, Shionyu M, Go M, Shimizu K, Shinomura T, Kimata K, Watanabe H (2003) Distinct interaction of versican/PG-M with hyaluronan and link protein. J Biol Chem 278 (42):41205–41212. https://doi.org/10.1074/jbc.M305060200

Matthews RT, Kelly GM, Zerillo CA, Gray G, Tiemeyer M, Hockfield S (2002) Aggrecan glycoforms contribute to the molecular heterogeneity of perineuronal nets. J Neurosci 22 (17):7536–7547

Melendez-Vasquez C, Carey DJ, Zanazzi G, Reizes O, Maurel P, Salzer JL (2005) Differential expression of proteoglycans at central and peripheral nodes of Ranvier. Glia 52(4):301–308. https://doi.org/10.1002/glia.20245

Midwood KS, Chiquet M, Tucker RP, Orend G (2016) Tenascin-C at a glance. J Cell Sci 129 (23):4321–4327. https://doi.org/10.1242/jcs.190546

Milev P, Fischer D, Haring M, Schulthess T, Margolis RK, Chiquet-Ehrismann R, Margolis RU (1997) The fibrinogen-like globe of tenascin-C mediates its interactions with neurocan and phosphacan/protein-tyrosine phosphatase-zeta/beta. J Biol Chem 272(24):15501–15509

Miyata S, Komatsu Y, Yoshimura Y, Taya C, Kitagawa H (2012) Persistent cortical plasticity by upregulation of chondroitin 6-sulfation. Nat Neurosci 15(3):414–422., S1-2. https://doi.org/10.1038/nn.3023

Morawski M, Bruckner G, Arendt T, Matthews RT (2012) Aggrecan: beyond cartilage and into the brain. Int J Biochem Cell Biol 44(5):690–693. https://doi.org/10.1016/j.biocel.2012.01.010

Murakami T, Tsubouchi Y, Tsubouchi M, Ohtsuka A, Taguchi T, Kikuta A (1993) The occurrence of rat spinal cord neurons with strongly negative-charged surface coats. Arch Histol Cytol 56 (5):501–504

Murdoch AD, Dodge GR, Cohen I, Tuan RS, Iozzo RV (1992) Primary structure of the human heparan sulfate proteoglycan from basement membrane (HSPG2/perlecan). A chimeric molecule with multiple domains homologous to the low density lipoprotein receptor, laminin, neural cell adhesion molecules, and epidermal growth factor. J Biol Chem 267(12):8544–8557

Nicholson C, Sykova E (1998) Extracellular space structure revealed by diffusion analysis. Trends Neurosci 21(5):207–215

Occhi S, Zambroni D, Del Carro U, Amadio S, Sirkowski EE, Scherer SS et al (2005) Both laminin and Schwann cell dystroglycan are necessary for proper clustering of sodium channels at nodes of Ranvier. J Neurosci 25(41):9418–9427. https://doi.org/10.1523/JNEUROSCI.2068-05.2005

Oegema TR Jr, Hascall VC, Dziewiatkowski DD (1975) Isolation and characterization of proteoglycans from the swarm rat chondrosarcoma. J Biol Chem 250(15):6151–6159

Ogawa T, Hagihara K, Suzuki M, Yamaguchi Y (2001) Brevican in the developing hippocampal fimbria: differential expression in myelinating oligodendrocytes and adult astrocytes suggests a dual role for brevican in central nervous system fiber tract development. J Comp Neurol 432 (3):285–295

Ogawa H, Oohashi T, Sata M, Bekku Y, Hirohata S, Nakamura K et al (2004) Lp3/Hapln3, a novel link protein that co-localizes with versican and is coordinately up-regulated by platelet-derived growth factor in arterial smooth muscle cells. Matrix Biol 23(5):287–298. https://doi.org/10.1016/j.matbio.2004.07.001

Oohashi T, Bekku Y (2007) Brain link proteins: neuromodulators for scaffolding the specialized hyaluronan-binding extracellular milieu in the brain. In: Maeda N (ed) Neural proteoglycans. Research Signpost, India, pp 67–83

Oohashi T, Hirakawa S, Bekku Y, Rauch U, Zimmermann DR, Su WD et al (2002) Bral1, a brain-specific link protein, colocalizing with the versican V2 isoform at the nodes of Ranvier in developing and adult mouse central nervous systems. Mol Cell Neurosci 19(1):43–57. https://doi.org/10.1006/mcne.2001.1061

Osborne-Lawrence SL, Sinclair AK, Hicks RC, Lacey SW, Eddy RL Jr, Byers MG et al (1990) Complete amino acid sequence of human cartilage link protein (CRTL1) deduced from cDNA clones and chromosomal assignment of the gene. Genomics 8(3):562–567

Pesheva P, Spiess E, Schachner M (1989) J1-160 and J1-180 are oligodendrocyte-secreted nonpermissive substrates for cell adhesion. J Cell Biol 109(4 Pt 1):1765–1778

Quick DC, Waxman SG (1977) Ferric ion, ferrocyanide, and inorganic phosphate as cytochemical reactants at peripheral nodes of Ranvier. J Neurocytol 6(5):555–570

Ratcliffe CF, Qu Y, McCormick KA, Tibbs VC, Dixon JE, Scheuer T, Catterall WA (2000) A sodium channel signaling complex: modulation by associated receptor protein tyrosine phosphatase beta. Nat Neurosci 3(5):437–444. https://doi.org/10.1038/74805

Rauch U, Gao P, Janetzko A, Flaccus A, Hilgenberg L, Tekotte H et al (1991) Isolation and characterization of developmentally regulated chondroitin sulfate and chondroitin/keratan sulfate proteoglycans of brain identified with monoclonal antibodies. J Biol Chem 266 (22):14785–14801

Rauch U, Karthikeyan L, Maurel P, Margolis RU, Margolis RK (1992) Cloning and primary structure of neurocan, a developmentally regulated, aggregating chondroitin sulfate proteoglycan of brain. J Biol Chem 267(27):19536–19547

Rauch U, Hirakawa S, Oohashi T, Kappler J, Roos G (2004) Cartilage link protein interacts with neurocan, which shows hyaluronan binding characteristics different from CD44 and TSG-6. Matrix Biol 22(8):629–639. https://doi.org/10.1016/j.matbio.2003.11.007

Ripellino JA, Margolis RU, Margolis RK (1989) Immunoelectron microscopic localization of hyaluronic acid-binding region and link protein epitopes in brain. J Cell Biol 108(5):1899–1907

Rodriguez E, Roughley P (2006) Link protein can retard the degradation of hyaluronan in proteoglycan aggregates. Osteoarthr Cartil 14(8):823–829. https://doi.org/10.1016/j.joca.2006.02.008

Roitbak T, Sykova E (1999) Diffusion barriers evoked in the rat cortex by reactive astrogliosis. Glia 28(1):40–48

Rossier J, Bernard A, Cabungcal JH, Perrenoud Q, Savoye A, Gallopin T, Hawrylycz M, Cuénod M, Do K, Urban A, Lein ES (2015) Cortical fast-spiking parvalbumin interneurons enwrapped in the perineuronal net express the metallopeptidases Adamts8, Adamts15 and Neprilysin. Mol Psychiatry 20(2):154–161. https://doi.org/10.1038/mp.2014.162

Saito F, Moore SA, Barresi R, Henry MD, Messing A, Ross-Barta SE et al (2003) Unique role of dystroglycan in peripheral nerve myelination, nodal structure, and sodium channel stabilization. Neuron 38(5):747–758

Schmalfeldt M, Bandtlow CE, Dours-Zimmermann MT, Winterhalter KH, Zimmermann DR (2000) Brain derived versican V2 is a potent inhibitor of axonal growth. J Cell Sci 113 (Pt 5):807–816

Scott JE (1989) Ion binding: patterns of 'affinity' depending on types of acid groups. Symp Soc Exp Biol 43:111–115

Seidenbecher CI, Richter K, Rauch U, Fässler R, Garner CC, Gundelfinger ED (1995) Brevican, a chondroitin sulfate proteoglycan of rat brain, occurs as secreted and cell surface glycosylphosphatidylinositol-anchored isoforms. J Biol Chem 270(45):27206–27212

Seidenbecher CI, Gundelfinger ED, Bockers TM, Trotter J, Kreutz MR (1998) Transcripts for secreted and GPI-anchored brevican are differentially distributed in rat brain. Eur J Neurosci 10 (5):1621–1630

Serwanski DR, Jukkola P, Nishiyama A (2017) Heterogeneity of astrocyte and NG2 cell insertion at the node of ranvier. J Comp Neurol 525(3):535–589

Seyfried NT, McVey GF, Almond A, Mahoney DJ, Dudhia J, Day AJ (2005) Expression and purification of functionally active hyaluronan-binding domains from human cartilage link protein, aggrecan and versican: formation of ternary complexes with defined hyaluronan oligosaccharides. J Biol Chem 280(7):5435–5448

Shi S, Grothe S, Zhang Y, O'Connor-McCourt MD, Poole AR, Roughley PJ, Mort JS (2004) Link protein has greater affinity for versican than aggrecan. J Biol Chem 279(13):12060–12066

Smith PD, Coulson-Thomas VJ, Foscarin S, Kwok JC, Fawcett JW (2015) "GAG-ing with the neuron": the role of glycosaminoglycan patterning in the central nervous system. Exp Neurol 274(Pt B):100–114. https://doi.org/10.1016/j.expneurol.2015.08.004

Spicer AP, Joo A, Bowling RA Jr (2003) A hyaluronan binding link protein gene family whose members are physically linked adjacent to chondroitin sulfate proteoglycan core protein genes: the missing links. J Biol Chem 278(23):21083–21091. https://doi.org/10.1074/jbc.M213100200

Sugiyama S, Di Nardo AA, Aizawa S, Matsuo I, Volovitch M, Prochiantz A, Hensch TK (2008) Experience-dependent transfer of Otx2 homeoprotein into the visual cortex activates postnatal plasticity. Cell 134(3):508–520. https://doi.org/10.1016/j.cell.2008.05.054

Susuki K, Chang KJ, Zollinger DR, Liu Y, Ogawa Y, Eshed-Eisenbach Y et al (2013) Three mechanisms assemble central nervous system nodes of Ranvier. Neuron 78(3):469–482. https://doi.org/10.1016/j.neuron.2013.03.005

Sykova E, Vorisek I, Mazel T, Antonova T, Schachner M (2005) Reduced extracellular space in the brain of tenascin-R- and HNK-1-sulphotransferase deficient mice. Eur J Neurosci 22 (8):1873–1880. https://doi.org/10.1111/j.1460-9568.2005.04375.x

Vigetti D, Andrini O, Clerici M, Negrini D, Passi A, Moriondo A (2008) Chondroitin sulfates act as extracellular gating modifiers on voltage-dependent ion channels. Cell Physiol Biochem 22 (1–4):137–146. https://doi.org/10.1159/000149791

Virgintino D, Perissinotto D, Girolamo F, Mucignat MT, Montanini L, Errede M, Kaneiwa T, Yamada S, Sugahara K, Roncali L, Perris R (2009) Differential distribution of aggrecan isoforms in perineuronal nets of the human cerebral cortex. J Cell Mol Med 13 (9B):3151–3173. https://doi.org/10.1111/j.1582-4934.2009.00694.x

Vorisek I, Sykova E (1997) Evolution of anisotropic diffusion in the developing rat corpus callosum. J Neurophysiol 78(2):912–919

Waxman SG, Quick DC (1978) Intra-axonal ferric ion-ferrocyanide staining of nodes of Ranvier and initial segments in central myelinated fibers. Brain Res 144(1):1–10

Weber P, Bartsch U, Rasband MN, Czaniera R, Lang Y, Bluethmann H et al (1999) Mice deficient for tenascin-R display alterations of the extracellular matrix and decreased axonal conduction velocities in the CNS. J Neurosci 19(11):4245–4262

Wintergerst ES, Fuss B, Bartsch U (1993) Localization of janusin mRNA in the central nervous system of the developing and adult mouse. Eur J Neurosci 5(4):299–310

Xiao ZC, Bartsch U, Margolis RK, Rougon G, Montag D, Schachner M (1997) Isolation of a tenascin-R binding protein from mouse brain membranes. A phosphacan-related chondroitin sulfate proteoglycan. J Biol Chem 272(51):32092–32101

Xiao ZC, Ragsdale DS, Malhotra JD, Mattei LN, Braun PE, Schachner M, Isom LL (1999) Tenascin-R is a functional modulator of sodium channel beta subunits. J Biol Chem 274 (37):26511–26517

Yamada H, Watanabe K, Shimonaka M, Yamaguchi Y (1994) Molecular cloning of brevican, a novel brain proteoglycan of the aggrecan/versican family. J Biol Chem 269(13):10119–10126

Yamada H, Fredette B, Shitara K, Hagihara K, Miura R, Ranscht B et al (1997) The brain chondroitin sulfate proteoglycan brevican associates with astrocytes ensheathing cerebellar glomeruli and inhibits neurite outgrowth from granule neurons. J Neurosci 17(20):7784–7795

Yamaguchi Y (2000) Lecticans: organizers of the brain extracellular matrix. Cell Mol Life Sci 57 (2):276–289. https://doi.org/10.1007/PL00000690

Zamecnik J, Vargova L, Homola A, Kodet R, Sykova E (2004) Extracellular matrix glycoproteins and diffusion barriers in human astrocytic tumours. Neuropathol Appl Neurobiol 30 (4):338–350. https://doi.org/10.1046/j.0305-1846.2003.00541.x

Oligodendrocyte Physiology Modulating Axonal Excitability and Nerve Conduction

9

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Abstract

Oligodendrocytes enable saltatory conduction by forming a myelin sheath around axons, dramatically boosts action potential conduction velocity. In addition to this canonical function of oligodendrocytes, it is now known that oligodendrocytes can respond to neuronal activity and regulate axonal conduction. Importantly, white matter plasticity, including adaptive responses by oligodendrocytes, has been shown to be involved in learning and memory. In this chapter, the role of oligodendrocytes in axonal conduction and axonal excitability will be reviewed. Focus will be paid to the mechanisms through which oligodendrocytes, including perineuronal oligodendrocytes, facilitate and suppress axonal conduction.

Keywords

Action potential · Conduction velocity · Hippocampus · Myelin

9.1 Introduction

All activity of the nervous system, from basic reflexes to cognition, depends on the propagation of action potentials, the principal method of carrying signals from one neuron to the next. A variety of information is encoded in the frequency of action potentials and by the arrangement and number of firing neurons within neuronal circuits. Changes in axonal conduction influence timing of synaptic transmission and signal processing in neuronal circuits. When action potentials from different neurons reach the same postsynaptic target within milliseconds of each other, the postsynaptic response is increased by temporal summation. Such simultaneous input is

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required for associative learning. Fine-tuning of action potential conduction, therefore, contributes to precise information processing. In addition to synaptic transmission, which is capable of plastic changes that are fundamental to the storage of information in the brain (Bliss and Collingridge 1993; Malenka and Bear 2004), axonal conduction of action potentials exhibits activity-dependent plasticity in both myelinated and unmyelinated fibers (Debanne et al. 2011; Chida et al. 2015; Yamazaki et al. 2014). Oligodendrocytes are the myelinating glial cells of the central nervous system. Each oligodendrocyte is capable of myelinating 10–30 axons. These cells play an essential role in axonal conduction plasticity in myelinated fibers and participate in fine temporal regulation of neuronal activities. Moreover, these functional changes in conduction velocity and axonal excitability, especially along myelinated fibers, could underlie the concept of white matter plasticity, which is critical for learning and higher-level cognitive function (Fields 2010).

In this chapter, we review the regulation of axonal excitability and conduction velocity in information processing, for both unmyelinated and myelinated axons, and summarize the evidence for the facilitation of axonal conduction by oligodendrocyte depolarization. We have expanded our investigation of the precise characteristics of facilitative effects of oligodendrocytes using optogenetics. Lastly, we have suggested a suppressive regulation of axonal conduction by oligodendrocytes and the involvement of perineuronal oligodendrocytes in the regulation of neuronal activities.

The properties of action potential propagation along axons are described using the following terms: axonal excitability, conduction velocity, and axonal conduction. Axonal excitability is defined as the threshold current to generate action potentials in axons. The conduction velocity is the speed at which action potentials travel along axons. Although changes in axonal excitability are correlated with changes in conduction velocity, the correlation is often non-linear, or negative in some cases. Throughout this chapter, axonal conduction is used in a broader sense, encompassing both axonal excitability and conduction velocity, to describe the propagation of action potential along axons.

9.2 Modulation of Axonal Excitability

The primary contributors to axonal excitability are (1) ion channel activation for action potential generation and (2) maintenance of the local ionic environment to allow for action potential propagation as needed (Kiernan and Kaji 2013). In myelinated axons, axonal excitability depends on the activity of both nodal and internodal ion channels. In all axons, axonal excitability is evaluated by measuring the threshold current that just evokes an action potential in an axon. Disrupted axonal excitability results in inadequate axonal conduction, which can alter action potential timing and impair information processing.

Axonal excitability is regulated in several different ways, including by activity-dependent mechanisms and neurotransmitter-mediated mechanisms. There are some forms of activity-dependent changes in axonal excitability, for example, the

supernormal and subnormal activity states that comprise the recovery cycle in response to neuronal stimulation. These well-known forms of short-term changes in axonal excitability are observed in both unmyelinated and myelinated axons in the peripheral and central nervous systems. The mechanisms of the recovery cycle are reviewed in detail elsewhere (Bucher and Goaillard 2011). The recovery cycle is intrinsically modulated, that is, axons regulate their excitability according to repetitively stimulation. In addition to these intrinsic mechanisms, axonal excitability is also affected by neurotransmitters and neuromodulators released from cells, which bind to the receptors expressed on the axon proper. It has long been known that the receptors for neurotransmitters, including glutamate, gamma-aminobutyric acid (GABA), serotonin, acetylcholine, and adenosine, are expressed on axons at some distance from the axonal initial segment and presynaptic terminal (i.e., axon proper). In-depth information about the evidence of neurotransmitter receptors on the axon proper has been discussed elsewhere (Bucher and Goaillard 2011; Debanne et al. 2011).

More recently, the effects of $GABA_A$ receptor activation on axonal conduction have been studied from a novel perspective. It was found that photolysis of caged GABA enhanced the amplitude of compound action potentials (CAPs) in parallel fibers in mouse cerebellum through activation of $GABA_A$ receptors (Dellal et al. 2012). This CAP enhancement was associated with the recruitment of more axons due to increases in firing probability. A simulation study using a Hodgkin-Huxley compartmental model suggested that $GABA_A$ receptor activation led to a decrease in action potential threshold that was dependent on the reversal potential of chloride. A conditioning stimulus, consisting of 20 pulses at 1 Hz, suppressed action potential conduction along rat hippocampal mossy fibers (Chida et al. 2015). Although the changes in axonal membrane potentials and firing probability induced by the conditioning stimulus were not directly examined, it is possible that the conditioning stimulus either hyperpolarized axonal membrane potentials as a result of the increase in intracellular Na^+ concentration and the subsequent increase in Na^+/K^+-ATPase activity or depolarized axonal membrane potentials by glutamate receptor activation. In the former case, the threshold for an action potential is increased by the membrane potential, while, in the latter case, sodium channel availability is decreased due to sodium channel inactivation; however, both result in the suppression of axonal conduction.

In myelinated axons in the central nervous system, GABA causes a reduction in amplitude and an increase in latency of CAPs through activating $GABA_A$ receptors in the spinal cord (Sakatani et al. 1991). Blockade of Cl^- transport by the diuretic furosemide significantly decreased the fiber volley and increased the latency to peak activity in field recordings of both Schaffer collaterals and alvear fibers in rat hippocampal slices, indicating a decrease in axonal excitability (Andreasen and Nedergaard 2017). This effect could be induced by mechanisms such as hyperpolarization of axonal membranes.

Axonal excitability is also modulated by the application of direct current to the brain (e.g., transcranial direct current stimulation, which is used to improve symptoms of neurological and psychiatric disorders) or spinal cord (e.g., trans-

spinal direct current stimulation, which is used to ameliorate disease sequelae locally at the spinal cord). Transcranial direct current stimulation of cerebral cortex in humans has been found to increase the corticospinal volleys, which are evoked by transcranial magnetic stimulation arranged to activate the corticospinal axons just below the motor cortex and are recorded at cervical epidural level (Di Lazzaro et al. 2013). Significant increase in corticospinal axonal activity is maintained for 15 min after the end of transcranial direct current stimulation, indicating that myelinated axons are capable of long-lasting facilitation of neuronal excitability. Trans-spinal direct current stimulation to the rostral portion of the lateral funiculus tract elicits a significant increase in CAPs that is immediate and lasting (Ahmed 2014). The magnitude of this increase in axonal excitability in white matter varies depending upon the polarity of trans-spinal direct current stimulation, suggesting that the polarity of the direct current could be a factor in determining the modulation of axonal excitability and its physiological effects. Epidurally applied direct current in rats induces a four- to sevenfold increase in the number of activated fibers in the dorsal column, which lasts for more than 1 h (Jankowska et al. 2017). This increase in excitability occurred in an activity-independent manner. Transcranial direct current stimulation releases noradrenaline, which enhances synaptic transmission in the cortex through the elevation of intracellular Ca^{2+} levels in astrocytes (Monai et al. 2016). Oligodendrocytes and myelin may also be involved in the effects of transcranial direct current stimulation on axonal excitability.

9.3 Modulation of Conduction Velocity

An action potential generated at one point of the axon causes a spread in depolarization that propagates the action potential. This phenomenon occurs like a chain reaction with action potentials being carried along axons by continuous spread in unmyelinated axons and by saltatory conduction in myelinated axons. Conduction velocity refers to the speed of propagation of an electrical impulse along an axon and is calculated by dividing the distance the signal traveled by the time it took to travel that distance. Conduction velocity is determined by how fast the membrane depolarizes and reaches threshold, and in myelinated fibers, it depends on axonal (diameter and axoplasmic conductance), nodal (area, capacitance, and conductance), and internodal (internodal length and myelin capacitance) parameters (Ritchie 1995). Clearly, spike conduction along axons is necessary for transmission between separate brain regions. Therefore, the conduction velocity must be carefully regulated.

Conduction velocity varies depending upon the neuronal region. In the sensory and motor pathways of the spinal cord, it is important to maintain fast conduction velocity for speedy detection of the environment and timely motor responses. In the auditory system, the interaural time difference that is used for precise sound localization is achieved by synchronizing action potential activity rather than necessarily permitting each neuron to signal action potentials at maximum conduction velocity. For associative learning processes, the action potentials that propagate individual

pieces of information should be time-matched to the same target. The conduction velocity, together with the length of the axon, also determines the conduction delay, which itself governs the timing of information transfer within neural circuits and has a function for temporal neural coding (Bucher 2016). In the bird auditory brain stem, the difference in conduction velocity between ipsilateral and contralateral fibers for a single axon has been demonstrated; the conduction velocity in the contralateral axon branch is approximately 2.3 times faster than the conduction velocity in the ipsilateral axonal branch of the same neuron (Seidl et al. 2014). This differential adjustment in conduction velocity helps to overcome the restrictions imposed by axon lengths and provides temporal accuracy of binaural activity onto the coincidence-detector neurons. Achievement of the adequate conduction velocity is established by local interactions of axon segments with oligodendrocytes, which relates to the spacing of the nodes of Ranvier (internodal distance).

In addition to axonal excitability, the conduction velocity is modulated by neuroactive substances. The conduction velocity of action potentials along rat hippocampal mossy fibers was decreased by repetitive low-frequency stimulation, which lasted for approximately 30 s (Chida et al. 2015). Although inactivation of voltage-dependent Na^+ channels is a possible mechanism in such activity-dependent modulation of conduction velocity, the Chida et al. (2015) study demonstrated that activation of GluK1-containing kainate receptors on the axon proper of mossy fibers is involved in the suppression of action potential conduction velocity. It is possible that repetitive axonal stimulation leads to excess glutamate release that is then leaked from the synaptic cleft and activates GluK1 glutamate receptors on the axon proper of nearby axons. In addition, an accumulation of evidence has demonstrated that glial cells release neurotransmitters and neuromodulators via various mechanisms. Moreover, substances could be released from the axon proper by vesicular (Kukley et al. 2007; Micu et al. 2016; Ziskin et al. 2007) and non-vesicular (Fields 2011) means. Glutamate released in this way might also activate GluK1-containing kainate receptors on mossy fibers.

Pharmacological blockage of gap junctions slows conduction velocity in hippocampal myelinated axons (Adriano et al. 2011). Similar suppression of conduction velocity is observed either in anoxic conditions or by application of a Na^+/K^+ ATPase blocker. Moreover, the effect of gap junction blockade is reversed by increasing the stored energy of the tissue by creatine treatment, indicating that oligodendrocytes synthesize ATP and transfer it to the axon through gap junctions and regulate the conduction velocity by affecting metabolic aspects.

The interaction between myelinated axons and oligodendrocytes has been shown to modulate conduction velocity in rats by our group (Yamazaki et al. 2007). The myelin sheaths of the axons of CA1 pyramidal cells are provided by oligodendrocytes located in the alveus. Simultaneous whole-cell recordings from oligodendrocytes and pyramidal cells were performed. During antidromic action potential recordings, oligodendrocytes were repetitively depolarized. The oligodendrocytes were depolarized to between −30 and −20 mVs with a train of current pulses (30 pulses at 1 Hz, duration of each pulse; 500 ms) through a patch electrode. In 4 out of 27 dual whole-cell recordings, significant changes in the latencies were

observed following oligodendrocyte depolarization. The latency of the action potentials was gradually shortened to approximately 90% of control levels and returned to control levels at approximately 20 s after the end of oligodendrocyte depolarization. The amplitude and half-width of the action potentials were not influenced by oligodendrocyte depolarization. This suggests that the conduction velocity could be increased by rapid and dynamic mechanisms involving oligodendrocyte depolarization. Because the extent of oligodendrocyte depolarization is dependent on neuronal function, this activity-dependent axon–oligodendrocyte communication has consequences for facilitation in neuronal circuits.

So far, several forms of conduction velocity modulation have been described. However, it is possible that the conduction velocity itself differs along the axon. In the peripheral nervous system, the conduction velocities vary between the distal and proximal segments of a limb because of differences in nerve diameter and temperature. Although such remarkable differences are unlikely in the central nervous system, it has been reported that, within a given axon, the lengths of the myelinated regions and unmyelinated regions also differ considerably as different neurons show different longitudinal distributions of myelin (Tomassy et al. 2014); however, others have reported that the nodal lengths are similar along a given axon within the optic nerve and cerebral cortex of rats (Arancibia-Cárcamo et al. 2017). The variation in the length of myelinated and unmyelinated regions might account for the non-uniform regulation of conduction velocity along axons. The conduction velocity along myelinated axons is optimal for information processing by adequate internodal length and Ranvier node diameter (Ford et al. 2015). In addition to these anatomical adjustments, the modulation of the conduction velocity by oligodendrocytes is required for fine regulation of axonal activity for proper information processing.

9.4 Magnitude of Oligodendrocyte Depolarization

Oligodendrocytes can respond to neuronal activity with prolonged depolarizations of membrane potential or an increase in the intracellular Ca^{2+} concentration. In addition, the depolarization of oligodendrocytes facilitates the conduction velocity of action potentials along myelinated axons in the hippocampus. Here, it is worth noting that the magnitude of oligodendrocyte depolarization has been observed in previous reports. In cultured oligodendrocytes prepared from mouse spinal cords, the application of 1 mM glutamate or 1 mM GABA induces depolarization from 2 to 5 mV (Kettenmann et al. 1984) or 2 to 8 mV, respectively (Gilbert et al. 1984). In these reports, the depolarization is considered to be an indirect effect, but later, it was found that the application of GABA directly depolarized oligodendrocytes in intact optic nerves in the mice to a similar degree (Butt and Tutton 1992). In that study, activation of $GABA_A$ receptors on oligodendrocytes induced depolarization due to a high intracellular concentration of Cl^- due to active Cl^- transport into oligodendrocytes.

Many studies have measured oligodendrocyte current responses to neurotransmitter receptor activation; however, few reports directly show the magnitude of

depolarization. Therefore, although it may not necessarily be reasonable in electrophysiology, we estimated the magnitude of approximate depolarization from the values of the current responses and the input resistance presented in the previous studies. In corpus callosum oligodendrocytes, application of glutamate, NMDA, and kainate results in a depolarization of approximately 5.5 mV, 4–8 mV, and 5–10 mV, respectively.

Here, it must be mentioned how oligodendrocytes depolarize in response to neuronal activities in physiological conditions. In the stratum radiatum of rat hippocampi, single electrical stimulation of Schaffer collaterals induces inward currents in oligodendrocytes, which results in approximately a 1–4 mV depolarization that is sensitive to Ba^{2+} (Zhou et al. 2007). In myelinating satellite oligodendrocytes in the somatosensory cortex of mice, five action potentials with a frequency of 100 Hz of host neurons resulted in depolarization by only 0.5 mV due to considerable low input resistance (Battefeld et al. 2016).

Although the alveus is classified as white matter, considerable glutamatergic and GABAergic fibers penetrate the alveus. Glutamate and GABA released from these terminals spill out from the synaptic cleft and activate oligodendrocytes. Thus, oligodendrocytes in the alveus can be significantly depolarized through multiple mechanisms. Both exogenous glutamate (applied by injection) and endogenous glutamate (released from axon terminals by electrical stimulation) induce depolarizing responses in alvear oligodendrocytes. Application of GABA onto oligodendrocytes also induces a depolarizing response (Yamazaki et al. 2010). Moreover, neuronal activity induces an increase in extracellular K^+ concentration, resulting in a shift in the equilibrium potential and activation of Ba^{2+}-sensitive K^+ channels, which induce depolarization of oligodendrocytes. Weak tetanic stimulation or theta stimulation protocol depolarizes rat alvear oligodendrocytes (Yamazaki et al. 2007). The depolarization from the theta stimulation protocol lasts for several tens of seconds. Similar results are seen in mouse hippocampal slices to theta stimulation, with an approximately 15 mV depolarization in alvear oligodendrocytes (Yamazaki et al. 2014). Thus, oligodendrocytes have the ability to become significantly depolarized in response to neuronal stimulation.

9.5 Modulatory Effects Oligodendrocyte Depolarization on Axonal Conduction Revealed by Using Optogenetics

9.5.1 Advantage of the Use of the Mice Expressing Channelrhodopsin-2 in Oligodendrocytes

In simultaneous recordings from oligodendrocytes and pyramidal cells (as described in Sect. 9.3) facilitative effects were detected in only four out of 27 recordings. In those four recordings, it was found that the pyramidal cell axons passed through the field where the recorded oligodendrocyte extended processes, which was revealed by post hoc biocytin staining. Further, there was no overlap in pyramidal cell axons and oligodendrocyte processes in any of the recordings where oligodendrocyte

depolarization was not observed. Because the area of oligodendrocyte depolarization is spatially limited to several nearby cells connected by gap junctions, it is difficult to investigate the properties and underlying mechanisms of the interaction between axons and oligodendrocytes. In addition, a more effective and precise technique to study oligodendrocyte depolarization is required since multiple oligodendrocytes align along an axon to form the myelin sheath of a single myelinated axon. Moreover, since oligodendrocyte depolarization is usually induced by electrical stimulation or the application of neurotransmitter receptor agonists and since these manipulations also excite axons, it has not been possible to evaluate properly the modulatory effects on axons by oligodendrocyte depolarization using such previous methods. To depolarize oligodendrocytes separately from neuronal activities, we used mice expressing channelrhodopsin-2 in oligodendrocytes (Tanaka et al. 2012). The C128S mutant of channelrhodopsin-2 was chosen because it has a higher sensitivity to blue light, which is expected to depolarize cells to physiological levels, and because its channel is closed by a pulse of yellow light, enabling the precise control of the duration of depolarization. Blue light pulses induced significant and reproducible depolarization in current-clamped oligodendrocytes in the alveus of the hippocampus. As the duration of the light pulse increased, the peak oligodendrocyte depolarization amplitude increased. The depolarization continued for several tens of seconds and then gradually returned to the baseline membrane potential (Yamazaki et al. 2014). These depolarization values are comparable to those induced by theta-burst stimulation, as described in the previous section, indicating that physiological levels of depolarization can be achieved by photostimulation.

9.5.2 Facilitation of Axonal Conduction by Oligodendrocyte Depolarization

To examine the effects of oligodendrocyte depolarization on the axonal conduction of action potentials, CAPs in the alveus were recorded extracellularly. Specifically, the amplitude of the CAP (an indicator of the number of activated axons, the duration of each action potential, and the timing of the conduction of multiple action potentials) was measured. The relationship between the magnitude of oligodendrocyte depolarization and the changes in CAP amplitude was obtained in order to study the properties of modulatory effects by oligodendrocytes. To manipulate the magnitude of depolarization, blue light pulses with different durations (5, 10, 25, 500 ms), or combinations of 500 ms blue light and yellow shutdown light (10 s) at different intervals (1, 10, 30, and 60 s) from the end of blue light were applied. As shown in Fig. 9.1, a large and prolonged depolarization of oligodendrocytes was required to induce the facilitative effects on axonal conduction. Further, the increase of CAP amplitude shows two different phases (early-onset short-lasting phase and late-onset long-lasting phase). When the oligodendrocyte depolarization was large enough (500 ms of photostimulation only or 500 ms of photostimulation followed by yellow light after a 60 s delay), the amplitude of the CAP showed both immediate increases (early phase which was measured 1–3 min after photostimulation; Fig. 9.1b) and

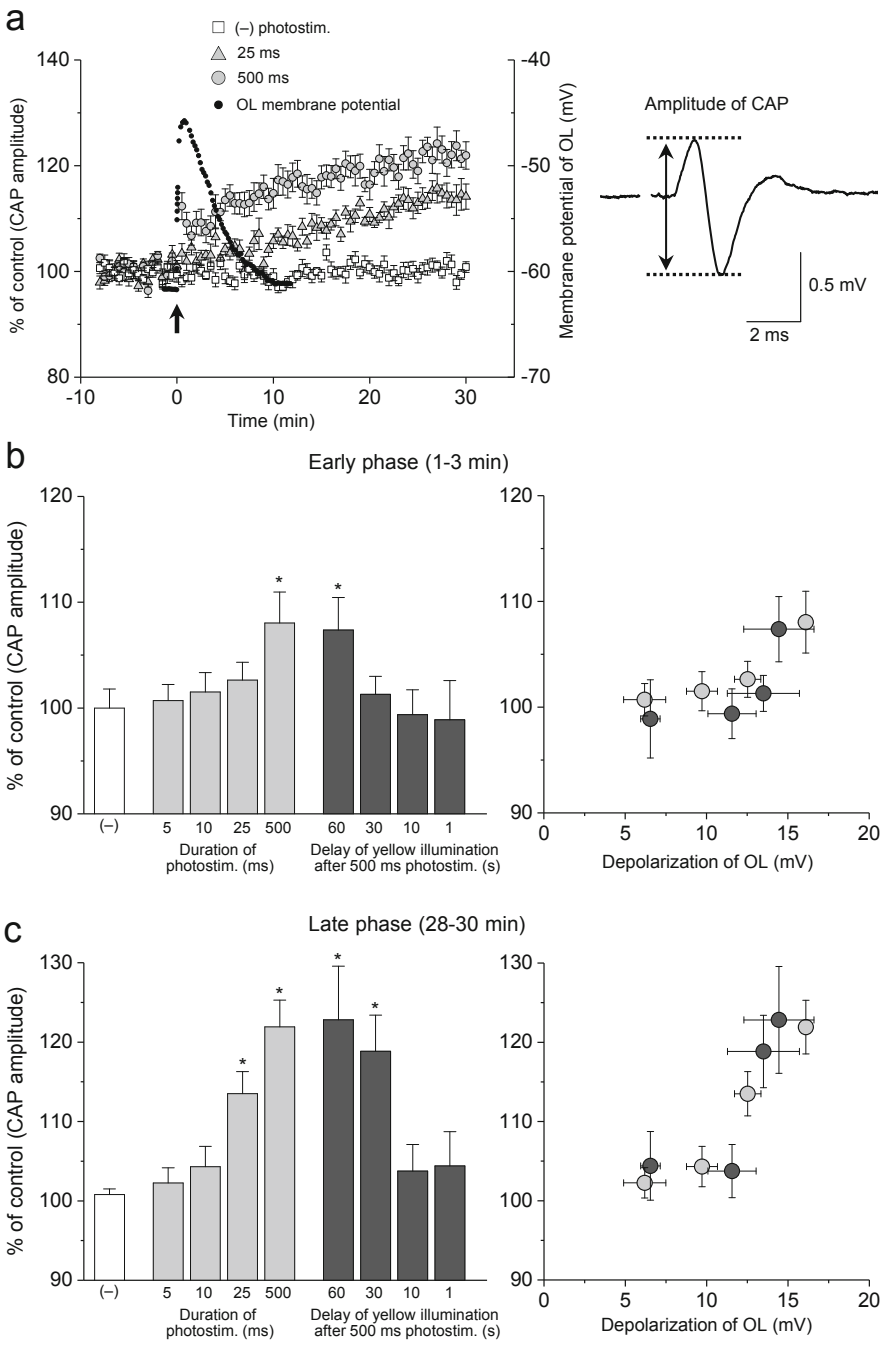

Fig. 9.1 Oligodendrocyte depolarization increases CAP amplitude in a manner dependent on the magnitude of oligodendrocyte depolarization in the alveus of the hippocampus in mice expressing channelrhodopsin-2 in oligodendrocytes. (**a**) Measurement of the amplitude of CAP for evaluation of axonal conduction (right). Changes in CAP amplitude induced by blue light photostimulation for

continuous increases, which reached a plateau after approximately 25 min (late phase which is measured after 28–30 min; Fig. 9.1c). On the other hand, when depolarization was moderate (25 ms photostimulation only or 500 ms photostimulation followed by yellow light after a 30 s delay), the CAP amplitude showed only late-phase increases. When depolarization was small, there was no significant change in the CAP amplitude.

9.5.3 Early-Onset and Short-Lasting Facilitation of Axonal Conduction Induced by Oligodendrocyte Depolarization

What happens in the early phase of axonal conduction? To address this issue, the CAP was analyzed for another parameter, the width of the CAP waveform. Using a 500-ms light pulse, a transient decrease in the width of the CAP waveform at the early phase was observed; the width then gradually returned to baseline levels after approximately 10 min (Fig. 9.2a). In the late phase of axonal conduction, there was no significant change in CAP width. The area of the CAP waveform, which reflects both the number of activated axons and the magnitude of each action potential, was also measured. The CAP area does not change immediately after light stimulation, but then gradually increases. As shown in Fig. 9.2a, these changes in the early phase of axonal conduction sharpened the waveform. It was speculated that the conduction velocity of each action potential is increased during this period and that this change sharpens the CAP waveform. To test this, the effects of oligodendrocyte depolarization on the conduction velocity of action potentials were examined. Antidromically conducted action potentials, which were evoked at axon proper by electrical stimulation, were recorded from CA1 pyramidal cells by performing whole-cell recordings. To evaluate the conduction velocities, the latencies of action potentials were measured. Initially, action potential latencies were decreased by light stimulation of oligodendrocytes that continued for approximately 10 min (Fig. 9.2b). Because a decrease in action potential latency mainly reflects an increase in the action potential conduction velocity, oligodendrocyte depolarization increased the conduction velocity during this period. The changes in CAP width and the latency of single action potentials showed a similar time course. During the early phase of CAP waveform changes, decreases in axonal latencies resulted in the simultaneous arrival

Fig. 9.1 (continued) 25 or 500 ms, starting at 0 min (arrow), expressed as a percentage of the mean CAP amplitude during the 8 min before delivery of the light pulse (left). The white squares are the data without photostimulation. The black plot shows a typical change in the oligodendrocyte (OL) membrane potential evoked by 500 ms of photostimulation. (**b**, **c**) Summary histograms for the change in CAP amplitude with no stimulation (−), after photostimulation for the indicated time, or after 500 ms photostimulation followed by yellow shutdown light (10 s) applied at the indicated time delay (left), and the relationship between peak depolarization of oligodendrocytes and the change in CAP amplitude (right) in 1–3 min (early phase of the change in axonal conduction; **b**) and in 28–30 min (late phase of the change in axonal conduction; **c**). $*p < 0.05$. Modified from Yamazaki et al. (2014), with permission

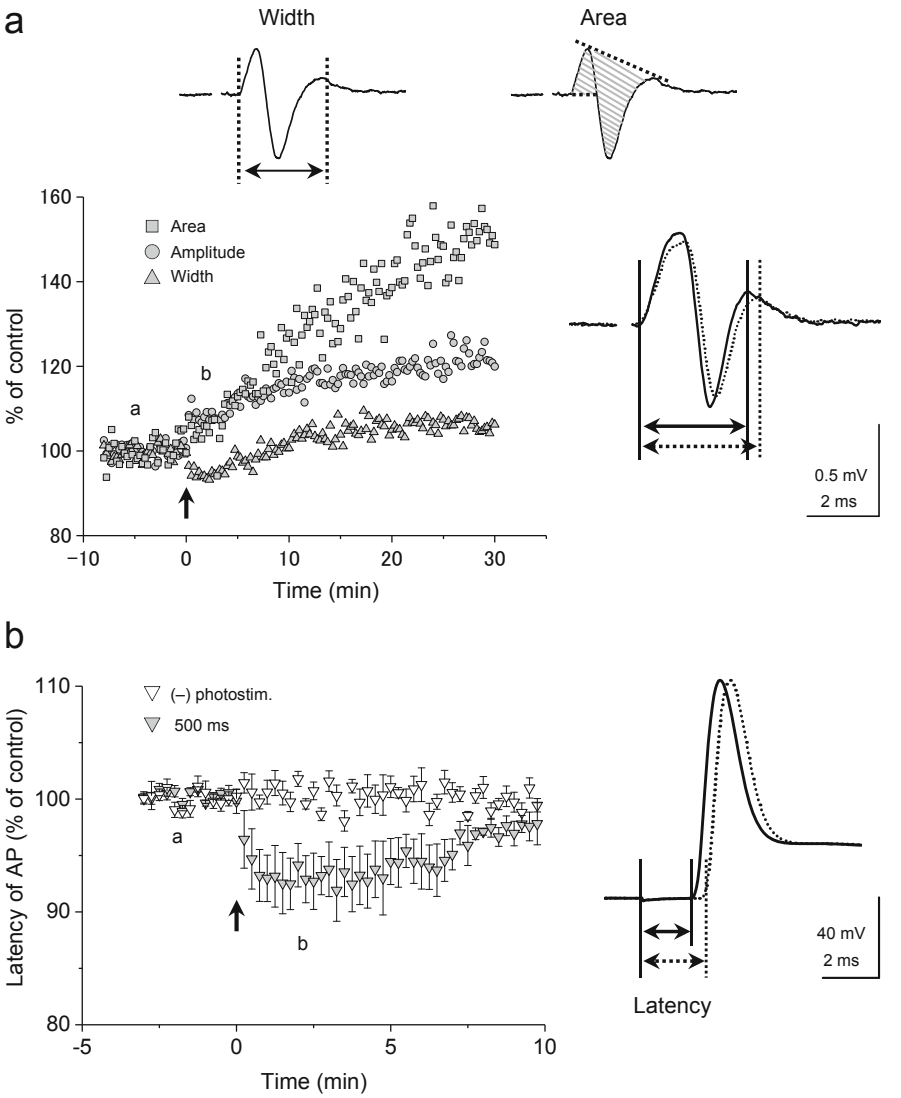

Fig. 9.2 Changes in CAP parameters and axonal latency induced by oligodendrocyte depolarization. (**a**) Measurement of the width and area of the CAP (top). Oligodendrocyte depolarization induced by 500 ms of photostimulation decreases CAP width, but not CAP area, in the early phase, while it increases CAP area, but not CAP width, in the late phase of the modification (bottom, left). CAP waveforms recorded at the times indicated by (**a**) (dotted line) and (**b**) (continuous line; bottom, right). (**b**) Measurement of the conduction latency of the antidromic action potential recorded from CA1 pyramidal cells, which are recorded at the times indicated by (**a**) (dotted line) or (**b**) (continuous line) in the left panel. Time-course of the latency of action potentials after 500 ms of photostimulation, expressed as a percentage of the mean value of the action potential (AP) latency recorded during the 3 min before delivery of the light pulse

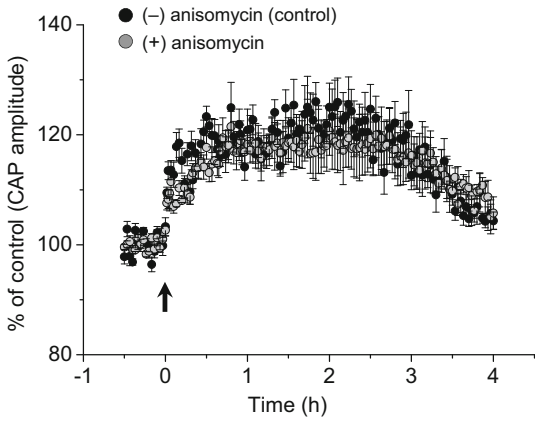

Fig. 9.3 Long-lasting facilitation of CAP amplitude induced by oligodendrocyte depolarization. Long-term plots of the change in CAP amplitude induced by photostimulation in the absence (control) and presence of 20 μM anisomycin, expressed as a percentage of the mean CAP amplitude during the 30 min before delivery of the light pulse. Photo stimulation for 500 ms is applied at 0 min, indicated by the arrow. Modified from Yamazaki et al. (2014), with permission

of multiple action potentials to the recording electrode without changing the number of activated axons, that is, synchronization of action potentials, leading to an increase in the CAP amplitude, a decrease in CAP width, and no change in CAP area. While the detailed mechanisms for this modulation have not yet been elucidated, since insulation at paranodal and internodal regions is important for saltatory conduction (Hirano and Llena 1995), a change in myelination resulting from the structural changes in oligodendrocyte processes by depolarization may be involved (Yamazaki et al. 2010) in conjunction with the change in K^+ channels (described in Sect. 9.5.6).

9.5.4 Late-Onset and Long-Lasting Facilitation of Axonal Conduction Induced by Oligodendrocyte Depolarization

Regarding the late-phase changes in axonal conduction, we investigated the duration of this type of modulation and considered possible mechanisms for changes during this period. For functional plasticity in the nervous system, it should be mentioned how long the plastic changes are maintained to consider its feature and importance for physiological function. We recorded CAPs for 4 h after blue light stimulation to address this issue. As shown in Fig. 9.3, long-term facilitation of axonal conduction is maintained for more than 3 h. Thus, similar to synaptic responses, axonal conduction shows long-term functional plasticity. Since long-term potentiation (LTP) at synapses, which is the most studied type of neural plasticity, can be divided into several temporal stages with late-phase LTP requiring new protein synthesis, we applied the protein translation inhibitor anisomycin throughout recordings. Long-

lasting increases in CAP amplitude following photostimulation were still observed, similar to controls. Thus, the long-term facilitation of axonal conduction did not require new protein synthesis.

During the late phase of axonal conduction, both amplitude and area of the CAP increased, while the width was about the same as before light stimulation (Fig. 9.2a). Because the decreased action potential latency returned to baseline levels after 10 min (Fig. 9.2b), it is not likely that an increase in conduction velocity was involved in the increase of the CAP waveform parameters at late phase. Since the magnitude of the CAP area reflects the number of activated axons and axonal excitability depends on the properties of nodal and internodal ion channels, which are influenced by myelination, it is possible that an increase in the number of axons activated by electrical stimulation at a given intensity is due to a decrease in firing threshold. The fact that myelination affects the firing rate also supports this rationale. An increase in amplitude or duration of each action potential due to changes in Na^+ and/or K^+ channel properties at nodes of Ranvier is also possible.

9.5.5 Oligodendrocyte Depolarization Facilitates Axonal Conduction in Different Brain Regions

There are more general issues concerning the modulation of axonal conduction induced by oligodendrocyte depolarization. It is worth noting whether oligodendrocyte depolarization exhibits similar facilitation of axonal conduction in other locations. To address this issue, similar recordings of CAPs were performed in the corpus callosum, the best brain region for studying axonal conduction. The oligodendrocytes in this region were depolarized by different durations of blue light pulses as were performed in the alveus (Fig. 9.4). In the corpus callosum, oligodendrocyte depolarization increased the CAP amplitude at both the early and late phases of axonal conduction as was seen in the alveus, although the magnitude of the increase tended to be smaller than that in the alveus (Fig. 9.4b, c). Thus, oligodendrocyte depolarization may have similar effects in myelinated axons of different brain regions.

9.5.6 Involvements of K^+ Channels in the Facilitative Effects on Axonal Conduction

In communication between neurons and glia, some chemicals released from glial cells (gliotransmitters) participate in the modulation of neuronal activities. While gliotransmitters are primarily released from astrocytes, it is reported that oligodendrocytes also release glutamate (Bagayogo and Dreyfus 2009; Frühbeis et al. 2013) and ATP (Domercq et al. 2010) to signal to axons. Therefore, we examined whether the modulation of axonal conduction is induced by the same mechanisms involved in the activation of neurotransmitter receptors. The pharmacological experiments are performed by applying various neurotransmitter receptor

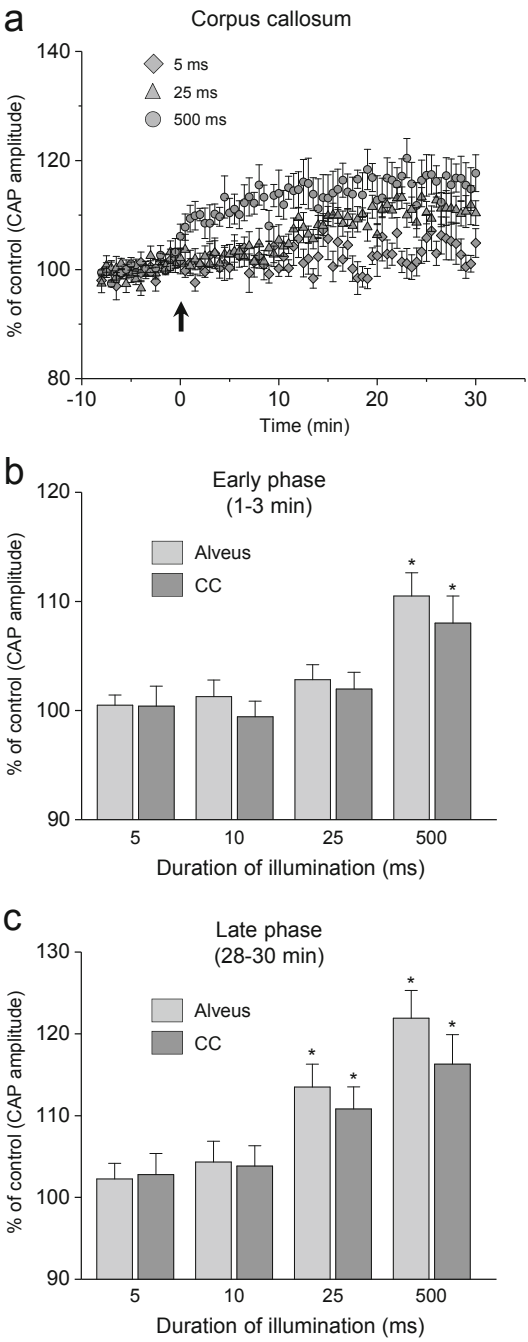

Fig. 9.4 Oligodendrocyte depolarization increases CAP amplitude in different brain regions. (**a**) Changes in CAP amplitude induced by blue light photostimulation for 5 ms, 25 ms, or 500 ms, starting at 0 min (arrow), expressed as a percentage of the mean CAP amplitude during the 8-min period before delivery of the light pulse to the corpus callosum. (**b**, **c**) Comparison of the changes in CAP amplitude after photostimulation for the indicated times at 1–3 min (early phase; **b**) and at 28–30 min (late phase; **c**) in the alveus and corpus callosum (CC). $*p < 0.05$

antagonists, including those for glutamate, GABA, and ATP receptors throughout CAP recordings. Antagonists of the $GABA_A$ receptor (bicuculline), the NMDA receptor (D-AP5 or D,L-AP5), and the P2 receptor (PPADS) show no significant effects on either the magnitude of oligodendrocyte depolarization or the increases in CAP parameters in both early and late-phase axonal conduction. Thus, it is unlikely that neurotransmitter receptor activation is involved in the facilitation of axonal conduction.

Since axonal conduction is considerably influenced by changes in the ionic environment, and since oligodendrocytes are depolarized in response to increased extracellular K^+ concentrations, which result from passing action potentials along the axon, it is possible that K^+ channels are involved in the observed axonal plasticity. Therefore, the influence of K^+ channel blockers on axonal conduction by oligodendrocyte depolarization was examined. Ba^{2+} or 4-AP that acts on different K^+ channels was applied throughout recordings. Ba^{2+} inhibited the increase in CAP amplitude at both early and late phases of axonal conduction, while 4-AP inhibited increases in axonal conduction only during the early phase (Fig. 9.5). Since 4-AP has no significant effect on light-evoked oligodendrocyte depolarization, the changes in 4-AP-sensitive K^+ channels expressed on axons, but not on oligodendrocytes, are involved in short-term early-phase axonal conduction plasticity. Although the expression of 4-AP-sensitive K^+ channels in myelinated axons is controversial, it is reported that field potentials that reflect the activities of myelinated axons are influenced by 4-AP application (Preston et al. 1983) in the corpus callosum. Thus, a change in 4-AP-sensitive K^+ channels on the axons of pyramidal cells is a possible mechanism for the early-onset short-term modulation of axonal conduction. In

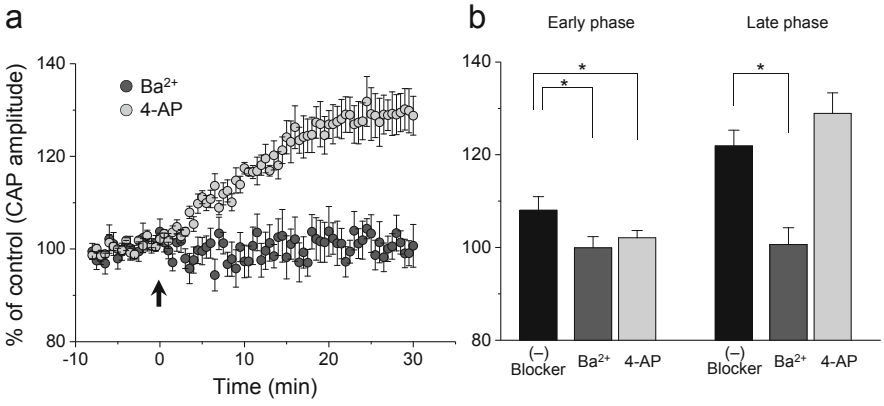

Fig. 9.5 Effects of K^+ channel blockers on the increase in CAP amplitude induced by oligodendrocyte depolarization. (**a**) Changes in CAP amplitude induced by 500 ms of photostimulation in the presence of 100 μM Ba^{2+} or 1 mM 4-AP expressed as a percentage of the mean value of the CAP amplitude recorded during the 8-min period before delivery of the light pulse, indicated by the arrow. (**b**) Summarized results for the effects of blockers on the changes in CAP amplitude at 1–3 min (early phase) and 28–30 min (late phase) after photostimulation. $*p < 0.05$. Modified from Yamazaki et al. (2014) with permission

regards to late-phase axonal conduction, the increase in CAP amplitude was inhibited by application of Ba^{2+}, but not by 4-AP. Ba^{2+} also significantly suppressed the light-evoked depolarization of oligodendrocytes, indicating two possible mechanisms: (1) the inhibition of oligodendrocyte depolarization caused by Ba^{2+} application results in inhibition of the increase in CAP amplitude, or (2) changes in Ba^{2+}-sensitive K^+ channels as a result of oligodendrocyte depolarization contribute to the increase in CAP amplitude. In either cases, Ba^{2+}-sensitive K^+ channels on oligodendrocytes are involved in late-onset long-lasting enhancement of axonal conduction. It has been confirmed that a transient increase in extracellular K^+ concentration itself does not contribute to the increase in CAP amplitude.

9.6 Suppressive Regulation of Axonal Conduction; Possible Involvement of Adenosine A_1 Receptors

For precise control of axonal function, suppressive regulation of the conduction velocity is required. Axonal conduction is decreased by the repetitive firing of action potentials in both peripheral (Wurtz and Ellisman 1986) and central nervous systems (Huff et al. 2011), suggesting intrinsic mechanisms for the suppression in an activity-dependent manner. This type of suppression includes not only short-term changes, such as a subnormal period in the recovery cycle of axonal excitability, but also long-term changes that are maintained for tens of minutes (Bakkum et al. 2008) to several months (Swadlow 1982, 1985).

In addition to the intrinsic mechanisms, the axonal conduction is suppressed by neurotransmitters and neuromodulators, as mentioned previously. It is generally accepted that endogenous adenosine, acting via A_1 receptors, exerts a global and tonic inhibitory modulation on neural activities in the brain (Benarroch 2008; Haas and Selbach 2000). The adenosine released from depolarized glial cells suppressed synaptic transmission and neuronal firing through the activation of A_1 receptors (Yamazaki et al. 2005). Increased adenosine levels facilitate the induction of LTP and attenuate the induction of depotentiation in mice expressing the mutant glial fibrillary acidic protein in astrocytes (Fujii et al. 2014; Lee et al. 2013). Adenosine also acts as an axon–oligodendrocyte transmitter, where the axonal release of adenosine promotes myelination in the nervous system (Stevens et al. 2002) and stimulates cellular migration via adenosine A_1 receptors on oligodendrocyte lineage cells in culture (Othman et al. 2003). While the CAPs recorded from mostly unmyelinated fibers in the corpus callosum are suppressed via application of A_1 receptor agonists (Swanson et al. 1998), the effect of adenosine on axonal conduction of myelinated axons is unclear. The subcellular expression of adenosine A_1 receptors, especially along axons, is contradicted (Swanson et al. 1995; Ochiishi et al. 1999), and the expression of adenosine A_1 receptors on oligodendrocytes in vivo is also obscure. In our recordings, oligodendrocytes are hyperpolarized by application of A_1 receptor agonists. Since depolarization of oligodendrocytes facilitates conduction velocity of action potentials (Yamazaki et al. 2007, 2014) as described above, it is possible that the hyperpolarization of oligodendrocytes shows

opposite effects on axonal conduction. However, hyperpolarization of oligodendrocytes by using optogenetics has no significant effect on the axonal conduction, suggesting that the oligodendrocyte hyperpolarization itself is not involved in the mechanism for the suppression of axonal conduction. Sustained high-frequency stimulation of axons causes morphological changes in paranodal myelin and decreases in the axonal conduction of action potentials in the spinal cord (Huff et al. 2011). Since activation of adenosine A_1 receptors reduce cAMP levels and suppress PKA activity and since PKA phosphorylates proteins related to the regulation of cell morphology, such as cytoskeleton regulatory proteins (Krause et al. 2003), it is possible that the reduction of cAMP levels by activation of adenosine A_1 receptors influence morphological regulation of oligodendrocytes, which affect the extent of the insulation of myelin, resulting in the suppression of axonal conduction.

9.7 Contribution of Perineuronal Oligodendrocytes to the Regulation of Axonal Activities

Oligodendrocytes are composed of a heterogeneous population of cells, which include both myelinating and non-myelinating oligodendrocytes (Del Río Hortega 1928). Although perineuronal oligodendrocytes, the cell bodies of which lie adjacent to neuronal cell bodies, are primarily non-myelinating, it has been reported that perineuronal oligodendrocytes adjacent to cell bodies of pyramidal neurons in somatosensory neocortical layer 5 form compact myelin. These cells limit neuronal excitability of adjacent pyramidal neurons through regulation of extracellular K^+ concentrations by gap–junction coupling with neighboring glial cells that form a syncytium around the soma of host pyramidal cells (Battefeld et al. 2016). It is possible that these perineuronal oligodendrocytes affect axonal excitability and thereby axonal conduction of the axons they provide myelin sheaths to by similar mechanisms.

The perineuronal oligodendrocytes are also found beside interneuronal soma in the hippocampus of rats. In the stratum radiatum, perineuronal oligodendrocytes only have a few processes, which are crooked, intricately twisted, and twined around the soma and proximal region of the dendrites of adjacent interneurons, indicating that these oligodendrocytes are non-myelinating. When interneurons fire at a high frequency, hyperpolarization by perineuronal oligodendrocytes suppress interneuron action potentials through their influence on K^+ channels, which are responsible for deep and fast after hyperpolarization (Yamazaki et al. 2018). In this type of perineuronal oligodendrocyte, there are other processes that seem to have no relationship with the host interneuron, but are probably in contact with the processes of other neurons, including axons. Since the Schaffer collaterals run through this region, it is possible that the perineuronal oligodendrocytes may have a role in modulating axonal excitability by influencing the axonal K^+ channels leading to regulation of the activities of the hippocampal area CA1. Other subclasses of interneurons in the hippocampus also accompany perineuronal oligodendrocytes.

Some oligodendrocytes in the alveus, which show morphological characteristics of mature myelinating oligodendrocytes lie adjacent to the cell bodies of oriens/lacunosum-moleculare (O-LM) neurons, which have a spine-like structure in their dendrites in the stratum oriens and extend their axons toward the stratum lacunosum-moleculare. The majority of the O-LM neurons accompanied by the perineuronal oligodendrocytes express non-desensitizing nicotinic acetylcholine receptors (probably α2 subunit-containing receptors; Jia et al. 2010). In the presence of nicotine, the O-LM neurons are consecutively evoked and influence hippocampal circuits in a layer-specific manner. Application of nicotine induced continuous firing in O-LM neurons, leading to depolarization of perineuronal oligodendrocytes to approximately 3 mV. Tetrodotoxin (TTX) application blocked this effect, indicating that the perineuronal oligodendrocyte depolarization was dependent on neuronal activity. When oligodendrocytes respond to repeated axonal signaling, the magnitude of the depolarization could become larger. Because depolarization of myelinating oligodendrocytes in this region facilitates axonal conduction of pyramidal cells, the perineuronal myelinating oligodendrocytes influence hippocampal output via their interaction with both axons and O-LM neurons.

9.8 Concluding Remarks

Oligodendrocytes play a variety of roles by modifying nerve conduction in addition to fast signal conduction (i.e., saltatory conduction) in information processing in the central nervous system. Oligodendrocytes communicate with axons closely and contribute to information processing by unique temporal and spatial behavior. Furthermore, because a single oligodendrocyte forms the myelin for 10–30 axons, the modulation by oligodendrocytes might coordinate the output of a subset of neurons and regulate different neuronal circuits simultaneously. Further research into a new type of structural and functional plasticity involving oligodendrocytes is needed. Since oligodendrocytes selectively myelinate a particular set of axons in white matter (Osanai et al. 2017), elucidation of the physiological significance of functional units consisting of an oligodendrocyte and a group of axons myelinated by a given oligodendrocyte (oligodendrocyte-bound axonal bale; de Hoz and Simons 2014) is also needed.

Although synaptic dysfunction is the cellular basis of many mental illnesses, slowing down or desynchronizing axonal conduction between different brain regions could also impair information processing in neurological and psychiatric disorders. Importantly, in mice expressing extra copies of the myelin-related PLP gene, while apparent demyelination is not observed, the conduction velocity of action potentials decreases due to abnormal paranodal structures (Tanaka et al. 2009). In addition, while mice overexpressing PLP have preserved motor and sensory functions, they exhibit behavioral changes, such as anxiety-like behavior, spatial learning deficits, and working memory deficits. These facts indicate that dysregulation of oligodendrocyte function, while not leading to demyelination, affects saltatory conduction, and furthermore is related to the disturbance of normal

brain function. It has also been found that abuse of addictive drugs causes structural changes in white matter and abnormalities related to oligodendrocytes and myelin (Berman et al. 2008; Kochunov et al. 2013; Kovalevich et al. 2012). Whether structural changes in, or gene abnormalities related to, oligodendrocyte function directly cause symptoms of psychiatric disorders or are the result of other changes caused by brain dysfunction remain; however, some psychiatric disorders are thought to be due to oligodendrocyte dysfunction. Investigating new roles of oligodendrocytes in normal information processing and cognitive functioning and the relationship between their abnormality and symptoms of psychiatric diseases has become a very attractive research area. An understanding of the dynamic regulation of nerve conduction by oligodendrocytes could lead to a better understanding of information processing in the central nervous system.

References

Adriano E, Perasso L, Panfoli I, Ravera S, Gandolfo C, Mancardi G, Morelli A, Balestrino M (2011) A novel hypothesis about mechanisms affecting conduction velocity of central myelinated fibers. Neurochem Res 36:1732–1739

Ahmed Z (2014) Trans-spinal direct current stimulation modifies spinal cord excitability through synaptic and axonal mechanisms. Physiol Rep. https://doi.org/10.14814/phy2.12157

Andreasen M, Nedergaard S (2017) Furosemide depresses the presynaptic fiber volley and modifies frequency-dependent axonal excitability in rat hippocampus. J Neurophysiol 117:1512–1523

Arancibia-Cárcamo IL, Ford MC, Cossell L, Ishida K, Tohyama K, Attwell D (2017) Node of Ranvier length as a potential regulator of myelinated axon conduction speed. elife. https://doi.org/10.7554/eLife.23329

Bagayogo IP, Dreyfus CF (2009) Regulated release of BDNF by cortical oligodendrocytes is mediated through metabotropic glutamate receptors and the PLC pathway. ASN Neuro. https://doi.org/10.1042/AN20090006

Bakkum DJ, Chao ZC, Potter SM (2008) Long-term activity-dependent plasticity of action potential propagation delay and amplitude in cortical networks. PLoS One. https://doi.org/10.1371/journal.pone.0002088

Battefeld A, Klooster J, Kole MH (2016) Myelinating satellite oligodendrocytes are integrated in a glial syncytium constraining neuronal high-frequency activity. Nat Commun. https://doi.org/10.1038/ncomms11298

Benarroch EE (2008) Adenosine and its receptors: multiple modulatory functions and potential therapeutic targets for neurologic disease. Neurology 70:231–236

Berman S, O'Neill J, Fears S, Bartzokis G, London ED (2008) Abuse of amphetamines and structural abnormalities in the brain. Ann N Y Acad Sci 1141:195–220

Bliss TVP, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39

Bucher D (2016) Contribution of axons to short-term dynamics of neuronal communication. In: Rockland K (ed) Axons and brain architecture. Academic, Cambridge, pp 245–263

Bucher D, Goaillard JM (2011) Beyond faithful conduction: short-term dynamics, neuromodulation, and long-term regulation of spike propagation in the axon. Prog Neurobiol 94:307–346

Butt AM, Tutton M (1992) Response of oligodendrocytes to glutamate and gamma-aminobutyric acid in the intact mouse optic nerve. Neurosci Lett 146:108–110

Chida K, Kaneko K, Fujii S, Yamazaki Y (2015) Activity-dependent modulation of the axonal conduction of action potentials along rat hippocampal mossy fibers. Eur J Neurosci 41:45–54

de Hoz L, Simons M (2014) The emerging functions of oligodendrocytes in regulating neuronal network behavior. BioEssays 37:60–69

Debanne D, Campanac E, Bialowas A, Carlier E, Alcaraz G (2011) Axon physiology. Physiol Rev 91:555–602

Del Río Hortega P (1928) Tercera aportación al conocimiento morfológico e interpretación funcional de la oligodendroglía. Mem R Soc Esp Hist Nat 14:5–122

Dellal SS, Luo R, Otis TS (2012) $GABA_A$ receptors increase excitability and conduction velocity of cerebellar parallel fiber axons. J Neurophysiol 107:2958–2970

Di Lazzaro V, Ranieri F, Profice P, Pilato F, Mazzone P, Capone F, Insola A, Oliviero A (2013) Transcranial direct current stimulation effects on the excitability of corticospinal axons of the human cerebral cortex. Brain Stimul 6:641–643

Domercq M, Perez-Samartin A, Aparicio D, Alberdi E, Pampliega O, Matute C (2010) P2X7 receptors mediate ischemic damage to oligodendrocytes. Glia 58:730–740

Fields RD (2010) Change in the brain's white matter. Science 330:768–769

Fields RD (2011) Nonsynaptic and nonvesicular ATP release from neurons and relevance to neuron–glia signaling. Semi Cell Devel Biol 22:214–219

Ford MC, Alexandrova O, Cossell L, Stange-Marten A, Sinclair J, Kopp-Scheinpflug C, Pecka M, Attwell D, Grothe B (2015) Tuning of Ranvier node and internode properties in myelinated axons to adjust action potential timing. Nat Commun. https://doi.org/10.1038/ncomms9073

Frühbeis C, Fröhlich D, Kuo WP, Amphornrat J, Thilemann S, Saab AS, Kirchhoff F, Möbius W, Goebbels S, Nave KA, Schneider A, Simons M, Klugmann M, Trotter J, Krämer-Albers EM (2013) Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication. PLoS Biol. https://doi.org/10.1371/journal.pbio.1001604

Fujii S, Tanaka KF, Ikenaka K, Yamazaki Y (2014) Increased adenosine levels in mice expressing mutant glial fibrillary acidic protein in astrocytes result in failure of induction of LTP reversal (depotentiation) in hippocampal CA1 neurons. Brain Res 1578:1–13

Gilbert P, Kettenmann H, Schachner M (1984) Gamma-aminobutyric acid directly depolarizes cultured oligodendrocytes. J Neurosci 4:561–569

Haas HL, Selbach O (2000) Functions of neuronal adenosine receptors. Naunyn Schmiedeberg's Arch Pharmacol 362:375–381

Hirano A, Llena JE (1995). Morphology of central nervous system axons. In: Waxman SG, Kocsis JD, Stys PK (eds) The axon. Oxford University Press, New York, p49–67

Huff TB, Shi Y, Sun W, Wu W, Shi R, Cheng JX (2011) Real-time CARS imaging reveals a calpain-dependent pathway for paranodal myelin retraction during high-frequency stimulation. PLoS One. https://doi.org/10.1371/journal.pone.0017176

Jankowska E, Kaczmarek D, Bolzoni F, Hammar I (2017) Long-lasting increase in axonal excitability after epidurally applied DC. J Neurophysiol 118:1210–1220

Jia Y, Yamazaki Y, Nakauchi S, Ito K, Sumikawa K (2010) Nicotine facilitates long-term potentiation induction in oriens-lacunosum moleculare cells via Ca^{2+} entry through non-alpha7 nicotinic acetylcholine receptors. Eur J Neurosci 31:463–476

Kettenmann H, Gilbert P, Schachner M (1984) Depolarization of cultured oligodendrocytes by glutamate and GABA. Neurosci Lett 47:271–276

Kiernan MC, Kaji R (2013) Physiology and pathophysiology of myelinated nerve fibers. In: Said G, Krarup C (eds) Peripheral nerve disorders. Handbook of clinical neurology, vol 115. Elsevier, Amsterdam, pp 43–53

Kochunov P, Du X, Moran LV, Sampath H, Wijtenburg SA, Yang Y, Rowland LM, Stein EA, Hong LE (2013) Acute nicotine administration effects on fractional anisotropy of cerebral white matter and associated attention performance. Front Pharmacol. https://doi.org/10.3389/fphar.2013.00117

Kovalevich J, Corley G, Yen W, Rawls SM, Langford D (2012) Cocaine-induced loss of white matter proteins in the adult mouse nucleus accumbens is attenuated by administration of a β-lactam antibiotic during cocaine withdrawal. Am J Pathol 181:1921–1927

Krause M, Dent EW, Bear JE, Loureiro JJ, Gertler FB (2003) Ena/VASP proteins: regulators of the actin cytoskeleton and cell migration. Annu Rev Cell Dev Biol 19:541–564

Kukley M, Capetillo-Zarate E, Dietrich D (2007) Vesicular glutamate release from axons in white matter. Nat Neurosci 10:311–320

Lee HU, Yamazaki Y, Tanaka KF, Furuya K, Sokabe M, Hida H, Takao K, Miyakawa T, Fujii S, Ikenaka K (2013) Increased astrocytic ATP release results in enhanced excitability of the hippocampus. Glia 61:210–224

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5–21

Micu I, Plemel JR, Lachance C, Proft J, Jansen A, Cummins K, van Minnen J, Stys PK (2016) The molecular physiology of the axo-myelinic synapse. Exp Neurol 276:41–50

Monai H, Ohkura M, Tanaka M, Oe Y, Konno A, Hirai H, Mikoshiba K, Itohara S, Nakai J, Iwai Y, Hirase H (2016) Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. Nat Commun. https://doi.org/10.1038/ncomms11100

Ochiishi T, Chen L, Yukawa A, Saitoh Y, Sekino Y, Arai T, Nakata H, Miyamoto H (1999) Cellular localization of adenosine A_1 receptors in rat forebrain: immunohistochemical analysis using adenosine A_1 receptor-specific monoclonal antibody. J Comp Neurol 411:301–316

Osanai Y, Shimizu T, Mori T, Yoshimura Y, Hatanaka N, Nambu A, Kimori Y, Koyama S, Kobayashi K, Ikenaka K (2017) Rabies virus-mediated oligodendrocyte labeling reveals a single oligodendrocyte myelinates axons from distinct brain regions. Glia 65:93–105

Othman T, Yan H, Rivkees SA (2003) Oligodendrocytes express functional A_1 adenosine receptors that stimulate cellular migration. Glia 44:166–172

Preston RJ, Waxman SG, Kocsis JD (1983) Effects of 4-aminopyridine on rapidly and slowly conducting axons of rat corpus callosum. Exp Neurol 79:808–820

Ritchie JM (1995) Physiology of axon. In: Waxman SG, Kocsis JD, Stys PK (eds) The axon. Oxford University Press, New York, pp 68–96

Sakatani K, Chesler M, Hassan AZ (1991) $GABA_A$ receptors modulate axonal conduction in dorsal columns of neonatal rat spinal cord. Brain Res 542:273–279

Seidl AH, Rubel EW, Barría A (2014) Differential conduction velocity regulation in ipsilateral and contralateral collaterals innervating brainstem coincidence detector neurons. J Neurosci 34:4914–4949

Stevens B, Porta S, Haak LL, Gallo V, Fields RD (2002) Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36:855–868

Swadlow HA (1982) Impulse conduction in the mammalian brain: physiological properties of individual axons monitored for several months. Science 218:911–913

Swadlow HA (1985) Physiological properties of individual cerebral axons studied in vivo for as long as one year. J Neurophysiol 54:1346–1362

Swanson TH, Drazba JA, Rivkees SA (1995) Adenosine A_1 receptors are located predominantly on axons in the rat hippocampal formation. J Comp Neurol 363:517–531

Swanson TH, Krahl SE, Liu YZ, Drazba JA, Rivkees SA (1998) Evidence for physiologically active axonal adenosine receptors in the rat corpus callosum. Brain Res 784:188–198

Tanaka H, Ma J, Tanaka KF, Takao K, Komada M, Tanda K, Suzuki A, Ishibashi T, Baba H, Isa T, Shigemoto R, Ono K, Miyakawa T, Ikenaka K (2009) Mice with altered myelin proteolipid protein gene expression display cognitive deficits accompanied by abnormal neuron-glia interactions and decreased conduction velocities. J Neurosci 29:8363–8371

Tanaka KF, Matsui K, Sasaki T, Sano H, Sugio S, Fan K, Hen R, Nakai J, Yanagawa Y, Hasuwa H, Okabe M, Deisseroth K, Ikenaka K, Yamanaka A (2012) Expanding the repertoire of optogenetically targeted cells with an enhanced gene expression system. Cell Rep. https://doi.org/10.1016/j.celrep.2012.06.011

Tomassy GS, Berger DR, Chen HH, Kasthuri N, Hayworth KJ, Vercelli A, Seung HS, Lichtman JW, Arlotta P (2014) Distinct profiles of myelin distribution along single axons of pyramidal neurons in the neocortex. Science 344:319–324

Wurtz CC, Ellisman MH (1986) Alterations in the ultrastructure of peripheral nodes of Ranvier associated with repetitive action potential propagation. J Neurosci 6:3133–3143
Yamazaki Y, Hozumi Y, Kaneko K, Li J, Fujii S, Miyakawa H, Kudo Y, Kato H (2005) Direct evidence for mutual interactions between perineuronal astrocytes and interneurons in the CA1 region of the rat hippocampus. Neurosci 134:791–802
Yamazaki Y, Hozumi Y, Kaneko K, Sugihara T, Fujii S, Goto K, Kato H (2007) Modulatory effects of oligodendrocytes on the conduction velocity of action potentials along axons in the alveus of the rat hippocampal CA1 region. Neuron Glia Biol 3:325–334
Yamazaki Y, Hozumi Y, Kaneko K, Fujii S, Goto K, Kato H (2010) Oligodendrocytes: facilitating axonal conduction by more than myelination. Neuroscientist 16:11–18
Yamazaki Y, Fujiwara H, Kaneko K, Hozumi Y, Xu M, Ikenaka K, Fujii S, Tanaka KF (2014) Short- and long-term functional plasticity of white matter induced by oligodendrocyte depolarization in the hippocampus. Glia 62:1299–1312
Yamazaki Y, Hozumi Y, Kaneko K, Fujii S (2018) Modulatory effects of perineuronal oligodendrocytes on neuronal activity in the rat hippocampus. Neurochem Res 43:18–31
Zhou W, Ge W, Zeng S, Duan S, Luo Q (2007) Identification and two-photon imaging of oligodendrocyte in CA1 region of hippocampal slices. Biochem Biophysi Res Comm 352:598–602
Ziskin JL, Nishiyama A, Rubio M, Fukaya M, Bergles DE (2007) Vesicular release of glutamate from unmyelinated axons in white matter. Nat Neurosci 10:321–330

Mitochondrial Dynamics in Physiology and Pathology of Myelinated Axons

10

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Abstract

Mitochondria play essential roles in neurons and abnormal functions of mitochondria have been implicated in neurological disorders including myelin diseases. Since mitochondrial functions are regulated and maintained by their dynamic behavior involving localization, transport, and fusion/fission, modulation of mitochondrial dynamics would be involved in physiology and pathology of myelinated axons. In fact, the integration of multimodal imaging in vivo and in vitro revealed that mitochondrial localization and transport are differentially regulated in nodal and internodal regions in response to the changes of metabolic demand in myelinated axons. In addition, the mitochondrial behavior in axons is modulated as adaptive responses to demyelination irrespective of the cause of myelin loss, and the behavioral modulation is partly through interactions with cytoskeletons and closely associated with the pathophysiology of demyelinating diseases. Furthermore, the behavior and functions of axonal mitochondria are modulated in congenital myelin disorders involving impaired interactions

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between axons and myelin-forming cells, and, together with the inflammatory environment, implicated in axonal degeneration and disease phenotypes. Further studies on the regulatory mechanisms of the mitochondrial dynamics in myelinated axons would provide deeper insights into axo–glial interactions mediated through myelin ensheathment, and effective manipulations of the dynamics may lead to novel therapeutic strategies protecting axonal and neuronal functions and survival in primary diseases of myelin.

Keywords
Axonal degeneration · Demyelination · Localization · Remyelination · Transport

10.1 Introduction

Myelin, the multilamellar ensheathment around axons, plays essential roles in facilitating fast saltatory conduction and conserves the space and possibly energy required for nerve conduction (Trapp and Stys 2009; Nave 2010a, b). The ensheathment by myelin-forming cells divides axons into multiple segments with differential morphology, molecular distribution, and accessibility to extracellular molecules, and alters axonal microenvironment and metabolism (Arroyo and Scherer 2000; Poliak and Peles 2003). In addition, myelin is essential for long-term maintenance of axonal integrity, and the loss as well as congenital defects of myelin lead to neurological disorders involving degeneration and loss of axons (Nave and Trapp 2008; Nave 2010a, b). In this context, the regulation of axonal metabolism, functions, and survival are closely associated with axonal mitochondria in physiology and pathology of the nervous system.

Mitochondria play essential roles in almost all cells in the nervous system and critical for cellular metabolisms such as energy production and Ca^{2+} homeostasis. Mitochondrial functions are important in neurons and associated with neurological disorders involving neuronal and axonal degeneration and loss (Wallace 2005; Lin and Beal 2006). Mitochondria are dynamic organelles and characterized in neurons by their characteristic distribution, transport, and fusion/fission (Chan 2006; Saxton and Hollenbeck 2012). The dynamic behavior is regulated by specialized molecules, and interaction with the other organelles including endoplasmic reticulum (ER) is also critical for maintenance of mitochondrial functions (Hayashi et al. 2009; Sheng and Cai 2012; Lamb et al. 2013; Friedman and Nunnari 2014; Tatsuta et al. 2014; Misgeld and Schwarz 2017). Therefore, the regulatory mechanisms of mitochondrial dynamics and organelle interactions have been implicated in pathophysiology of myelin diseases.

This chapter will focus on the characteristic behavior of mitochondria in axons, and provide overview of recent studies on the dynamics of mitochondria in myelinated axons as well as their alterations in myelin diseases. Furthermore, the mechanisms and roles of the behavior and alterations in physiology of myelinated axons and pathophysiology of myelin diseases are also discussed.

10.2 General Behavior of Axonal Mitochondria

Mitochondrial behavior in axons is different from that in the soma of neurons and the other cells, and characterized by two major populations, stationary and motile. The majority of axonal mitochondria are stationary, immobile during entire observation period, and enriched in regions such as growth cones and synaptic terminals, which are considered to have higher metabolic demand (Fig. 10.1a) (Saxton and Hollenbeck 2012; Sheng and Cai 2012). Other mitochondria are motile, which are generally small, and their movement is in anterograde and retrograde directions (Fig. 10.1a). It has been established that these two populations are interchangeable. Motile mitochondria stop and accumulate at the specific regions and static mitochondria start moving when the axonal metabolic states are altered. For example, mitochondria were enriched near the growth cones under axonal growth, while stopping of the axonal growth diminished the mitochondrial accumulation in the distal axons (Morris and Hollenbeck 1993). Therefore, the overall regulation of the two motile and stationary populations is critical to determine mitochondrial distribution within the axons.

The movement of axonal mitochondria is mediated by specific motor proteins and their adaptor molecules, which regulate direction and cessation of their movement (Fig. 10.1b). The motor proteins include kinesin and dynein with distinct or common

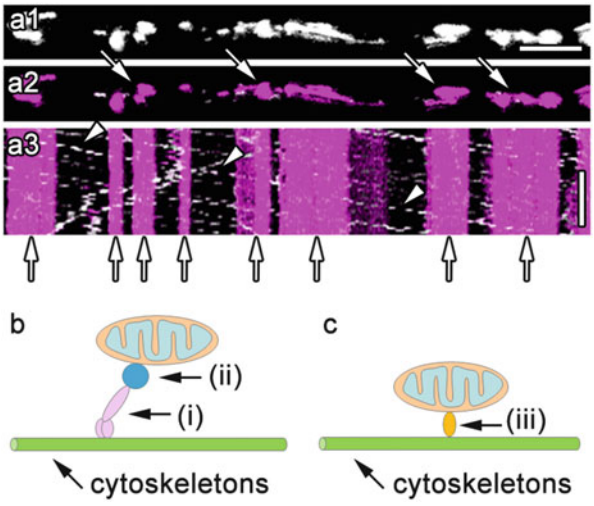

Fig. 10.1 Motile and stationary mitochondria in axons. The first image of the time-lapse imaging showing fluorescently labeled mitochondria in an axon of cultured dorsal root ganglion (**a1**). The stationary mitochondrial profiles are colored magenta (**a2**, arrows) and the kymograph of the time-lapse imaging (**a3**) shows vertically appear stationary mitochondria (**a3**, arrows) and diagonal trajectories of motile mitochondria (**a3**, arrowheads). Bars: 10 μm (horizontal) or 20 sec (vertical). Schemes showing a motile mitochondrion with the motor (**b**, i) and adaptor (**b**, ii) proteins on a track of cytoskeletons, and a stationary mitochondrion with a tethering molecule (**c**, iii) on the scaffold of cytoskeletons

adaptor complex such as Miro and Milton in *Drosophila* and RhoT1/2 and trafficking protein kinesin-binding (TRAK) 1/2 in mammals (Schwarz 2013). While kinesins and dyneins are motors on microtubule tracks, myosins are responsible for the slower bidirectional movement on actin filaments, although myosins may be associated with tethering rather than transport of axonal mitochondria (Morris and Hollenbeck 1995; Pathak et al. 2010). The mitochondrial movement is modulated by multiple mechanisms, including Ca^{2+} and glucose, and cytosolic Ca^{2+} binds to EF-hands of Miro or RhoT1/2, induces mitochondrial detachment from microtubule tracks and stops mitochondrial movement (Saotome et al. 2008; Wang and Schwarz 2009; Pekkurnaz et al. 2014). The local inhibition of mitochondrial movement results in increase of mitochondrial sizes in axons (Chada and Hollenbeck 2004; Macaskill et al. 2009).

Mitochondrial localization is mediated by regulation of mitochondrial docking as well as the local inhibition of mitochondrial movement. Apart from the role of myosin as a potential mitochondrial tether to the actin filament, syntaphilin is another mitochondrial docking molecule, which binds mitochondria to microtubule scaffolds (Fig. 10.1c) (Kang et al. 2008; Pathak et al. 2010). The loss of syntaphilin significantly increased mitochondrial motility while its overexpression decreased number of motile mitochondria (Kang et al. 2008). The impairment in mitochondrial tethering via syntaphilin perturbed axonal arborization, and increased the variability of synaptic transmission (Courchet et al. 2013; Sun et al. 2013). These results indicate that mitochondrial tethering plays significant roles in axonal functions and morphogenesis.

In addition to the transport and localization, mitochondria undergo dynamic morphological changes via fusion and fission. The fusion of two mitochondrial segments generates one large daughter mitochondrion where free exchange of soluble and membrane-bound molecules takes place within the daughter mitochondrion (Busch et al. 2006; Liu et al. 2009). Mitochondrial fission generates two small daughter mitochondria from one large mitochondrion, and one of the two daughter mitochondria could be dysfunctional and selectively degraded for mitochondrial quality control (Twig et al. 2008). The fusion and fission of mitochondria are regulated by distinct sets of GTPases, including mitofusins (Mfn1, Mfn2) and dynamin-related protein 1 (Drp1) on outer membranes and optic atrophy 1 (OPA1) on inner membranes (Hoppins et al. 2007). The fusion and fission of mitochondria are generally critical for neurological disorders, and genetic mutations of Mfn2 and OPA1 cause Charcot–Marie–Tooth type 2 and autosomal dominant optic atrophy, respectively (Alexander et al. 2000; Delettre et al. 2000; Zuchner et al. 2004). Loss of Drp1 in rodents was embryonic lethal, and mutation in human caused severe neurodevelopmental defects (Waterham et al. 2007; Ishihara et al. 2009).

Collectively, regulation of mitochondrial movement, localization, and morphology are critical for functional maintenance and survival of axons and neurons. Understanding the dynamic aspects of mitochondria in myelinated axons would provide insights regarding how myelination affects axonal homeostasis both in physiology and in pathology of the nervous system.

10.3 Mitochondrial Regulation in Myelinated Axons

The ensheathment by myelin in the nervous system of vertebrate causes extreme specialization of axons (Poliak and Peles 2003). The gaps of the insulating compact myelin sheath are the highly specialized domains called nodes of Ranvier. Voltage-dependent Na^+ channels are clustered on the axolemma of the nodes and required for saltatory conduction. The nodes are flanked by paranodes, which have unique cytoplasmic loops of myelin-forming cells. The paranodes contain the junctional complex between axons and myelin-forming cells and limit diffusion of extracellular as well as axolemmal molecules (Mackenzie et al. 1984; Bhat et al. 2001; Boyle et al. 2001; Perkins et al. 2008; Rosenbluth 2009; Mierzwa et al. 2011; Shroff et al. 2011). K^+ channels are enriched in the juxtaparanodal regions, which are about 15 μm long, adjacent to the paranodal regions, and sometimes with invaginations of plasma membranes into the axons (Spencer and Thomas 1974; Griffin and Price 1981).

Conventionally, it was believed that axonal mitochondria were in general accumulated in the nodes with some variance in different tracts (Hollenbeck and Saxton 2005; Chen and Chan 2006). However, the recent development of the methods for three dimensional (3D) ultrastructural analyses enabled high-throughput acquisition of serial electron microscopic images and complete reconstruction of all mitochondria in individual axons (Briggman and Bock 2012; Ohno et al. 2015; Nguyen et al. 2016; Thai et al. 2016). Application of these advanced methods revealed that mitochondria in myelinated axons were enriched in internodal regions rather than the nodes (Fig. 10.2a, b) (Ohno et al. 2011). The abundant mitochondria in the internodal regions are consistent with the notion that the energy substrate for axonal mitochondria is provided through myelin sheath as a form of lactate or glucose and thereby myelin-forming cells maintain axonal integrity (Nave 2010a, b; Brown et al. 2012; Saab and Nave 2017). The transport of lactate may be mediated by distinct transporters such as monocarboxylate transporter 1 (MCT1), whose deficiency in oligodendrocytes impaired axonal survival in the central nervous system (Funfschilling et al. 2012; Lee et al. 2012). In addition, the Na^+/K^+ ATPase, which maintains ion gradient of Na^+ and K^+ in an energy-dependent manner, was enriched on axolemma of the internodes (Young et al. 2008; Trapp and Stys 2009). Therefore, enrichment of axonal mitochondria in the internodal regions suggests that mitochondrial energy production in myelinated axons predominates in regions with the supply of energy substrate and higher metabolic demand under basal conditions (Perge et al. 2009). The molecular mechanisms regulating mitochondrial distribution in internodes are unclear. However, time-lapse observation in vitro demonstrated stationary mitochondria were substantially increased during myelin formation (Kiryu-Seo et al. 2010). It is possible that molecules such as syntaphilin, which support mitochondrial docking and increase of stationary mitochondria, are involved in the internodal enrichment. Such modulation of mitochondria-associated proteins in different segments of myelinated axons may be associated with signal transduction between axons and myelinating glia, which involves posttranslational modification of cytoskeletal proteins in axons (Sousa and Bhat 2007).

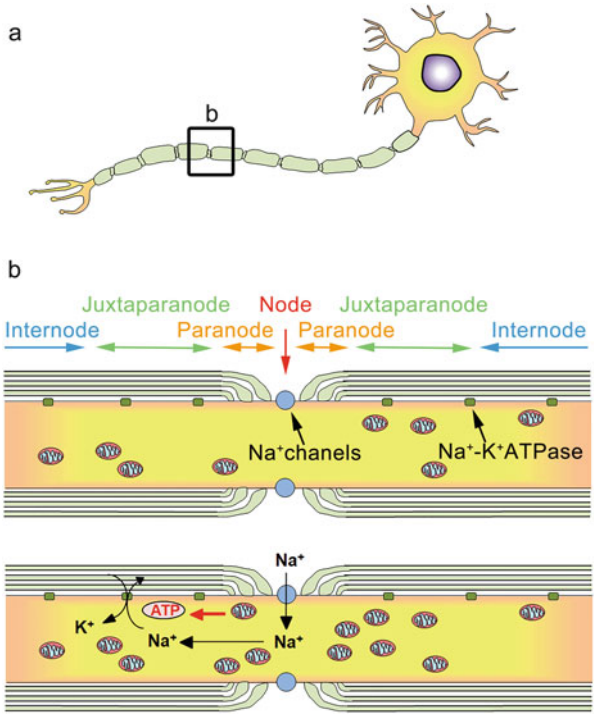

Fig. 10.2 Mitochondrial behavior in myelinated axons. The myelinated axon of a neuron (**a**) is marked with a rectangle and magnified (**b**). There are multiple segments called nodes, paranodes, juxtaparanodes, and internodes (**b**). Mitochondria are enriched in juxtaparanodes and internodal regions (**b**, upper panel), while mitochondria are stopped and increased near the node upon nerve conduction (**b**, lower panel)

In addition to the mitochondrial enrichment in internodal regions, small and short mitochondria were accumulated in a fraction of nodal regions, and the nodal accumulation was mediated by stopping of motile mitochondria in a manner dependent on axonal electrical activity and Ca^{2+} (Fig. 10.2b) (Zhang et al. 2010; Ohno et al. 2011). The metabolism of myelinated axons upon nerve conduction is distinct from that in unmyelinated axons. Myelin ensheathment enables rapid saltatory conduction by concentrating Na^+ channels in the nodal regions and is considered to conserve energy as well as space for nerve conduction (Ritchie 1995). Activation of nodal Na^+ channels upon nerve conduction increased Na^+ concentration in nodal axoplasm (Fleidervish et al. 2010). Repetitive axonal conduction requires an energy-dependent exchange of axoplasmic Na^+ for extracellular K^+ through Na^+/K^+ ATPases, which was enriched in the juxtaparanodal and internodal regions in human brain tissues (Young et al. 2008). Given that nerve conduction was impaired in genetic disorders of mitochondrial functions caused by mutations of mitochondrial DNA (Kaufmann et al. 2006; Horga et al. 2014), activity-dependent mitochondrial stopping and localization in the nodal and paranodal axoplasm would be important in order to meet the energy demand of saltatory nerve conduction.

The distribution and motility of mitochondria in myelinated axons appear to be regulated in response to the metabolic alterations of the axons. The adaptive response of mitochondrial behavior is considered to be more important in myelin diseases particularly in demyelinating disorders. The alterations, mechanisms, and

the roles of mitochondrial dynamics in demyelinating axons will be discussed in the next section.

10.4 Mitochondrial Alterations in Demyelinated Axons

Axonal degeneration, commonly observed in demyelinating diseases (Fig. 10.3a, b), is characterized by axonal swelling and loss and contributes to permanent neurological deficits (Trapp and Nave 2008). The mitochondrial alterations have been implicated in the pathophysiology of demyelinating diseases (Mahad et al. 2015). Demyelination poses a major challenge to axons since axons lose support from myelin-forming cells. Upon demyelination, Na^+ channels were redistributed along the axons, expression of Na^+ channel isoforms was changed, and confinement of Na^+ influx was modified (Craner et al. 2004a; b). The redistribution of Na^+ channels may increase energy consumption necessary for the exchange of Na^+ and K^+ upon nerve conduction (Waxman 2008). In addition, the demyelinated axons are exposed to inflammatory environment, which is common in demyelinating diseases such as multiple sclerosis or animal models such as experimental autoimmune encephalomyelitis (EAE), and often includes toxic mediators including nitric oxide (NO). NO can diffuse into demyelinated axons, and inhibit mitochondrial ATP production. All these factors perturb the energy metabolism of demyelinated axons and would lead

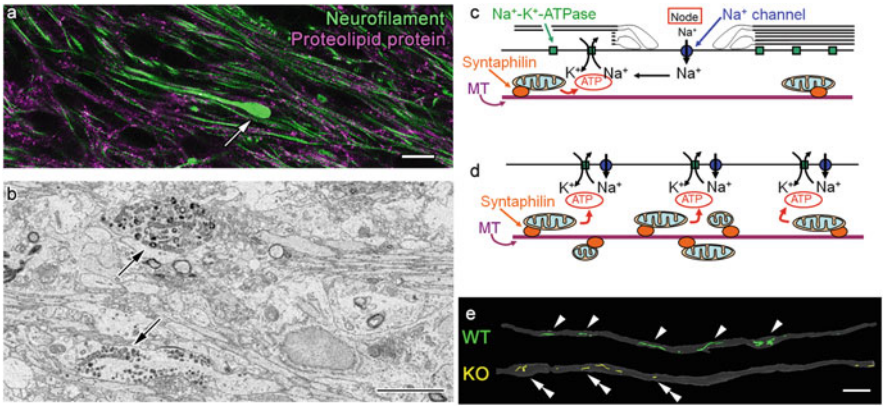

Fig. 10.3 Axons and mitochondria in demyelinated lesions. Immunostaining for an axonal marker, neurofilament, and a myelin marker, proteolipid protein (**a**), and an electron micrograph (**b**) in mouse corpus callosum demyelinated with cuprizone feeding shows axonal swellings (**a**, **b**, arrows) with numerous axoplasmic organelles (**b**, arrows). A scheme showing stationary mitochondria, enriched in internodal regions of myelinated axons (**c**) and increased in demyelinated axons (**d**), are tethered to microtubules with syntaphilin. The three-dimensional reconstruction of serial electron microscopic images, which are obtained from axons demyelinated under cuprizone feeding shows mitochondrial number and volume in a syntaphilin knockout axon (KO, double arrowheads) is less than that in a wild-type axon (WT, arrowheads). Bars 5 μm

to axonal degeneration mediated by the accumulation of Na^+ and Ca^{2+} (Trapp and Stys 2009).

The mitochondrial distribution, behavior, and lifecycle are modulated upon demyelination in order to maintain axonal integrity and functions. In human brain tissues of patients with demyelinating diseases, the mitochondrial size and numbers were significantly increased (Mahad et al. 2009; Witte et al. 2009; Zambonin et al. 2011). The increase was prominent compared with adjacent normal-appearing white matter, and also the consistent increase of mitochondrial mass was observed in demyelinated axons of animal models (Mutsaers and Carroll 1998; Sathornsumetee et al. 2000; Zambonin et al. 2011). The live imaging studies of fluorescently labeled mitochondria in demyelinated axons in vitro revealed that the majority of increased mitochondrial profiles in demyelinated axons were stationary and these static population increased in their sizes (Kiryu-Seo et al. 2010; Ohno et al. 2014). The expression of mitochondrial tethering molecule, syntaphilin, was increased in demyelinated axons of human multiple sclerosis patients and a demyelinating mouse model produced by cuprizone feeding (Fig. 10.3c, d), and the genetic ablation of syntaphilin impaired volume increase of mitochondria in demyelinated axons and led to exacerbation of axonal degeneration (Mahad et al. 2009; Ohno et al. 2014). Since the lack of syntaphilin did not augment neurological symptoms in the inflammatory demyelination model of EAE, the mitochondrial tethering could be more beneficial in demyelination with less inflammation (Joshi et al. 2015). Indeed, the impairment of mitochondrial respiratory chain components in acute demyelinating lesions was implicated in the tissue damages (Mahad et al. 2008). These studies support the concept that the enrichment of functional mitochondria is the common adaptive response of axons against demyelination, mediated by molecular interaction between mitochondria and cytoskeletons, and beneficial for the survival of demyelinated axons.

In addition, mitochondrial fusion and fission play essential roles for functional regulation and maintenance of mitochondria (Youle and van der Bliek 2012; Friedman and Nunnari 2014). The mitochondrial sizes were increased in demyelinated axons of human tissues and animal models at the light microscopic level (Zambonin et al. 2011). This observation was supported by detailed 3D electron microscopic observation showing that the volume of individual mitochondria was increased in demyelination model produced by cuprizone feeding (Ohno et al. 2014). These results suggested that the increase of mitochondrial volume in demyelinated axons is at least partly mediated by mitochondrial fusion. Although the balanced mitochondrial fusion/fission is critical for neuronal functions and survival, aberrant activation of mitochondrial fission was observed in neurological disorders, and inhibition of mitochondrial fission led to neuroprotection in models of neurological diseases (Barsoum et al. 2006; Grohm et al. 2012; Cho et al. 2013). In fact, inhibition of mitochondrial fission with overexpression of dominant-negative Drp1 protected axons from degeneration induced by aberrant activation of the axonal cation channel, transient receptor potential vanilloid receptor 1 (TRPV1) (Chiang et al. 2015). Since aberrant axoplasmic Ca^{2+} increase may

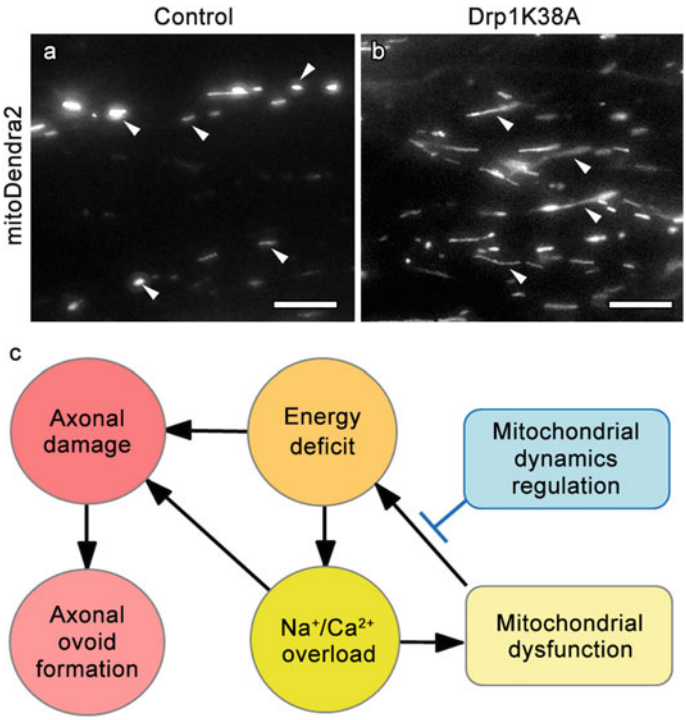

Fig. 10.4 Mitochondrial fission and axonal degeneration. Mitochondria-targeted fluorescent Dendra2 (mitoDendra2) in cultured rat dorsal root ganglion axons shows overexpression of dominant-negative dynamin-related protein1 (Drp1K38A) elongate mitochondrial profiles (**b**, arrowheads) compared with control (**a**, arrowheads). Bars: 5 μm. Regulation of mitochondrial dynamics would ameliorate mitochondrial dysfunction and support survival of axons (**c**)

contribute to the degeneration of demyelinated axons, inhibition of mitochondrial fission could be beneficial for the survival of demyelinated axons (Fig. 10.4).

The inflammatory toxic mediators cause damages in mitochondrial molecules, and mitochondrial segments with the damaged molecules would require turnover and functional maintenance, involving fusion/fission and transport (Smith et al. 1999; Chang and Reynolds 2006; Twig et al. 2008; Saxton and Hollenbeck 2012). Mitochondrial transport was substantially impaired by inflammatory reactions and oxidative stress in demyelinated tissues, as observed in an inflammatory demyelination model (Sorbara et al. 2014). On the other hand, in live imaging studies of demyelinated axons in vitro under the presence of less inflammation and oxidative stress, demyelination increased speed and number of axonal mitochondria (Kiryu-Seo et al. 2010; Ohno et al. 2014). The increased mitochondrial transport in demyelinated axons was at least partly mediated by the expression of activating transcription factor 3 (ATF3) (Kiryu-Seo et al. 2010). The increased mitochondrial transport supported renewal of mitochondrial proteins in the distal portion of neurites, and perturbed axonal transport of mitochondria in demyelinated axons is

associated with accumulation of oxidative stress and impaired axonal integrity (Ferree et al. 2013; Sorbara et al. 2014). Alterations of mitochondrial transport upon demyelination may be regulated as an adaptive response against mitochondrial damage under the noxious environment of demyelinated axons and involves unidentified retrograde signaling toward nuclei from demyelinated axons. Furthermore, environmental perturbation of the response would exacerbate the axonal pathology.

Remyelination, the restoration of the myelin sheath, is protective for axonal survival and neurological deficits (Franklin and Ffrench-Constant 2008). The remyelination reverses the mitochondrial alterations upon demyelination, and the sizes of mitochondrial profiles were decreased compared with demyelinated axons in brain tissues of multiple sclerosis patients (Zambonin et al. 2011). The stationary mitochondria were decreased while more mitochondria were mobile in remyelinated axons compared with demyelinated axons in vitro (Kiryu-Seo et al. 2010; Zambonin et al. 2011). However, when compared with myelinated axons, mitochondrial mass in remyelinated axons is still slightly larger. Further studies are required for elucidating the metabolic states and demands of remyelinated axons, which will provide further insights about the molecular mechanisms associated with the incomplete reversal of mitochondrial behavior upon remyelination.

10.5 Alterations of Axonal Mitochondria in Models of Congenital Myelin Disorders

Congenital defects of myelin structures and functions lead to various phenotypes from almost normal development and aging to severe neurodevelopmental defects and premature death. These phenotypes are at least partly attributable to the abnormal myelin ensheathment, which results in impaired nerve conduction and alterations of axo–glial interactions affecting organelle dynamics and metabolism (Nave 2010a, b). Abnormal dynamics of mitochondria are often implicated in the pathophysiology of the congenital myelin disorders.

The severe phenotypes are observed among the disorders caused by mutations in proteolipid protein (PLP). The types of mutations in the Plp gene on X-chromosome cause wide ranges of symptoms including severe disorders of Pelizaeus-Merzbacher disease (PMD) to the much milder form of spastic paraplegia type 2 (SPG2) (Willard and Riordan 1985; Saugier-Veber et al. 1994). Animal models for these PLP-associated disorders are also established and contributed to our understanding of the pathological mechanisms (Yool et al. 2000; Inoue 2005). In myelin-deficient (MD) rats, a model of PMD, where myelin formation is disrupted by a point mutation in the Plp gene (Csiza and de Lahunta 1979; Boison and Stoffel 1989), oligodendrocytes ensheath axons but fail to produce compact myelin sheath. Although these axons in MD rats had node-like regions with concentrated Na^+ channels, molecular distribution of paranodal proteins, such as contactin and contactin-associated proteins (Caspr), and saltatory conduction were significantly affected (Waxman et al. 1990; Arroyo et al. 2002). In the electron microscopic analyses, mitochondrial densities and areas occupied by mitochondria were

significantly increased in axons of MD rats (Dentinger et al. 1985). In addition, live imaging analyses in organotypic slice cultures revealed that the stationary mitochondria were increased in these axons of MD rats, and the mitochondrial motility was not affected by the axonal electrical activity around the node-like regions (Ohno et al. 2011). These findings are consistent with the concept that increased mitochondrial mass in demyelinated axons is largely stationary, and myelin formation and saltatory conduction is critically involved in the modulation of mitochondrial transport and localization at the nodal regions (Mahad et al. 2009; Kiryu-Seo et al. 2010).

PMD is also caused by the duplication of the Plp gene, and animals with extra copies of Plp are informative to understand the role of PLP as well as the pathophysiology of PMD (Griffiths et al. 1998a, b). The animal models with different dosages of Plp gene demonstrated that higher PLP dosage causes severer and often lethal phenotypes involving impaired myelin formation and oligodendrocyte degeneration, and lower dosage leads to progressive demyelination with axonal degeneration (Kagawa et al. 1994; Readhead et al. 1994). In demyelinated axons of mice overexpressing PLP, mitochondrial density was significantly increased in axons of optic nerves, and mitochondrial respiratory functions were also upregulated in the demyelinated axons of PLP mutants (Hogan et al. 2009). Mutation in the other myelin genes also causes a severe deficit of myelin formation in the nervous system, and myelin basic protein (MBP) is partially deleted and myelin formation is impaired in *shiverer* mice (Roach et al. 1985). Analyses with electron microscopy and enzyme histochemistry revealed increased mitochondrial density and mitochondrial cytochrome c activity in spinal cord axons of *shiverer* mice lacking myelin chronically (Andrews et al. 2006). These findings are consistent with the findings in demyelinated axons of human multiple sclerosis patients (Mahad et al. 2009), and increased mitochondrial volume and functions would be the common response against the loss of myelin irrespective of the cause.

Axons of specific tracts in the PLP mutants have impaired axonal transport, and accumulation of axoplasmic organelles including mitochondria was observed in retinal ganglion axons (Edgar et al. 2010; Ip et al. 2012). The accumulation of organelles was prominent in paranodal regions, and the perturbation of axonal transport was associated with neuroinflammation, since the disruption of transport was rescued in the absence of cytotoxic T cells (Ip et al. 2012). The massive neuroinflammation may be associated with the mitochondrial abnormality in PLP mutant model as well (Nave et al. 1986; Macklin et al. 1987; Moriguchi et al. 1987; Huttemann et al. 2009; Tatar et al. 2010). On the other hand, in the nerve fibers of Caspr mutants, axonal mitochondria with abnormal morphology were accumulated near the nodes (Einheber et al. 2006; Sun et al. 2009). Mutations of Caspr cause impaired paranodal septate-like junctions, abnormal expression or distribution of paranodal and juxtaparanodal molecules, and severe decrease of conduction velocity (Bhat et al. 2001). The abnormal transport, distribution, and functions of mitochondria in myelin mutants would be affected by not only inflammation but also abnormal metabolism derived from impaired myelin structures.

Genetic ablation of myelin-related molecules, such as PLP, 2′,3′-cyclic nucleotide phosphodiesterase (CNP) and myelin-associated glycoprotein (MAG), caused progressive axonal degeneration following myelin formation in the central and peripheral nervous system (Griffiths et al. 1998a, b; Yin et al. 1998; Lappe-Siefke et al. 2003; Nguyen et al. 2009). In these mutant models of myelin-related proteins, axonal swelling with accumulated axoplasmic organelles was commonly observed (Griffiths et al. 1998a, b; Lappe-Siefke et al. 2003; Yin et al. 2006). Biochemical analyses revealed fast axonal transport was impaired (Edgar et al. 2004), and abnormal organelle accumulation in the distal regions of the nodes was often observed in degenerating axons (Griffiths et al. 1998a, b; Yin et al. 2006). In a myelin mutant model with axonal swelling in the distal side of the nodes, time-lapse imaging of live axons in slice cultures indicated impaired transport of mitochondria in distal regions of the nodes, and this transport defects accompanied destruction of microtubules and abnormal microtubule stability in axons (Yin et al. 2006, 2016). Posttranslational modification of axonal cytoskeletons could be perturbed in myelin mutants such as *shiverer* and MAG-deficient mice (Rosenbluth 1980; Inoue et al. 1981; Windebank et al. 1985; Shine et al. 1992; Colello et al. 1994; Kirkpatrick and Brady 1994; Yin et al. 1998; Brady et al. 1999; Nguyen et al. 2009), and therefore the trophic support of myelin ensheathment may include signal transduction, which modulates axonal cytoskeletons and is required for transport of axonal organelles including mitochondria.

Axonal pathology that is independent of the formation and maintenance of myelin ensheathment also involves morphological alterations of axonal mitochondria and associated organelles. The detailed ultrastructural analyses using the 3D reconstruction of serial electron microscopic images revealed that mitochondria in the myelinated axons of optic nerves had an extension of outer membranes, which had close contacts with tubular smooth ER membranes (Yin et al. 2016). In the myelin mutant model with progressive axonal degeneration following myelination, the intimate contacts between mitochondria and ER were decreased when the mitochondria became shorter and the extension was diminished. These structural changes in organellar interactions accompanied abnormal cristae structures and impaired functions of axonal mitochondria (Yin et al. 2016). These results indicate that axonal degeneration in dysmyelinated axons of myelin mutants also involves organelle interactions necessary for mitochondrial homeostasis.

10.6 Summary and Conclusions

The integration of multimodal imaging along with animal and in vitro models enabled more detailed analyses of mitochondrial behavior in myelinated, demyelinated, and dysmyelinated axons. These analyses started to reveal that stationary and motile pools of axonal mitochondria are regulated in nodal and internodal regions in response to metabolic alterations of myelinated axons. In addition, adaptive responses involving cytoskeletal interactions and genetic transcription regulate the behavioral alterations of mitochondria in demyelinated axons

and affect the pathophysiology of demyelinating diseases. Furthermore, congenital myelin disorders modulate behavior and functions of axonal mitochondria, which influence axonal survival and disease phenotypes and are associated with impaired interactions between axons and myelin-forming cells as well as the inflammatory environment of the nervous system. The regulatory mechanisms and effective manipulations of mitochondrial dynamics and functions would provide deeper insights into axo–glial interactions mediated through myelin sheath, and may lead to novel therapeutic strategies protecting axons and neurons in primary diseases of myelin.

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References

Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, Rodriguez M, Kellner U, Leo-Kottler B, Auburger G, Bhattacharya SS, Wissinger B (2000) OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. Nat Genet 26(2):211–215

Andrews H, White K, Thomson C, Edgar J, Bates D, Griffiths I, Turnbull D, Nichols P (2006) Increased axonal mitochondrial activity as an adaptation to myelin deficiency in the Shiverer mouse. J Neurosci Res 83(8):1533–1539

Arroyo EJ, Scherer SS (2000) On the molecular architecture of myelinated fibers. Histochem Cell Biol 113:1–18

Arroyo EJ, Xu T, Grinspan J, Lambert S, Levinson SR, Brophy PJ, Peles E, Scherer SS (2002) Genetic dysmyelination alters the molecular architecture of the nodal region. J Neurosci 22:1726–1737

Barsoum MJ, Yuan H, Aa G, Liot G, Kushnareva Y, Gräber S, Kovacs I, Lee WD, Waggoner J, Cui J, White AD, Bossy B, Martinou J-C, Youle RJ, Sa L, Ellisman MH, Ga P, Bossy-Wetzel E (2006) Nitric oxide-induced mitochondrial fission is regulated by dynamin-related GTPases in neurons. EMBO J 25:3900–3911

Bhat MA, JC Rios YL, Garcia-Fresco GP, Ching W, St Martin M, Li J, Einheber S, Chesler M, Rosenbluth J, Salzer JL, Bellen HJ (2001) Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. Neuron 30(2):369–383

Boison D, Stoffel W (1989) Myelin-deficient rat: a point mutation in exon III (A----C, Thr75----Pro) of the myelin proteolipid protein causes dysmyelination and oligodendrocyte death. EMBO J 8:3295–3302

Boyle ME, Berglund EO, Murai KK, Weber L, Peles E, Ranscht B (2001) Contactin orchestrates assembly of the septate-like junctions at the paranode in myelinated peripheral nerve. Neuron 30 (2):385–397

Brady ST, Witt AS, Kirkpatrick LL, de Waegh SM, C Readhead PHT, Lee VM (1999) Formation of compact myelin is required for maturation of the axonal cytoskeleton. J Neurosci 19 (17):7278–7288

Briggman KL, Bock DD (2012) Volume electron microscopy for neuronal circuit reconstruction. Curr Opin Neurobiol 22:154–161

Brown AM, Evans RD, Black J, Ransom BR (2012) Schwann cell glycogen selectively supports myelinated axon function. Ann Neurol 72(3):406–418

Busch KB, Bereiter-Hahn J, Wittig I, Schagger H, Jendrach M (2006) Mitochondrial dynamics generate equal distribution but patchwork localization of respiratory complex I. Mol Membr Biol 23:509–520

Chada SR, Hollenbeck PJ (2004) Nerve growth factor signaling regulates motility and docking of axonal mitochondria. Curr Biol 14(14):1272–1276
Chan DC (2006) Mitochondria: dynamic organelles in disease, aging, and development. Cell 125 (7):1241–1252
Chang DT, Reynolds IJ (2006) Mitochondrial trafficking and morphology in healthy and injured neurons. Prog Neurobiol 80(5):241–268
Chen H, Chan DC (2006) Critical dependence of neurons on mitochondrial dynamics. Curr Opin Cell Biol 18(4):453–459
Chiang H, Ohno N, Hsieh YL, Mahad DJ, Kikuchi S, Komuro H, Hsieh ST, Trapp BD (2015) Mitochondrial fission augments capsaicin-induced axonal degeneration. Acta Neuropathol 129 (1):81–96
Cho B, Choi SY, Cho HM, Kim HJ, Sun W (2013) Physiological and pathological significance of dynamin-related protein 1 (drp1)-dependent mitochondrial fission in the nervous system. Experimental neurobiology 22(3):149–157
Colello RJ, Pott U, Schwab ME (1994) The role of oligodendrocytes and myelin on axon maturation in the developing rat retinofugal pathway. J Neurosci 14(5. Pt 1):2594–2605
Courchet J, Lewis TL Jr, Lee S, Courchet V, Liou DY, Aizawa S, Polleux F (2013) Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture. Cell 153(7):1510–1525
Craner MJ, Hains BC, Lo AC, Black J, Waxman SG (2004a) Co-localization of sodium channel Nav1.6 and the sodium-calcium exchanger at sites of axonal injury in the spinal cord in EAE. Brain 127:294–303
Craner MJ, Newcombe J, Black JA, Hartle C, Cuzner ML, Waxman SG (2004b) Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na^+/Ca^{2+} exchanger. Proc Natl Acad Sci U S A 101(21):8168–8173
Csiza CK, de Lahunta A (1979) Myelin deficiency (md): a neurologic mutant in the Wistar rat. Am J Pathol 95:215–224
Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquellec L, Arnaud B, Ducommun B, Kaplan J, Hamel CP (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nat Genet 26(2):207–210
Dentinger MP, Barron KD, Csiza CK (1985) Glial and axonal development in optic nerve of myelin deficient rat mutant. Brain Res 344(2):255–266
Edgar JM, McLaughlin M, Yool D, Zhang SC, Fowler JH, Montague P, Ja B, McCulloch MC, Duncan ID, Garbern J, Nave K, Griffiths IR (2004) Oligodendroglial modulation of fast axonal transport in a mouse model of hereditary spastic paraplegia. J Cell Biol 166:121–131
Edgar JM, McCulloch MC, Montague P, Brown AM, Thilemann S, Pratola L, Gruenenfelder FI, Griffiths IR, Nave KA (2010) Demyelination and axonal preservation in a transgenic mouse model of Pelizaeus-Merzbacher disease. EMBO Mol Med 2:42–50
Einheber S, Bhat MA, Salzer JL (2006) Disrupted axo-glial junctions result in accumulation of abnormal mitochondria at nodes of ranvier. Neuron Glia Biol 2(3):165–174
Ferree AW, Trudeau K, Zik E, Benador IY, Twig G, Gottlieb RA, Shirihai OS (2013) MitoTimer probe reveals the impact of autophagy, fusion, and motility on subcellular distribution of young and old mitochondrial protein and on relative mitochondrial protein age. Autophagy 9 (11):1887–1896
Fleidervish I, Lasser-Ross N, Gutnick MJ, Ross WN (2010) Na^+ imaging reveals little difference in action potential-evoked Na^+ influx between axon and soma. Nat Neurosci 13:852–860
Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9(11):839–855
Friedman JR, Nunnari J (2014) Mitochondrial form and function. Nature 505(7483):335–343
Funfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Mobius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes

CT, Frahm J, Goebbels S, Nave KA (2012) Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature 485(7399):517–521

Griffin JW, Price DL (1981) Schwann cell and glial responses in beta, beta'-iminodipropionitrile intoxication. I. Schwann cell and oligodendrocyte ingrowths. J Neurocytol 10(6):995–1007

Griffiths I, Klugmann M, Anderson T, Thomson C, Vouyiouklis D, Nave KA (1998a) Current concepts of PLP and its role in the nervous system. Microsc Res Tech 41(5):344–358

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave KA (1998b) Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science 280(5369):1610–1613

Grohm J, Kim SW, Mamrak U, Tobaben S, Cassidy-Stone A, Nunnari J, Plesnila N, Culmsee C (2012) Inhibition of Drp1 provides neuroprotection in vitro and in vivo. Cell Death Differ 19 (9):1446–1458

Hayashi T, Rizzuto R, Hajnoczky G, Su TP (2009) MAM: more than just a housekeeper. Trends Cell Biol 19(2):81–88

Hogan V, White K, Edgar J, McGill A, Karim S, McLaughlin M, Griffiths I, Turnbull D, Nichols P (2009) Increase in mitochondrial density within axons and supporting cells in response to demyelination in the Plp1 mouse model. J Neurosci Res 87(2):452–459

Hollenbeck PJ, Saxton WM (2005) The axonal transport of mitochondria. J Cell Sci 118 (Pt 23):5411–5419

Hoppins S, Lackner L, Nunnari J (2007) The machines that divide and fuse mitochondria. Annu Rev Biochem 76:751–780

Horga A, Pitceathly RD, Blake JC, Woodward CE, Zapater P, Fratter C, Mudanohwo EE, Plant GT, Houlden H, Sweeney MG, Hanna MG, Reilly MM (2014) Peripheral neuropathy predicts nuclear gene defect in patients with mitochondrial ophthalmoplegia. Brain 137 (Pt 12):3200–3212

Huttemann M, Zhang Z, Mullins C, Bessert D, Lee I, Nave KA, Appikatla S, Skoff RP (2009) Different proteolipid protein mutants exhibit unique metabolic defects. ASN Neuro 1(3)

Inoue K (2005) PLP1-related inherited dysmyelinating disorders: Pelizaeus-Merzbacher disease and spastic paraplegia type 2. Neurogenetics 6(1):1–16

Inoue Y, Nakamura R, Mikoshiba K, Tsukada Y (1981) Fine structure of the central myelin sheath in the myelin deficient mutant Shiverer mouse, with special reference to the pattern of myelin formation by oligodendroglia. Brain Res 219(1):85–94

Ip CW, Kroner A, Groh J, Huber M, Klein D, Spahn I, Diem R, Williams SK, Nave KA, Edgar JM, Martini R (2012) Neuroinflammation by cytotoxic T-lymphocytes impairs retrograde axonal transport in an oligodendrocyte mutant mouse. PLoS One 7:1–9

Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, Otera H, Nakanishi Y, Nonaka I, Goto Y-I, Taguchi N, Morinaga H, Maeda M, Takayanagi R, Yokota S, Mihara K (2009) Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. Nat Cell Biol 11:958–966

Joshi DC, Zhang CL, Lin TM, Gusain A, Harris MG, Tree E, Yin Y, Wu C, Sheng ZH, Dempsey RJ, Fabry Z, Chiu SY (2015) Deletion of mitochondrial anchoring protects dysmyelinating shiverer: implications for progressive MS. J Neurosci 35(13):5293–5306

Kagawa T, Ikenaka K, Inoue Y, Kuriyama S, Tsujii T, Nakao J, Nakajima K, Aruga J, Okano H, Mikoshiba K (1994) Glial cell degeneration and hypomyelination caused by overexpression of myelin proteolipid protein gene. Neuron 13:427–442

Kang JS, Tian JH, Pan PY, Zald P, Li C, Deng C, Sheng ZH (2008) Docking of axonal mitochondria by syntaphilin controls their mobility and affects short-term facilitation. Cell 132(1):137–148

Kaufmann P, Pascual JM, Anziska Y, Gooch CL, Engelstad K, Jhung S, DiMauro S, De Vivo DC (2006) Nerve conduction abnormalities in patients with MELAS and the A3243G mutation. Arch Neurol 63(5):746–748

Kirkpatrick LL, Brady ST (1994) Modulation of the axonal microtubule cytoskeleton by myelinating Schwann cells. J Neurosci 14:7440–7450

Kiryu-Seo S, Ohno N, Kidd GJ, Komuro H, Trapp BD (2010) Demyelination increases axonal stationary mitochondrial size and the speed of axonal mitochondrial transport. J Neurosci 30 (19):6658–6666

Lamb CA, Yoshimori T, Tooze SA (2013) The autophagosome: origins unknown, biogenesis complex. Nat Rev Mol Cell Biol 14(12):759–774

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA (2003) Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet 33(3):366–374

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ, Rothstein JD (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature 487(7408):443–448

Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787–795

Liu X, Weaver D, Shirihai O, Hajnóczky G (2009) Mitochondrial 'kiss-and-run': interplay between mitochondrial motility and fusion-fission dynamics. EMBO J 28:3074–3089

Macaskill AF, Rinholm JE, Twelvetrees AE, Arancibia-Carcamo IL, Muir J, Fransson A, Aspenstrom P, Attwell D, Kittler JT (2009) Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. Neuron 61(4):541–555

Mackenzie ML, Shorer Z, Ghabriel MN, Allt G (1984) Myelinated nerve fibres and the fate of lanthanum tracer: an in vivo study. J Anat 138(Pt 1):1–14

Macklin WB, Gardinier MV, King KD, Kampf K (1987) An AG----GG transition at a splice site in the myelin proteolipid protein gene in jimpy mice results in the removal of an exon. FEBS Lett 223(2):417–421

Mahad D, Ziabreva I, Lassmann H, Turnbull D (2008) Mitochondrial defects in acute multiple sclerosis lesions. Brain 131(Pt 7):1722–1735

Mahad DJ, Ziabreva I, Campbell G, Lax N, White K, Hanson PS, Lassmann H, Turnbull DM (2009) Mitochondrial changes within axons in multiple sclerosis. Brain 132(Pt 5):1161–1174

Mahad DH, Trapp BD, Lassmann H (2015) Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol 14:183–193

Mierzwa A, Shroff S, Rosenbluth J (2011) Permeability of the paranodal junction of myelinated nerve fibers. J Neurosci 30(47):15962–15968

Misgeld T, Schwarz TL (2017) Mitostasis in neurons: maintaining mitochondria in an extended cellular architecture. Neuron 96(3):651–666

Moriguchi A, Ikenaka K, Furuichi T, Okano H, Iwasaki Y, Mikoshiba K (1987) The fifth exon of the myelin proteolipid protein-coding gene is not utilized in the brain of jimpy mutant mice. Gene 55(2–3):333–337

Morris RL, Hollenbeck PJ (1993) The regulation of bidirectional mitochondrial transport is coordinated with axonal outgrowth. J Cell Sci 104(Pt 3):917–927

Morris RL, Hollenbeck PJ (1995) Axonal transport of mitochondria along microtubules and F-actin in living vertebrate neurons. J Cell Biol 131(5):1315–1326

Mutsaers SE, Carroll WM (1998) Focal accumulation of intra-axonal mitochondria in demyelination of the cat optic nerve. Acta Neuropathol 96(2):139–143

Nave KA (2010a) Myelination and support of axonal integrity by glia. Nature 468(7321):244–252

Nave KA (2010b) Myelination and the trophic support of long axons. Nat Rev Neurosci 11 (4):275–283

Nave KA, Trapp BD (2008) Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci 31:535–561

Nave K, Lai C, Bloom FE, Milner RJ (1986) Jimpy mutant mouse: a 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in RNA splicing. Proc Natl Acad Sci U S A 83:9264–9268

Nguyen T, Mehta NR, Conant K, Kim KJ, Jones M, Calabresi PA, Melli G, Hoke A, Schnaar RL, Ming GL, Song H, Keswani SC, Griffin JW (2009) Axonal protective effects of the myelin-associated glycoprotein. J Neurosci 29(3):630–637

Nguyen HB, Thai TQ, Saitoh S, Wu B, Saitoh Y, Shimo S, Fujitani H, Otobe H, Ohno N (2016) Conductive resins improve charging and resolution of acquired images in electron microscopic volume imaging. Sci Rep 6:23721

Ohno N, Kidd GJ, Mahad D, Kiryu-Seo S, Avishai A, Komuro H, Trapp BD (2011) Myelination and axonal electrical activity modulate the distribution and motility of mitochondria at CNS nodes of Ranvier. J Neurosci 31(20):7249–7258

Ohno N, Chiang H, Mahad DJ, Kidd GJ, Liu L, Ransohoff RM, Sheng ZH, Komuro H, Trapp BD (2014) Mitochondrial immobilization mediated by syntaphilin facilitates survival of demyelinated axons. Proc Natl Acad Sci U S A 111(27):9953–9958

Ohno N, Katoh M, Saitoh Y, Saitoh S, Ohno S (2015) Three-dimensional volume imaging with electron microscopy toward connectome. Microscopy (Oxf) 64(1):17–26

Pathak D, Sepp KJ, Hollenbeck PJ (2010) Evidence that myosin activity opposes microtubule-based axonal transport of mitochondria. J Neurosci 30:8984–8992

Pekkurnaz G, Trinidad JC, Wang X, Kong D, Schwarz TL (2014) Glucose regulates mitochondrial motility via Milton modification by O-GlcNAc transferase. Cell 158:54–68

Perge JA, Koch K, Miller R, Sterling P, Balasubramanian V (2009) How the optic nerve allocates space, energy capacity, and information. J Neurosci 29(24):7917–7928

Perkins GA, Sosinsky GE, Ghassemzadeh S, Perez A, Jones Y, Ellisman MH (2008) Electron tomographic analysis of cytoskeletal cross-bridges in the paranodal region of the node of Ranvier in peripheral nerves. J Struct Biol 161(3):469–480

Poliak S, Peles E (2003) The local differentiation of myelinated axons at nodes of Ranvier. Nat Rev Neurosci 4(12):968–980

Readhead C, Schneider A, Griffiths I, Nave KA (1994) Premature arrest of myelin formation in transgenic mice with increased proteolipid protein gene dosage. Neuron 12:583–595

Ritchie JM (1995) Physiology of axons. In: Waxman SG, Kocsis JD, Stys PK (eds) The axon: structure, function and pathophysiology. Oxford University Press, Oxford, pp 68–96

Roach A, Takahashi N, Pravtcheva D, Ruddle F, Hood L (1985) Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice. Cell 42(1):149–155

Rosenbluth J (1980) Central myelin in the mouse mutant shiverer. J Comp Neurol 194(3):639–648

Rosenbluth J (2009) Multiple functions of the paranodal junction of myelinated nerve fibers. J Neurosci Res 87(15):3250–3258

Saab AS, Nave KA (2017) Myelin dynamics: protecting and shaping neuronal functions. Curr Opin Neurobiol 47:104–112

Saotome M, Safiulina D, Szabadkai G, Das S, Fransson A, Aspenstrom P, Rizzuto R, Hajnoczky G (2008) Bidirectional Ca^{2+}-dependent control of mitochondrial dynamics by the Miro GTPase. Proc Natl Acad Sci U S A 105(52):20728–20733

Sathornsumetee S, McGavern DB, Ure DR, Rodriguez M (2000) Quantitative ultrastructural analysis of a single spinal cord demyelinated lesion predicts total lesion load, axonal loss, and neurological dysfunction in a murine model of multiple sclerosis. Am J Pathol 157 (4):1365–1376

Saugier-Veber P, Munnich A, Bonneau D, Rozet JM, Le Merrer M, Gil R, Boespflug-Tanguy O (1994) X-linked spastic paraplegia and Pelizaeus-Merzbacher disease are allelic disorders at the proteolipid protein locus. Nat Genet 6:257–262

Saxton WM, Hollenbeck PJ (2012) The axonal transport of mitochondria. J Cell Sci 125 (Pt 9):2095–2104

Schwarz TL (2013) Mitochondrial trafficking in neurons. Cold Spring Harb Perspect Biol 5(6)

Sheng ZH, Cai Q (2012) Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. Nat Rev Neurosci 13(2):77–93

Shine HD, Readhead C, Popko B, Hood L, Sidman RL (1992) Morphometric analysis of normal, mutant, and transgenic CNS: correlation of myelin basic protein expression to myelinogenesis. J Neurochem 58(1):342–349

Shroff S, Mierzwa A, Scherer SS, Peles E, Arevalo JC, Chao MV, Rosenbluth J (2011) Paranodal permeability in "myelin mutants". Glia 59(10):1447–1457
Smith KJ, Kapoor R, Felts PA (1999) Demyelination: the role of reactive oxygen and nitrogen species. Brain Pathol 9(1):69–92
Sorbara CD, Wagner NE, Ladwig A, Nikic I, Merkler D, Kleele T, Marinkovic P, Naumann R, Godinho L, Bareyre FM, Bishop D, Misgeld T, Kerschensteiner M (2014) Pervasive axonal transport deficits in multiple sclerosis models. Neuron 84(6):1183–1190
Sousa AD, Bhat MA (2007) Cytoskeletal transition at the paranodes: the Achilles' heel of myelinated axons. Neuron Glia Biol 3(2):169–178
Spencer PS, Thomas PK (1974) Ultrastructural studies of the dying-back process. II. The sequestration and removal by Schwann cells and oligodendrocytes of organelles from normal and diseases axons. J Neurocytol 3(6):763–783
Sun XY, Takagishi Y, Okabe E, Chishima Y, Kanou Y, Murase S, Mizumura K, Inaba M, Komatsu Y, Hayashi Y, Peles E, Oda S, Murata Y (2009) A novel Caspr mutation causes the shambling mouse phenotype by disrupting axoglial interactions of myelinated nerves. J Neuropathol Exp Neurol 68(11):1207–1218
Sun T, Qiao H, Pan PY, Chen Y, Sheng ZH (2013) Motile axonal mitochondria contribute to the variability of presynaptic strength. Cell Rep 4(3):413–419
Tatar CL, Appikatla S, Da B, Paintlia AS, Singh I, Skoff RP (2010) Increased Plp1 gene expression leads to massive microglial cell activation and inflammation throughout the brain. ASN Neuro 2:219–231
Tatsuta T, Scharwey M, Langer T (2014) Mitochondrial lipid trafficking. Trends Cell Biol 24 (1):44–52
Thai TQ, Nguyen HB, Saitoh S, Wu B, Saitoh Y, Shimo S, Elewa YH, Ichii O, Kon Y, Takaki T, Joh K, Ohno N (2016) Rapid specimen preparation to improve the throughput of electron microscopic volume imaging for three-dimensional analyses of subcellular ultrastructures with serial block-face scanning electron microscopy. Med Mol Morphol 49(3):154–162
Trapp BD, Nave KA (2008) Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci 31:247–269
Trapp BD, Stys PK (2009) Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol 8(3):280–291
Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 27 (2):433–446
Wallace DC (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 39:359–407
Wang X, Schwarz TL (2009) The mechanism of Ca^{2+}-dependent regulation of kinesin-mediated mitochondrial motility. Cell 136(1):163–174
Waterham HR, Koster J, van Roermund CWT, Mooyer PAW, Wanders RJA, Leonard JV (2007) A lethal defect of mitochondrial and peroxisomal fission. N Engl J Med 356:1736–1741
Waxman SG (2008) Mechanisms of disease: sodium channels and neuroprotection in multiple sclerosis-current status. Nat Clin Pract Neurol 4(3):159–169
Waxman SG, Ja B, Duncan ID, Ransom BR (1990) Macromolecular structure of axon membrane and action potential conduction in myelin deficient and myelin deficient heterozygote rat optic nerves. J Neurocytol 19:11–28
Willard HF, Riordan JR (1985) Assignment of the gene for myelin proteolipid protein to the X chromosome: implications for X-linked myelin disorders. Science 230(4728):940–942
Windebank AJ, Wood P, Bunge RP, Dyck PJ (1985) Myelination determines the caliber of dorsal root ganglion neurons in culture. J Neurosci 5(6):1563–1569
Witte ME, Bo L, Rodenburg RJ, Belien JA, Musters R, Hazes T, Wintjes LT, Smeitink JA, Geurts JJ, De Vries HE, van der Valk P, van Horssen J (2009) Enhanced number and activity of mitochondria in multiple sclerosis lesions. J Pathol 219(2):193–204

Yin X, Crawford TO, Griffin JW, Tu P, Lee VM, Li C, Roder J, Trapp BD (1998) Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. J Neurosci 18(6):1953–1962

Yin X, Baek RC, Kirschner DA, Peterson A, Fujii Y, Nave KA, Macklin WB, Trapp BD (2006) Evolution of a neuroprotective function of central nervous system myelin. J Cell Biol 172 (3):469–478

Yin X, Kidd GJ, Ohno N, Perkins GA, Ellisman MH, Bastian C, Brunet S, Baltan S, Trapp BD (2016) Proteolipid protein-deficient myelin promotes axonal mitochondrial dysfunction via altered metabolic coupling. J Cell Biol 215(4):531–542

Yool DA, Edgar JM, Montague P, Malcolm S (2000) The proteolipid protein gene and myelin disorders in man and animal models. Hum Mol Genet 9(6):987–992

Youle RJ, van der Bliek AM (2012) Mitochondrial fission, fusion, and stress. Science 337:1062–1065

Young EA, Fowler CD, Kidd GJ, Chang A, Rudick R, Fisher E, Trapp BD (2008) Imaging correlates of decreased axonal Na^+/K^+ ATPase in chronic multiple sclerosis lesions. Ann Neurol 63(4):428–435

Zambonin JL, Zhao C, Ohno N, Campbell GR, Engeham S, Ziabreva I, Schwarz N, Lee SE, Frischer JM, Turnbull DM, Trapp BD, Lassmann H, Franklin RJ, Mahad DJ (2011) Increased mitochondrial content in remyelinated axons: implications for multiple sclerosis. Brain 134 (Pt 7):1901–1913

Zhang CL, Ho PL, Kintner DB, Sun D, Chiu SY (2010) Activity-dependent regulation of mitochondrial motility by calcium and Na/K-ATPase at nodes of Ranvier of myelinated nerves. J Neurosci 30(10):3555–3566

Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E, Patitucci A, Senderek J, Parman Y, Evgrafov O, Jonghe PD, Takahashi Y, Tsuji S, Pericak-Vance MA, Quattrone A, Battaloglu E, Polyakov AV, Timmerman V, Schroder JM, Vance JM (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. Nat Genet 36(5):449–451

The Role of Sulfatides in Axon–Glia Interactions

11

Hiroko Baba and Tomoko Ishibashi

Abstract

Myelin is heavily enriched in lipids (comprising approximately 70% of its dry weight), and the amount of cholesterol and glycolipids is higher than in any other cell membrane. Galactocerebroside (GalC) and its sulfated form, sulfatide, comprise the major glycolipid components of myelin. Their functional significance has been extensively studied using membrane models, cell culture, and in vivo experiments in which either GalC/sulfatide or sulfatide is deficient. From these studies, GalC and sulfatide have been distinctly localized within oligodendrocytes and their specific function in myelin has been elucidated. Here, the function of sulfatide in axo–glial interactions in myelin-forming cells as well as within myelin and its potential mechanisms of action are discussed.

Keywords

Sulfatide · Galactocerebroside · Myelin · Oligodendrocyte · Microdomain

11.1 Introduction

Myelin is a specialized multi-lamellar membranous structure formed by oligodendrocytes in the central nervous system (CNS) and by Schwann cells in the peripheral nervous system (PNS). The main function of myelin is to insulate most of the axonal surface, except for the nodes of Ranvier, and to facilitate saltatory conduction and ensure axonal integrity.

Myelinated axons consist of four functional domains, which include the node of Ranvier, paranode, juxtaparanode, and internode. At the paranode, paranodal myelin

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loops attach to the axonal surface to form the paranodal axo–glial junction. On the glial side, neurofascin 155 binds to an axonal protein complex consisting of contactin-associated protein (Caspr) and contactin to form the paranodal axo–glial junction. This acts as a lateral diffusion barrier to separate voltage-gated sodium channels at the node from voltage-gated potassium channels at the juxtaparanode. For details on the molecular mechanisms regulating the formation and maintenance of these domain structures (See Chap. 2).

Compared with other biological membranes, myelin is heavily enriched in lipids, which comprises approximately 70% of its total dry weight. Among these lipids, the proportion of cholesterol (27–28%) and glycolipids (approximately 30%) is significantly higher than in any other cell membrane (Taylor et al. 2004; Ozgen et al. 2016). The most abundant glycolipid is galactocerebroside (GalC), which comprises 22.7 or 23.3% of the total myelin lipids (dry weight), while sulfatide (SO_3-3Gal-ceramide), a sulfated form of GalC, comprises 3.8 or 7.1% of the myelin lipid content in human and rat CNS, respectively (Ozgen et al. 2016). GalC is produced from ceramide by the transfer of galactose from UDP-galactose and is catalyzed by the enzyme ceramide galactosyltransferase (CGT, EC 2.4.1.45) (Morell and Radin 1969; Schulte and Stoffel 1993; Bosio et al. 1996b), which is localized to the lumen of the ER in myelin-forming cells (Sprong et al. 1998; van Meer and Lisman 2002). The activated form of the sulfate group, PAPS (3′-phosphoadenosine 5′-phosphosulfate), is then transferred to position 3 of the galactose residue of GalC by the enzyme cerebroside sulfotransferase (CST, EC2.8.2.11) in the lumen of the Golgi (Fig. 11.1) (Benjamins et al. 1982; Tennekoon et al. 1983; for a review, also see Honke 2013, 2017). Sulfatide is negatively charged by the addition of sulfate onto its neutral precursor, GalC, suggesting its unique function from GalC. Although GalC and sulfatide are not myelin-specific lipids, they are extremely abundant within myelin sheaths in both the CNS and the PNS compared to other tissues. They are present mainly in the outer leaflet of the plasma membrane, and are involved in various functions, including formation of membrane microdomains, called lipid rafts, where these glycosphingolipids play roles in myelin along with cholesterol and raft-associated proteins (Taylor et al. 2004; Boggs et al. 2010; Ozgen et al. 2016).

In this chapter, the roles of sulfatides in myelin sheaths or in myelin-forming cells, and their possible mechanisms of action will be discussed.

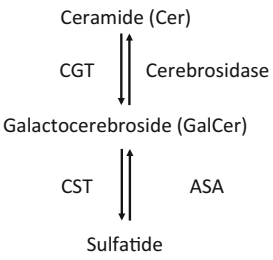

Fig. 11.1 Biosynthesis and degradation of GalC and sulfatide. *CGT* cerebroside galactosyl transferase (EC 2.4.1.45), *CST* cerebroside sulfotransferase (EC 2.8.2.11), *ASA* arylsulfatase A (EC 3.1.6.8)

11.2 Glycosphingolipids in Cultured Oligodendrocytes

Both GalC and sulfatide are detected in cultured oligodendrocytes using specific monoclonal antibodies against these lipids, O1 and O4, respectively (Bansal et al. 1989). The differentiation process of oligodendrocyte lineage cells can be observed in oligodendrocyte cultures prepared from rodent brain primary glia without neurons, and each developmental stage of oligodendrocyte differentiation can be identified by stage-specific markers (Pfeiffer et al. 1993; Taylor et al. 2004). Early oligodendrocyte progenitor cells (OPCs) are characterized by their bipolar shape and expression of GD3 and A2B5 antigens. Late OPCs (pro-oligodendroblasts, OLs) contain multiple processes. These cells display the A2B5 antigen and pro-OL antigen (POA) that can be visualized by specific mouse monoclonal antibodies. As development progresses, immature oligodendrocytes containing many processes express GalC, sulfatide, and some myelin-specific proteins, including 2′3′-cyclic-nucleotide 3′-phosphodiesterase (CNP) and myelin-associated glycoprotein (MAG). In the final stage, mature oligodendrocytes in culture express myelin basic protein (MBP), proteolipid protein (PLP) and myelin-oligodendrocyte glycoprotein (MOG) and form myelin-like membrane sheets in culture without neurons. Both GalC and sulfatide are present on the cell surface of mature oligodendrocytes; however, their distributions are somewhat different. GalC, stained by an O1 antibody, is localized to the myelin-like membrane sheets that also express myelin-specific proteins, whereas sulfatide, visualized with an O4 antibody, is excluded from these sheets (Maier et al. 2008).

Within OPCs, POA appears earlier than GalC, which is a precursor of sulfatide. For this reason, until recently, POA was thought to be a separate sulfated glycolipid from sulfatide, although the O4 monoclonal antibody recognizes both sulfatide and POA and they are both entirely absent in CGT knockout (CGT-KO) (Bansal et al. 1999) and CST knockout (CST-KO) mice (Hirahara et al. 2004). Recently, Hirahara et al. (2017) used imaging mass spectrometry to identify POA as a sulfatide with various short-chain fatty acids, including C16 non-hydroxylated fatty acids (C16), C18 non-hydroxylated (C18), or C18 hydroxylated fatty acids (C18-OH). During development, C22-OH sulfatide predominates during the later stages, after which non-hydroxylated and hydroxylated C24 fatty acids, with or without a double bond, become the most abundant in the adult brain and mature oligodendrocytes (Marbois et al. 2000; Schmitt et al. 2015; Ozgen et al. 2016; Hirahara et al. 2017; Boggs 2017). Thus, sulfatide species change during development and each may have unique roles in oligodendrocyte maturation and myelin function.

In addition to fatty acid length, the proportion of hydroxy to non-hydroxy galactolipids increases with age, correlating with the expression of myelin genes (Hoshi et al. 1973; Alderson et al. 2006). More than 50% of GalC and sulfatide contain very long-chain fatty acids hydroxylated at C2 (2-hydroxy fatty acids) (Alderson et al. 2004; Eckhardt et al. 2005). Mutations in the gene encoding fatty acid 2-hydroxylase, which is highly expressed in oligodendrocytes in the brain and peripheral myelin and catalyzes 2-hydroxylation of GalC and sulfatide, are associated with childhood-onset leukodystrophy with spastic paraparesis

(Edvardson et al. 2008). These patients show normal early development and present with lower-limb spasticity at 4–6 years of age. Homozygous mutations in this gene have also been associated with other neurodegenerative disorders, including spastic paraplegia 35 (SPG35) and neurodegeneration with brain iron accumulation. Therefore, these are now considered to be part of the phenotypic spectrum of fatty acid hydroxylase-associated neurodegeneration (FAHN) (Dick et al. 2010; Kruer et al. 2010, 2011; Finsterer et al. 2012). Taken together, this suggests that the difference in the length of fatty acids and hydroxyl group composition of glycosphingolipids between immature and mature oligodendrocytes may influence their function.

11.3 Role of Glycosphingolipids as Negative Regulators of Oligodendrocyte Differentiation in Culture and In Vivo

Glycosphingolipids appear during relatively early stages of oligodendrocyte differentiation, and thus their roles have been extensively examined in culture using blocking antibodies, including R-mAb (GalC/sulfatide), O1 (GalC), and O4 (POA, sulfatide) (Ranscht et al. 1982; Bansal et al. 1989). Since negatively charged sulfatide is known to interact with adhesion molecules, extracellular matrix proteins, blood coagulation factors, and microorganisms (Vos et al. 1994; Pesheva et al. 1997; Honke 2013; Grassi et al. 2016), it may act as a sensor/transmitter of environmental information in oligodendrocyte lineage cells (Bansal and Pfeiffer 1989, 1994). To identify the functional role of the glycosphingolipids in oligodendrocyte terminal differentiation, function-blocking monoclonal antibodies were added to cell culture media. R-mAb and O4 caused the arrest of oligodendrocyte differentiation, but O1 did not have this effect, indicating that sulfatide, but not GalC, affects oligodendrocyte lineage progression perhaps by mimicking an endogenous ligand (Bansal and Pfeiffer 1989; Bansal et al. 1999). The effects of these antibodies were reversible, and cells were able to complete differentiation after antibody removal from the media. Interestingly, the terminal differentiation of oligodendrocytes was accelerated in cultures prepared from CGT-KO mice, in which both GalC and sulfatide are absent (Bansal et al. 1999), and CST-KO mice which have GalC but lack sulfatide (Hirahara et al. 2004), suggesting that sulfatide acts as a negative regulator of oligodendrocyte differentiation, likely by interactions with external ligands. In vivo studies of immature CST-KO mice have also demonstrated the presence of enhanced populations of oligodendrocytes in the forebrain, medulla, cerebellum (Hirahara et al. 2004), and optic nerve (Kajigaya et al. 2011), and this increased number of oligodendrocytes is maintained through 7 months of age (Shroff et al. 2009). Thus, sulfatide, but not GalC, has a role in the regulation of oligodendrocyte terminal differentiation both in vitro and in vivo.

11.4 Roles of Glycosphingolipids in Myelin Morphology and Function In Vivo

To determine the function of these glycosphingolipids in myelin-forming cells, mice lacking both GalC and sulfatide were generated by knocking out the gene encoding the CGT enzyme, which adds galactose to ceramide to produce GalC (Coetzee et al. 1996, 1998; Bosio et al. 1996a, 1998; Dupree et al. 1998). These mice are completely devoid of both glycosphingolipids, while hydroxy fatty acid-containing glucocerebroside and sphingomyelin are upregulated within myelin (Coetzee et al. 1996). The CGT-KO mice also lack seminolipid and its precursor in the testes, which causes the arrest of spermatogenesis (Fujimoto et al. 2000). These animals start to display mild neurological symptoms around postnatal day 12 (P12), and later exhibit severe tremors and hindlimb paralysis with age and die as early as P18 to 30, although some survive past P90 (Coetzee et al. 1996). Nerve conduction studies using excised spinal cord showed reduced amplitudes of the compound action potentials and conduction deficits. In contrast, histological analysis showed that while the compact myelin was slightly thinner, it was relatively preserved when examined in transverse sections (Coetzee et al. 1996, 1998). These mice develop progressive hindlimb paralysis, which corresponds with extensive vacuolation in the ventral column of the P43 spinal cord. When longitudinal sections of myelinated axons were carefully examined, striking changes were observed around the nodal regions, including altered nodal lengths, an abundance of heminodes, the absence of transverse bands, and the presence of reversed lateral loops of myelin within the CNS (Dupree et al. 1998). As described in the introduction, cytoplasm from each layer of myelin appears and forms a paranodal loop at the paranode. The tips of these loops attach to the axonal surface and form the axo–glial paranodal junction, which is similar to the septate junction found in insects. In CGT-KO mice, these axo–glial junctions are widely disrupted, and paranodal myelin loops are detached from the axonal surface and often inverted, especially in the CNS. Immunohistological studies showed that clusters of the paranodal proteins Caspr, contactin, and neurofascin 155 were partially or completely absent from the paranodes (Dupree et al. 1999; Poliak et al. 2001). Since paranodal axo–glial junctions act as lateral diffusion barriers, loss of the barrier results in aberrant localization of the juxtaparanodal Kv channels to the paranode in CGT-KO mice. Studies with this animal model clearly show that glycosphingolipids are not significant contributors to compact myelin formation but are essential for the formation of axo–glial junctions at the paranode. The progressive demyelination often found in the CNS of CGT-KO mice suggests that glycosphingolipids are critical for the maintenance of compact myelin. Moreover, oligodendrocyte-specific CGT expression rescued the phenotype of CGT-KO mice, indicating that loss of CGT in oligodendrocytes, but not in other cells, is responsible for the myelin abnormalities and neurological deficits found in these mice (Zöller et al. 2005). Thus, the use of CGT-KO mice enabled us to identify the functional importance of the major myelin glycosphingolipids; however, the results obtained with this animal model did not clearly discriminate between the importance of the various glycolipids.

In order to answer this question, Honke et al. (2002) generated mice by homologous recombination of the *CST* gene. This gene encodes the enzyme that catalyzes the transfer of sulfate onto GalC to form sulfatide. These CST-KO mice are born healthy but begin to display tremor and hindlimb weakness by 6 weeks of age (Honke et al. 2002). These mice completely lack sulfatide and seminolipid, although their precursors are present, indicating that a single gene is responsible for the production of both lipids. Neurological symptoms including muscle weakness, pronounced tremor, and ataxia are progressive with age, although these mice can survive for more than one year. Thus, their phenotype is milder than that of CGT-KO mice, including the age of onset and severity. Histological analysis showed that compact myelin formed normally; however, similar to the CGT-KO mice, clusters of paranodal proteins, including Caspr, contactin, and neurofascin 155, were either partially or completely absent. This is consistent with results from EM studies in which paranodal axo–glial junctions were shown to be disrupted in both the CNS and the PNS (Ishibashi et al. 2002; Suzuki et al. 2004; Marcus et al. 2006; Hoshi et al. 2007). Interestingly, Nav channel clusters are observed at the nodes of Ranvier in the developing CNS, although the lengths of these clusters are somewhat longer than those of wild-type mice. These Nav clusters then progressively disappear with age, which corresponds with the timing of neurological abnormalities (Ishibashi et al. 2002). Clusters of Kv channels are also aberrantly formed at the paranode during development, then disappear with age in the CNS. Deterioration of the paranodal axo–glial junction was detected by magnetic resonance imaging (MRI) and diffusion tensor MRI (DTI), and was characterized by significantly lower T1 times and higher T2 times by MRI, and lower axial diffusivity and higher radial diffusivity by DTI in the spinal cord of 8-week-old CST-KO mice (Takano et al. 2012). Since the aberrant paranodal accumulation of Kv channels, as well as severely disrupted Caspr cluster formation, was already present in the developing optic nerve, this suggests that sulfatide is important for the formation of paranodal axo–glial junctions (Ishibashi et al. 2002).

EM analysis performed by Marcus et al. (2006) on CST-KO mice demonstrated the abundant formation of thinner compact myelin sheaths with moderately altered paranodal structure in the CNS. However, deterioration of the nodal structure, myelin vacuolar degeneration, and reduced axonal caliber and circularity become more pronounced with age. Furthermore, demyelination is not as prominent as in CGT-KO mice that display extensive demyelination by 45 days of age (Coetzee et al. 1996; Dupree et al. 2004). Based on comparisons between CGT-KO and CST-KO mice, the authors concluded that sulfatide is not critical for myelin development but is required for proper maintenance of myelinated fibers in the CNS, whereas GalC is critical for myelin development. The difference between the CGT-KO and CST-KO phenotypes may be related to their distinct localization. A fractionation study using myelin membranes from rat brain primarily recovered GalC in the compact myelin fraction, whereas sulfatide was enriched in the non-compact myelin fraction, consistent with the immunostaining patterns of GalC in myelin-like membrane sheets and the exclusion of sulfatide from these sheets in cultured oligodendrocytes (Maier et al. 2008).

A recent analysis of CNS myelin in CST-KO mice of different ages showed a one-third reduction in compact myelin proteins, MBP and PLP, and an equivalent post-developmental loss of major myelin lipids (Palavicini et al. 2016). This study also demonstrated that sulfatide deficiency affects the proteins in non-compact myelin, including neurofascin 155 at the paranode and MAG, restricted to the inner tongue. Furthermore, high molecular weight PLP complexes are progressively decreased in CST-KO mice, suggesting that the interaction between adjacent extracellular PLP domains in compact myelin may be affected. Taken together, these changes may be responsible for the phenotype observed in CST-KO mice (Palavicini et al. 2016). Two-dimensional analysis of the myelin from CGT-KO and CST-KO brains revealed significant alterations in proteins that regulate cytoskeletal dynamics, energy metabolism, vesicular trafficking, and adhesion (Fewou et al. 2010), although it is not clear what causes these alterations nor their involvement in the phenotypes of these mutants.

In contrast to the progressive CNS changes, the number of channel clusters did not change with age, although in the PNS of CST-KO mice Caspr and neurofascin 155 were completely absent in half of the paranodal regions and short clusters of these molecules remained in the other paranodes (Hoshi et al. 2007). Nav clusters at the nodes were longer than in wild type controls, and Kv channels were aberrantly localized to the paranodal regions. The nerve conduction velocities were also significantly reduced (Hayashi et al. 2013). The differences in the damage to the CNS and PNS in aged mice may be explained by the different roles of the paranodal axo–glial junctions in these two systems. In the PNS, gliomedin is produced by Schwann cells and localized to the surface of the microvilli at the nodes (Eshed et al. 2005). Both the cleaved and the membranous forms of gliomedin bind to axonal adhesion molecules at the node and protect the nodal characteristics including Nav channels in the PNS (Maertens et al. 2007; Eshed et al. 2007; Feinberg et al. 2010). However, the significance of the paranodal axo–glial junction as a diffusion barrier in the PNS remains controversial (Nelson and Jenkins 2017). In contrast, the paranodal axo–glial junction in the CNS is one of the key components for nodal assembly along with the ECM complex and the axonal scaffolding protein ankyrin-G (Nelson and Jenkins 2017; Griggs et al. 2017).

Changes in sulfatide levels are also associated with various human diseases. Arylsulfatase A (ASA) is an enzyme that catalyzes sulfatide degradation in lysosomes (Fig. 11.1). Accumulation of sulfatide by deficiencies in ASA activity or lack of its activator protein, saposin B, causes metachromatic leukodystrophy (MLD), an inherited lysosomal storage disorder characterized by various neurological symptoms with progressive demyelination in the CNS and PNS (van Rappard et al. 2015; Cesani et al. 2016). Thus, excessive amounts of sulfatide in cells can also cause myelin damage in humans and in MLD model mice (Ramakrishnan et al. 2007), although a study in mice with abnormal neuronal sulfatide storage suggests that neuronal accumulation also affects the disease phenotype (Eckhardt et al. 2007). In contrast, sulfatide levels in the brain of patients with Alzheimer's disease (AD) of all stages, including preclinical AD patients, are significantly reduced (Svennerholm and Gottfries 1994; Han et al. 2002; Cheng et al. 2013) and these changes may be

related to the white matter pathology in AD. Alterations in apolipoprotein E-mediated sulfatide trafficking may be involved in the sulfatide depletion in AD brains (Han et al. 2003; Han 2007). Therefore, the maintenance of appropriate sulfatide levels may be important for healthy brain function.

11.5 Potential Mechanism of Sulfatide Function in Myelin

From the previous studies, we know that sulfatide is present at specialized membrane microdomains called rafts where cholesterol is enriched and glycosylphosphatidylinositol (GPI)-anchored proteins, as well as signal proteins, are concentrated and initiate cell signaling. At these specific regions, sulfatide may also affect sorting, lateral assembly of myelin molecules, and membrane dynamics (for a review, see Ozgen et al. 2016; Grassi et al. 2016). For example, an in vitro study suggested the involvement of sulfatide in transcytotic PLP transport by triggering its reallocation between different microdomains (Baron et al. 2015). In the dynamics of other major myelin proteins, MBP was also affected by changes in galactosphingolipids (Boggs et al. 2010; Ozgen et al. 2014), suggesting their indirect interaction despite their different distribution, MBP is associated with the inner leaflet while these lipids are found in the outer leaflet of the myelin membrane. According to a fractionation study of the membrane homogenates obtained from the brain and optic nerve of CGT-KO mice, neurofascin 155 was present in different fractions than in the wild type, suggesting that myelin galactosphingolipids may stabilize axo–glial contacts by the formation of neurofascin 155-containing lipid rafts at the paranode (Schafer et al. 2004). Since sulfatide is a major negatively charged lipid in the outer leaflet of the myelin membrane, its negative charge may also be important for maintaining myelin (Palavicini et al. 2016). Furthermore, Boggs et al. (2010) introduced the intriguing theory that GalC and sulfatide form glycosynapses by trans carbohydrate–carbohydrate interactions between apposed oligodendrocyte membranes or extracellular surfaces of compact myelin, and signals mediated by these glycosynapses may be important for myelination and/or myelin function. In various cells, sulfatide responds to extracellular signaling processes, such as the initiation of basement membrane assembly by binding to laminin (Li et al. 2005), inhibition of platelet aggregation by binding to von Willebrand factors (Borthakur et al. 2003), and promotion of metastasis by binding to P-selectin (Garcia et al. 2007). This type of mechanism may be related to the regulation of oligodendrocyte differentiation or inhibition of axonal outgrowth reported by Winzeler et al. (2011). Therefore, studies aimed at addressing the functional mechanisms of GalC/sulfatide or sulfatide itself, as well as the identification of interacting molecules related to their function should be pursued.

11.6 Importance of Paranodal Axo–Glial Junctions and/or Sulfatides in Axonal Homeostasis

In the PNS of CST-KO mice, axonal swellings at the nodes of Ranvier were often observed by immunostaining of Nav channels as well as in EM studies. These nodal swellings frequently contained swollen mitochondria with disorganized cristae (Hoshi et al. 2007). The accumulation of large, swollen, abnormal mitochondria is also present in the PNS of Caspr knockout mice, in which paranodal axo–glial junctions are disrupted (Einheber et al. 2006). However, such nodal changes are not prominent in the CNS. Instead, axonal swellings are often found in the axons of the Purkinje cells in CST-KO cerebella (Honke et al. 2002; Ishibashi et al. 2015). These axonal swellings in the CNS are usually covered with PLP-positive compact myelin sheaths, suggesting that they are mostly formed in the internode instead of the node of Ranvier (Ishibashi et al. 2015). Focal axonal swellings or axonal spheroids are commonly found in other animals with disrupted paranodal axo–glial junctions (Garcia-Fresco et al. 2006; Pillai et al. 2007; Teigler et al. 2009; Watanabe et al. 2010; Takagishi et al. 2016). Therefore, the disturbance in proper formation and maintenance of the paranodal axo–glial junction through sulfatide-mediated mechanisms may be involved in the Purkinje axon pathology observed in CST-KO mice.

During development, swellings in Purkinje axons become prominent only after myelin is formed, and they progressively increase in number and size with age in the cerebellum of CST-KO mice (Ishibashi et al. 2015). Initially, when the swellings are small, the entire swelling is labeled with anti-calbindin antibody, and the cytoskeletal organization visualized by antibodies against neurofilament is usually restricted to the axonal center. Over time, the accumulation of mitochondrial COX IV becomes prominent in the swellings, followed sequentially by neurofilament and finally amyloid precursor protein (APP), which is often used as a marker for axonal damage (Cochran et al. 1991; Gentleman et al. 1993). The accumulation of mitochondria in the axonal swellings of Purkinje cells, indicated by cytochrome c and neurofilament staining, have also been reported in Neurexin IV/Capsr1/paranodin (NCP1)-KO as well as CGT-KO mice (Garcia-Fresco et al. 2006), suggesting that these swellings are associated with paranodal abnormalities rather than sulfatide deficiency. An EM study showed that numerous membrane organelles, dense bodies, and tubular structures closely resembling smooth endoplasmic reticulum (sER) were found within the swellings. However, similar swellings were not found in the optic nerves nor other CNS regions including the cerebral cortex and spinal cord, indicating that they are characteristic to Purkinje cells (Ishibashi et al. 2015). Since the accumulation of ER-like structures was often found in the swellings, and Purkinje cells contain various proteins related to Ca^{2+} homeostasis, the distribution of inositol trisphosphate receptor 1 (IP3R1), which is abundant in these cells (Satoh et al. 1990; Mikoshiba 2007), was examined in the CST-KO cerebellum. Interestingly, IP3R1 accumulation was even found in small swellings that were still negative for neurofilament immunostaining at 6 weeks of age, whereas IP3R1-positive swellings were completely absent in wild type controls. Ether lipid-deficient mice also show

similar axonal swellings with the accumulation of IP3R1-containing sER-like tubuli (Teigler et al. 2009), suggesting that they may be common features of myelinated axons with paranodal disruption. During development, IP3R1-positive swellings appear in the CST-KO cerebellum at P12 when MBP-positive myelin is formed in the granule cell layer. Axonal swellings first occur after myelination, and accumulation of IP3R1 begins in the early stage of this axonal change. IP3R1 is a Ca^{2+} channel in the sER membrane and activation of this channel by binding IP3 results in the release of Ca^{2+} from the ER to the cytoplasm as a part of the IP3 signaling cascade. This receptor is also responsible for intra-axonal Ca^{2+} release under certain pathological conditions (for a review, see Stirling and Stys 2010). Interestingly, overexpression of IP3R1 in transfected cells in vitro showed stacks of membranous ER (Takei et al. 1994), similar to that found in CST-KO mice. The mechanism of IP3R1 accumulation in the internodes of Purkinje axons, and the focal development of axonal swellings under sulfatide null conditions likely resulting from the disruption of the paranodal axo–glial junction, as well as the influence of regional swellings on axonal function, should be investigated further.

Previous EM studies in tissues of the CNS and PNS in CST-null mice clearly indicated that the location of axonal swellings differed completely; for example, in the internodal region in Purkinje axons of the CNS, and in the nodal region in PNS axons (Ishibashi et al. 2015; Hoshi et al. 2007, respectively). Similar patterns were also found in the distribution of mitochondria and focally elevated Ca^{2+} in myelinated axons between the CNS and PNS (for reviews, see Stirling and Stys 2010; Chiu 2011). In the PNS, mitochondria are present at a higher concentration at or near the nodes of Ranvier, possibly regulating their movements by nodal constriction, action potentials, elevation in cytosolic Ca^{2+}, and local energy demand by Na/K-ATPase concentrated at the node (for a review, see Chiu 2011). In contrast, in the myelinated axons of the CNS, mitochondria are distributed diffusely over the internodal region but avoid the nodal region (Chiu 2011; Ohno et al. 2011). Since Ca^{2+} arrests mitochondrial motility, the difference in mitochondrial distribution between the PNS and CNS may be related to the differences in localization of Ca^{2+} elevation by local action potentials: at the node of Ranvier in the PNS, and in the internode in the CNS (Chiu 2011). Of importance, these patterns correspond to the local energy demands. Thus, it is possible that insufficient sealing of the paranodal barrier in CST-KO mice may affect local axonal Ca^{2+} homeostasis, which in turn causes focal axonal swellings accompanied by the accumulation of abnormally large mitochondria at the PNS node and accumulation of IP3R1 in the internode of Purkinje axons.

11.7 Conclusion

GalC and sulfatide are two major glycolipids within myelin sheaths. The functional roles of each glycolipid in myelin and its molecular mechanism have been extensively studied using non-cellular or cellular in vitro studies as well as animal studies, including CGT-KO and CST-KO mice. Based on comparisons of these two mice,

sulfatide was shown to be essential for the formation of paranodal axo–glial junctions and for proper maintenance of CNS myelin. Sulfatide also regulates the terminal differentiation of oligodendrocytes. Focal axonal swellings (spheroids) in Purkinje cells of CST-KO mice are similar to the early axonal changes found in various pathological conditions in animals as well as humans. Moreover, the dysregulation of focal Ca^{2+} levels may be involved in subsequent axonal degeneration (Stirling and Stys 2010). Thus, CST-KO mice will be a useful tool for identifying the molecular mechanisms governing the interaction between myelin and axons in the maintenance of Ca^{2+} homeostasis and protection against degeneration.

References

Alderson NL, Rembiesa BM, Walla MD, Bielawska A, Bielawski J, Hama H (2004) The human FA2H gene encodes a fatty acid 2-hydroxylase. J Biol Chem 279:485622–448568

Alderson NL, Maldonado EN, Kern MJ, Bhat NR, Hama H (2006) FA2H-dependent fatty acid 2-hydroxylation in postnatal mouse brain. J Lipid Res 47:2772–2780

Bansal R, Pfeiffer SE (1989) Reversible inhibition of oligodendrocyte progenitor differentiation by a monoclonal antibody against surface galactolipids. Proc Natl Acad Sci U S A 86:6181–6185

Bansal R, Pfeiffer SE (1994) Inhibition of protein and lipid sulfation in oligodendrocytes blocks biological responses to FGF-2 and retards cytoarchitectural maturation, but not developmental lineage progression. Dev Biol 162:511–524

Bansal R, Warrington AE, Gard AL, Ranscht B, Pfeiffer SE (1989) Multiple and novel specificities of monoclonal antibodies O1, O4, and R-mAb used in the analysis of oligodendrocyte development. J Neurosci Res 24:548–557

Bansal R, Winkler S, Bheddah S (1999) Negative regulation of oligodendrocyte differentiation by galactosphingolipids. J Neurosci 19:7913–7924

Baron W, Ozgen H, Klunder B, de Jonge JC, Nomden A, Plat A, Trifillieff E, de Vries H, Hoekstra D (2015) The major myelin-resident protein PLP is transported to myelin membranes via a transcytotic mechanism: involvement of Sulfatide. Mol Cell Biol 35:288–302

Benjamins JA, Hadden T, Skoff RP (1982) Cerebroside sulfotransferase in Golgi-enriched fractions from rat brain. J Neurochem 38:233–241

Boggs JM (2017) Early glycolipid (POA) in pro-oligodendroblasts revealed to be sulfatide. J Neurochem 140:356–358

Boggs JM, Gao W, Zhao J et al (2010) Participation of galactosylceramide and sulfatide in glycosynapses between oligodendrocyte or myelin membranes. FEBS Lett 584:1771–1778

Borthakur G, Cruz MA, Dong JF, McIntire L, Li F, López JA, Thiagarajan P (2003) Sulfatides inhibit platelet adhesion to von Willebrand factor in flowing blood. J Thromb Haemost 1:1288–1295

Bosio A, Binczek E, Stoffel W (1996a) Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. Proc Natl Acad Sci U S A 93:13280–13285

Bosio A, Binczek E, Le Beau MM, Fernald AA, Stoffel W (1996b) The human gene CGT encoding the UDP-galactose ceramide galactosyl transferase (cerebroside synthase): cloning, characterization, and assignment to human chromosome 4, band q26. Genomics 34:69–75

Bosio A, Büssow H, Adam J, Stoffel W (1998) Galactosphingolipids and axono-glial interaction in myelin of the central nervous system. Cell Tissue Res 292:199–210

Cesani M, Lorioli L, Grossi S, Amico G, Fumagalli F, Spiga I, Filocamo M, Biffi A (2016) Mutation update of ARSA and PSAP genes causing metachromatic leukodystrophy. Hum Mutat 37:16–27

Cheng H, Wang M, Li JL, Cairns NJ, Han X (2013) Specific changes of sulfatide levels in individuals with pre-clinical Alzheimer's disease: an early event in disease pathogenesis. J Neurochem 127:733–738

Chiu SY (2011) Matching mitochondria to metabolic needs at nodes of Ranvier. Neuroscientist 17:343–350

Cochran E, Bacci B, Chen Y, Patton A, Gambetti P, Autilio-Gambetti L (1991) Amyloid precursor protein and ubiquitin immunoreactivity in dystrophic axons is not unique to Alzheimer's diseases. Am J Pathol 139:485–489

Coetzee T, Fujita N, Dupree J et al (1996) Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. Cell 86:209–219

Coetzee T, Suzuki K, Popko B (1998) New perspectives on the function of myelin galactolipids. Trends Neurosci 21:126–130

Dick KJ, Eckhardt M, Paisán-Ruiz C, Alshehhi AA, Proukakis C, Sibtain NA, Maier H, Sharifi R, Patton MA, Bashir W, Koul R, Raeburn S, Gieselmann V, Houlden H, Crosby AH (2010) Mutation of FA2H underlies a complicated form of hereditary spastic paraplegia (SPG35). Hum Mutat 31:E1251–E1260

Dupree JL, Coetzee T, Suzuki K et al (1998) Myelin abnormalities in mice deficient in galactocerebroside and sulfatide. J Neurocytol 27:649–659

Dupree JL, Girault JA, Popko B (1999) Axo-glial interactions regulate the localization of axonal paranodal proteins. J Cell Biol 147:1145–1152

Dupree JL, Mason JL, Marcus JR, Stull M, Levinson R, Matsushima GK, Popko B (2004) Oligodendrocytes assist in the maintenance of sodium channel clusters independent of the myelin sheath. Neuron Glia Biol 1:179–192

Eckhardt M, Yaghootfam A, Fewou SN, Zöller I, Gieselmann V (2005) A mammalian fatty acid hydroxylase responsible for the formation of α-hydroxylated galactosylceramide in myelin. Biochem J 388:245–254

Eckhardt M, Hedayati KK, Pitsch J, Lüllmann-Rauch R, Beck H, Fewou SN, Gieselmann V (2007) Sulfatide storage in neurons causes hyperexcitability and axonal degeneration in a mouse model of metachromatic leukodystrophy. J Neurosci 27:9009–9021

Edvardson S, Hama H, Shaag A et al (2008) Mutations in the fatty acid 2-hydroxylase gene are associated with leukodystrophy with spastic paraparesis and dystonia. Am J Hum Genet 83:643–648

Einheber S, Bhat MA, Salzer JL (2006) Disrupted axo-glial junctions result in accumulation of abnormal mitochondria at nodes of Ranvier. Neuron Glia Biol 2:165–174

Eshed Y, Feinberg K, Poliak S, Sabanay H, Sarig-Nadir O, Spiegel I, Bermingham JR, Peles E (2005) Gliomedin mediates Schwann cell-axon interaction and the molecular assembly of the nodes of Ranvier. Neuron 47:215–229

Eshed Y, Feinberg K, Carey DJ, Peles E (2007) Secreted gliomedin is a perinodal matrix component of peripheral nerves. J Cell Biol 177:551–562

Feinberg K, Eshed-Eisenbach Y, Frechter S, Amor V, Salomon D, Sabanay H, Dupree JL, Brumet M, Brophy PJ, Shrager P, Peles E (2010) A glial signal consisting of gliomedin and NrCAM clusters axonal Na^+ channels during the formation of nodes of Ranvier. Neuron 65:490–502

Fewou SN, Fernandes A, Stockdale K et al (2010) Myelin protein composition is altered in mice lacking either sulfated or both sulfated and non-sulfated galactolipids. J Neurochem 112:599–610

Finsterer J, Löscher W, Quasthoff S, Wanschitz J, Auer-Grumbach M, Stevanin G (2012) Hereditary spastic paraplegias with autosomal dominant, recessive, X-linked, or maternal trait of inheritance. J Neurol Sci 318:1–18

Fujimoto H, Tadano-Aritomi K, Tokumasu A, Ito K, Hikita T, Suzuki K, Ishizuka I (2000) Requirement of seminolipid in spermatogenesis revealed by UDP-galactose:ceramide galactosyltransferase-deficient mice. J Biol Chem 275:22623–22626

Garcia J, Callewaert N, Borsig L (2007) P-selectin mediated metastatic progression through binding to sulfatides on tumor cells. Glycobiology 17:185–196

Garcia-Fresco GP, Sousa AD, Pillai AM, Moy SS, Crawley JN, Tessarollo L, Dupree JL, Bhat MA (2006) Disruption of axo-glial junctions causes cytoskeletal disorganization and degeneration of Purkinje neuron axons. Proc Natl Acad Sci U S A 103:513705142

Gentleman SM, Nash MJ, Sweeting CJ, Graham DI, Roberts GW (1993) β-Amyloid precursor protein (βAPP) as a marker for axonal injury after head injury. Neurosci Lett 160:139–144

Grassi S, Prioni S, Cabitta L et al (2016) The role of 3-O-sulfogalactosylceramide, sulfatide, in the lateral organization of myelin membrane. Neurochem Res 41:130–143

Griggs RB, Yermakov LM, Susuki K (2017) Formation and disruption of functional domains in myelinated CNS axons. Neurosci Res 116:77–87

Han X, Holtzman DM, McKeel DW Jr, Kelley J, Morris JC (2002) Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. J Neurochem 82:809–818

Han X (2007) Potential mechanisms contributing to sulfatide depletion at the earliest clinically recognizable stage of Alzheimer's disease: a tale of shotgun lipidomics. J Neurochem 103 (s1):171–179

Han X, Cheng H, Fryer JD, Fagan AM, Holtzman DM (2003) Novel role for apolipoprotein E in the central nervous system. Modulation of sulfatide content. J Biol Chem 278:8043–8051

Hayashi A, Kaneko N, Tomihira C et al (2013) Sulfatide decrease in myelin influences formation of the paranodal axo-glial junction and conduction velocity in the sciatic nerve. Glia 61:466–474

Hirahara Y, Bansal R, Honke K et al (2004) Sulfatide is a negative regulator of oligodendrocyte differentiation: development in sulfatide-null mice. Glia 45:269–277

Hirahara Y, Wakabayashi T, Mori T et al (2017) Sulfatide species with various fatty acid chains in oligodendrocytes at different developmental stages determined by imaging mass spectrometry. J Neurochem 140:435–450

Honke K (2013) Biosynthesis and biological function of sulfoglycolipids. Proc Jpn Acad Ser B Phys Biol Sci 89:129–138

Honke K (2017) Biological functions of sulfoglycolipids and the EMARS method for identification of co-cultured molecules in the membrane microdomains. J Biochem 163:253–263

Honke K, Hirahara Y, Dupree J et al (2002) Paranodal junction formation and spermatogenesis require sulfoglycolipids. Proc Natl Acad Sci U S A 99:4227–4232

Hoshi M, Williams M, Kishimoto Y (1973) Characterization of brain cerebrosides at early stages of development in the rat. J Neurochem 21:709–712

Hoshi T, Suzuki A, Hayashi S et al (2007) Nodal protrusions, increased Schmidt-Lanterman incisures, and paranodal disorganization are characteristic features of sulfatide-deficient peripheral nerves. Glia 55:584–594

Ishibashi T, Dupree JL, Ikenaka K et al (2002) A myelin galactolipid, sulfatide, is essential for maintenance of ion channels on myelinated axon but not essential for initial cluster formation. J Neurosci 22:6507–6514

Ishibashi T, Kodama A, Baba H (2015) Disruption of paranodal axo-glial interaction and/or absence of sulfatide causes irregular type I inositol 1,4,5-trisphosphate receptor deposition in cerebellar Purkinje neuron axons. J Neurosci Res 93:19–27

Kajigaya H, Tanaka KF, Hayashi A et al (2011) Increased numbers of oligodendrocyte lineage cells in the optic nerves of cerebroside sulfotransferase knockout mice. Proc Jpn Acad Ser B 87:415–424

Kruer MC, Paisán-Ruiz C, Boddaert N, Yoon MY, Hama H, Gregory A, Malandrini A, Woltjer RL, Munnich A, Gobin S, Polster BJ, Palmeri S, Edvardson S, Hardy J, Houlden H, Hayflick SJ (2010) Defective FA2H leads to a novel form of neurodegeneration with brain iron accumulation (NBIA). Ann Neurol 68:611–618

Kruer MC, Gregory A, Hayflick SJ (2011) Fatty acid hydroxylase-associated neurodegeneration. In: Adam MP et al (eds) GeneReviews [internet]. University of Washington, Seattle. [updated 2012]

Li S, Liquari P, McKee KK, Harrison D, Patel R, Lee S, Yurchenco PD (2005) Laminin-sulfatide binding initiates basement membrane assembly and enables receptor signaling in Schwann cells and fibroblasts. J Cell Biol 169:179–189

Maertens B, Hopkins D, Franzke CW, Keene DR, Bruckner-Tuderman L, Greenspan DS, Koch M (2007) Cleavage and oligomerization of gliomedin, a transmembrane collagen required for node of Ranvier formation. J Biol Chem 282:10647–10659

Maier O, Hoekstra D, Baron W (2008) Polarity development in oligodendrocytes: sorting and trafficking of myelin components. J Mol Neurosci 35:35–53

Marbois BN, Faull KF, Fluharty AL, Raval-Fernandes S, Rome LH (2000) Analysis of sulfatide from rat cerebellum and multiple sclerosis white matter by negative ion electrospray mass spectrometry. Biochim Biophys Acta 1484:59–70

Marcus J, Honigbaum S, Shroff S et al (2006) Sulfatide is essential for the maintenance of CNS myelin and axon structure. Glia 53:372–381

Mikoshiba K (2007) IP_3 receptor/Ca^{2+} channel: from discovery to new signaling concepts. J Neurochem 102:1426–1446

Morell P, Radin NS (1969) Synthesis of cerebroside by brain from uridine diphosphate galactose and ceramide containing hydroxy fatty acid. Biochemistry 8:506–512

Nelson AD, Jenkins PM (2017) Axonal membranes and their domains: assembly and function of the axon initial segment and node of Ranvier. Front Cell Neurosci 11:136

Ohno N, Kidd GJ, Mahad D, Kiryu-Seo S, Avishai A, Komuro H, Trapp BD (2011) Myelination and axonal electrical activity modulate the distribution and motility of mitochondria at CNS nodes of Ranvier. J Neurosci 31:7249–7258

Ozgen H, Schrimpf W, Hendrix J et al (2014) The lateral membrane organization and dynamics of myelin proteins PLP and MBP are dictated by distinct galactolipids and the extracellular matrix. PLoS One 7:e101834

Ozgen H, Baron W, Hoekstra D et al (2016) Oligodendroglial membrane dynamics in relation to myelin biogenesis. Cell Mol Life Sci 73:3291–3310

Palavicini JP, Wang C, Chen L, Ahmar S, Higuera JD, Dupree JL, Han X (2016) Novel molecular insights into the critical role of sulfatide in myelin maintenance/function. J Neurochem 139:45–54

Pesheva P, Gloor S, Schachner M et al (1997) Tenascin-R is an intrinsic autocrine factor for oligodendrocyte differentiation and promotes cell adhesion by a sulfatide-mediated mechanism. J Neurosci 17:4642–4651

Pfeiffer SE, Warrington AE, Bansal R (1993) The oligodendrocyte and its many cellular processes. Trends Cell Biol 3:191–197

Pillai AM, German P, Garcia-Fresco GP, Sousa AD, Dupree JL, Philpot BD, Bhat MA (2007) No effect of genetic deletion of contactin-associated protein (CASPR) on axonal orientation and synaptic plasticity. J Neurosci Res 85:2318–2331

Poliak S, Gollan L, Salmon D, Berglund EO, Ohara R, Ranscht B, Peles E (2001) Localization of Caspr2 in myelinated nerves depends on axon-glia interactions and the generation of barriers along the axon. J Neurosci 21:7568–7575

Ramakrishnan H, Hedayati KK, Lüllmann-Rauch R, Wessig C, Fewou SN, Maier H, Goebel HH, Gieselmann V, Eckhardt M (2007) Increasing sulfatide synthesis in myelin-forming cells of arylsulfatase A-deficient mice causes demyelination and neurological symptoms reminiscent of human metachromatic leukodystrophy. J Neurosci 27:9482–9490

Ranscht B, Clapshaw PA, Price J, Noble M, Seifert W (1982) Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. Proc Natl Acad Sci U S A 79:2709–2713

Satoh T, Ross CA, Villa A, Supattapone S, Pozzan T, Snyder SH, Meldolesi J (1990) The inositol 1,4,5,-trisphosphate receptor in cerebellar Purkinje cells: quantitative immunogold labeling reveals concentration in an ER subcompartment. J Cell Biol 111:615–624

Schafer DP, Bansal R, Hedstrom KL, Pfeiffer SE, Rasband MN (2004) Does paranode formation and maintenance require partitioning of neurofascin 155 into lipid rafts? J Neurosci 24:3176–3185

Schmitt S, Castelvetri LC, Simons M (2015) Metabolism and functions of lipids in myelin. Biochim Biophys Acta 1851:999–1005

Schulte S, Stoffel W (1993) Ceramide UDP galactosyltransferase from myelinating rat brain: purification, cloning, and expression. Proc Natl Acad Sci U S A 90:10265–10269

Shroff S, Pomicter AD, Fox MA et al (2009) Adult sulfatide null mice maintain an increased number of oligodendrocytes. J Neurosci Res 87:3403–3414

Sprong H, Kruithof B, Leijendekker R, Slot JW, van Meer G, van der Sluijs P (1998) UDP-galactose:ceramide galactosyltransferase is a class I integral membrane protein of the endoplasmic reticulum. J Biol Chem 273:25880–25888

Stirling DP, Stys PK (2010) Mechanisms of axonal injury: internodal nanocomplexes and calcium deregulation. Trends in Mo Med 16:160–170

Suzuki A, Hoshi T, Ishibashi T et al (2004) Paranodal axoglial junction is required for the maintenance of the Nav1.6-type sodium channel in the node of Ranvier in the optic nerves but not in peripheral nerve fibers in the sulfatide-deficient mice. Glia 46:274–283

Svennerholm L, Gottfries C-G (1994) Membrane lipids, selectively diminished in Alzheimer brains, suggest synapse loss as a primary event in early-onset form (type I) and demyelination in late-onset form (type II). J Neurochem 62:1039–1047

Takagishi Y, Katanosaka K, Mizoguchi H, Murata Y (2016) Disrupted axon-glia interactions at the paranode in myelinated nerves cause axonal degeneration and neuronal cell death in the aged Caspr mutant mouse shambling. Neurobiol Aging 43:34–46

Takano M, Hikishima K, Fujiyoshi K et al (2012) MRI characterization of paranodal junction failure and related spinal cord changes in mice. PLoS One 7:e52904

Takei K, Mignery GA, Mugnaini E, Südohof TC, De Camilli P (1994) Inositol 1,4,5-trisphosphate receptor causes formation of ER cisternal stacks in transfected fibroblasts and in cerebellar Purkinje cells. Neuron 12:327–342

Taylor CM, Marta CB, Bansal R et al (2004) The transport, assembly, and function of myelin lipids. In: Lazzarini RA (ed) Myelin biology and disorders, vol 2. Elsevier, London, pp 57–88

Teigler A, Komljenovic D, Draguhn A et al (2009) Defects in myelination, paranode organization and Purkinje cell innervation in the ether lipid-deficient mouse cerebellum. Hum Mol Genet 18:1897–1908

Tennekoon G, Zaruba M, Wolinsky J (1983) Topography of cerebroside sulfotransferase in Golgi-enriched vesicles from rat brain. J Cell Biol 97:1107–1112

van Meer G, Lisman Q (2002) Sphingolipid transport: rafts and translocators. J Biol Chem 277:25855–25858

van Rappard DF, Boelens JJ, Wolf NI (2015) Metachromatic leukodystrophy: disease spectrum and approaches for treatment. Best Pract Res Clin Endocrinol Metab 29:261–273

Vos JP, Lopes-Cardozo M, Cadella BM (1994) Metabolic and functional aspects of sulfogalactolipids. Biochim Biophys Acta 1211:125–149

Watanabe S, Endo S, Oshima E, Hoshi T, Higashi H, Yamada K, Tohyama K, Yamashita T, Hirabayashi Y (2010) Glycosphingolipid synthesis in cerebellar Purkinje neurons: roles in myelin formation and axonal homeostasis. Glia 58:1197–1207

Winzeler AM, Mandemarkers WJ, Sun MZ, Stafford M, Phillips CT, Barres BA (2011) The lipid sulfatide is a novel myelin-associated inhibitor of CNS axon outgrowth. J Neurosci 31:6481–6492

Zöller I, Büssow H, Gieselmann V, Eckhardt M (2005) Oligodendrocyte-specific ceramide galactosyltransferase (CGT) expression phenotypically rescues CGT-deficient mice and demonstrates that CGT activity does not limit brain galactosylceramide level. Glia 52:190–198

Structures and Molecular Composition of Schmidt–Lanterman Incisures

12

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Abstract

Schmidt–Lanterman incisure (SLI) is a circular-truncated cone shape in the myelin internode that is a specific feature of myelinated nerve fibers formed in Schwann cells in the peripheral nervous system (PNS). The SLI circular-truncated cones elongate like spring at the narrow sites of beaded appearance nerve fibers under the stretched condition. In this chapter, we demonstrate various molecular complexes in SLI, and especially focus on membrane skeleton, protein 4.1G–membrane protein palmitoylated 6 (MPP6)–cell adhesion molecule 4 (CADM4). 4.1G was essential for the molecular targeting of MPP6 and

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CADM4 in SLI. Motor activity and myelin ultrastructures were abnormal in 4.1G-deficient mice, indicating the 4.1G function as a signal for proper formation of myelin in PNS. Thus, SLI probably has potential roles in the regulation of adhesion and signal transduction as well as in structural stability in Schwann cell myelin formation.

Keywords
Schmidt–Lanterman incisure · Schwann cell · Membrane skeleton · Protein 4.1 family · Membrane palmitoylated protein family · Cell adhesion molecule · Charcot–Marie–Tooth neuropathy

12.1 Schmidt–Lanterman incisure (SLI)

12.1.1 Structure of SLI

The Schmidt–Lanterman incisures (SLIs) are characteristic funnel-shaped structures in the internodes of myelinated nerve fibers in the PNS, formed in Schwann cell myelin, as demonstrated in the teased nerve fibers of the mouse sciatic nerves by the protein 4.1G immunostaining (Fig. 12.1a). Each SLI has three specific structural features, as schematically shown in Fig. 12.1b; (1) a coil spring shape due to its cytoplasmic continuity in the myelin roll, (2) the adhesion of membranes with adjacent membranes, and (3) swelling of the cytoplasm, which differs from the tight interactions of adjacent membranes in compact myelin (Terada et al. 2016). These three combinations indicate that SLIs work in a similar manner to a volute spring.

12.1.2 Shape Change of SLI During Nerve Stretch

In vivo cryotechnique (IVCT) reveals the dynamic characteristics of living animal organs (Ohno et al. 2017). The IVCT was applied to evaluate morphological changes in the mouse PNS, as demonstrated in Fig. 12.2a (Kamijo et al. 2014). Structures of nerve fibers in PNS showed a beaded appearance during stretching (Fig. 12.2b), whereas they were mostly cylindrical under non-stretched conditions. Many SLIs in the stretched nerve fibers were elongated (Fig. 12.2b, c) and localized at narrow sites and transitional regions from the narrow to wide areas of the beaded appearance (Fig. 12.2b). These findings indicate that SLI function as bumper structures against mechanical forces, demonstrated as a model in Fig. 12.2d. Interestingly, SLI has been suggested to be cleavage site of the myelin sheath during Wallerian degeneration, and newly formed F-actin is related to the RAS-related C3 botulinus toxin substrate-1 (Rac1) signaling (Jung et al. 2011).

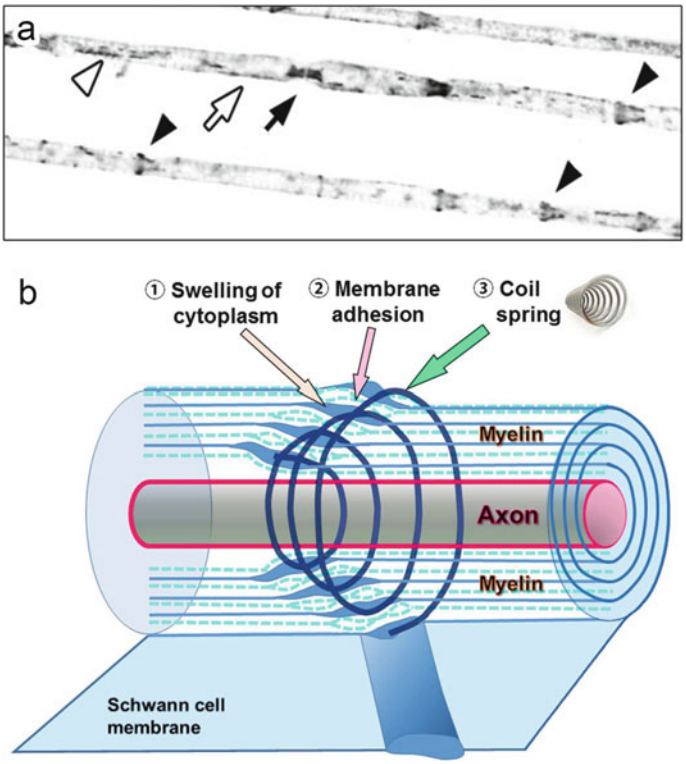

Fig. 12.1 Structure of SLIs. (**a**) Light microscopically, SLIs are funnel shaped or truncated cone appearance in internodes (black arrowheads), demonstrated by the protein 4.1G immunostaining in the teased mouse sciatic nerve. Protein 4.1G is also localized in paranodes (black arrow). White arrowhead and arrow indicate Cajar bands. (**b**) Schematic model of SLI, which demonstrates three structural features: (1) swelling of cytoplasm, (2) adhesion of membranes, and (3) a coil spring. Some images were adapted from previous papers (Ohno et al. 2006; Terada et al. 2016), with permission

12.1.3 Molecular Components in SLI

Inside SLI, various molecules are incorporated, as listed in Table 12.1. Main components of the membrane skeleton, actin and spectrin, have been reported (Susuki et al. 2011; Trapp et al. 1989). Gap junctions are formed by connexins (Freidin et al. 2009; Zhao et al. 1999), and Na^+-K^+-$2Cl^-$ cotransporter-1 (NKCC1) (Alvarez-Leefmans et al. 2001), potassium channels (Rash et al. 2016; Zhou et al. 1999), and aquaporin-1 (Segura-Anaya et al. 2015) were also identified, indicating ion transport and water entry in SLI. Various tight and adherens junctional molecules have also been identified as adhesion structures in SLI; including claudin (Poliak et al. 2002), occludin (Alanne et al. 2009), zonula occludens-1/2 (ZO1/2) (Poliak et al. 2002), membrane-associated guanylate kinase, WW and PDZ domain-

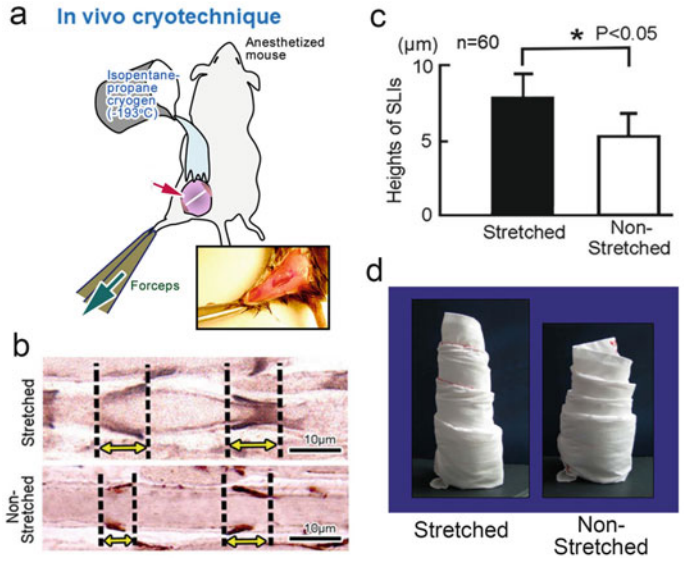

Fig. 12.2 Dynamic structural change of SLIs. (**a**) Schematic representation of in vivo cryotechnique for the mouse sciatic nerve. Anesthetized mouse sciatic nerve (red arrow) is stretched by pulling leg (blue arrow) and cryofixed with isopentane-propane cryogen (−193 °C). Inset demonstrates the actual photo of the nerve under stretching. (**b**) Light microscopic images of the 4.1G-immunostained paraffin sections of stretched or non-stretched nerve fiber, demonstrating the appearance of SLIs (yellow arrows). (**c**) Statistic analysis of heights of truncated cones in SLIs under stretched or non-stretched condition. (**d**) Model of dynamic structural changes of SLI by wrapping cloth. Edge of the cloth is sewed not to detach easily. Some images are adapted from previous paper (Kamijo et al. 2014), with permission

containing protein-2 (MAGI2) (Poliak et al. 2002), tricellurin (Kikuchi et al. 2010), E-cadherin and related catenins (Tricaud et al. 2005; Fannon et al. 1995; Perrin-Tricaud et al. 2007), erbin (Rangwala et al. 2005; Tao et al. 2009), multi-PDZ domain protein-1 (MUPP1) (Poliak et al. 2002), and junctional adhesion molecule (JAM)-C (Scheiermann et al. 2007). In addition, myelin-associated glycoprotein (MAG) has been a well-known adhesion molecule localized in SLI (Trapp et al. 1989; Yin et al. 1998). We have demonstrated additional membrane skeletal proteins and adhesion molecules in SLI, such as 4.1G (Ohno et al. 2006), membrane protein palmitoylated-6 (MPP6) (Terada et al. 2012), Lin7 (Saitoh et al. 2017), and the intramembranous adhesion molecule, cell adhesion molecule 4 (CADM4) (Ivanovic et al. 2012), as schematically demonstrated in Fig. 12.3a. Regarding membrane-associated guanylate kinase (MAGUK) family proteins including MPP6, we will precisely describe in the following sections.

Table 12.1 List of proteins localized in SLI

Protein name	Category	Changes under knockout and/or knockdown (KO/KD)	Interaction proteins	References
Actin	Membrane skeleton: filamentous formation		Spectrin; Protein 4.1	Trapp et al. (1989)
Annexin II	Calcium-dependent actin- and phospholipid-binding			Hayashi et al. (2007)
Aquaporin-1 (AQP1)	Water channel			Segura-Anaya et al. (2015)
β-catenin	Adherens junction		E-cadherin	Fannon et al. (1995)
Cell adhesion molecule-4 (CADM4)	Adhesion	Focal hypermyelination in conditional KO mouse; Delay in the initiation of myelination	Protein 4.1G	Spiegel et al. (2007) and Golan et al. (2013)
Claudin-5	Tight junction			Poliak et al. (2002)
Connexin-29 (Cx29)	Gap junction	Auditory neuropathy		Tang et al. (2006) and Rash et al. (2016)
Connexin-32 (Cx32)	Gap junction	Demyelination	Dlg1	Duffy et al. (2007) and Scherer et al. (1998)
Choline transporter-like protein-1 (CTL1)	Choline transport		CADM4	Heffernan et al. (2017)
Discs large homolog-1 (Dlg1)/Synapse-associate protein-97 (SAP97)	Membrane-associated guanylate kinase (MAGUK)	Transient increase in myelin thickness during development by conditional inactivation	Myotubularin-related-2 (MTMR2); phosphatase and tensin homolog (PTEN)	Bolis et al. (2009), Cotter et al. (2010) and Noseda et al. (2013)
E-cadherin	Adherens junction	Perturbation of incisure	β-catenin; p120-catenin	Fannon et al. (1995) and Tricaud et al. (2005)
Erbin	Adherens junction	Decrease myelination	ErbB2	Rangwala et al. (2005) and Tao et al. (2009)

(continued)

Table 12.1 (continued)

Protein name	Category	Changes under knockout and/or knockdown (KO/KD)	Interaction proteins	References
Junctional adhesion molecule-C (JAM-C)	Adherens junction	Tomacula formation		Scheiermann et al. (2007)
Voltage-gated K^+ channels-1.1, 1.2 (Kv1.1., Kv1.2)	Ion channel (narrow end of SLI)	Abnormal excitation	Cx29	Zhou et al. (1999) and Rash et al. (2016)
Lin7 (Veli; MALS)	Scaffolding		MPP6	Saitoh et al. (2017)
Membrane-associated guanylate kinase, WW and PDZ domain-containing protein-2 (MAGI2)	MAGUK; Tight and adherens junctions			Poliak et al. (2002)
Myelin-associated glycoprotein (MAG)	Adhesion	Tomacula formation		Trapp et al. (1989) and Yin et al. (1998)
Merlin (Schwanomin)	Scaffolding and signaling	Transient hypomyelination, improved by loss of Yes-associated protein (YAP); Rescue Rac1-deficient phenotype	MPP1	Seo et al. (2009), Guo et al. (2012) and Mindos et al. (2017)
Membrane protein palmitoylated-5 (MPP5; Pals1)	Tight junction	Reduction of myelin sheath thickness by silencing; Delayed myelination, reduced conduction velocities during early development		Ozcelik et al. (2010) and Zollinger et al. (2015)
MPP6 (Pals2)	MAGUK		Protein 4.1G; Lin7	Terada et al. (2012)
multi-PDZ domain protein-1 (MUPP1)	Tight and adherens junctions		JAM; Claudin; MPP5	Poliak et al. (2002)

(continued)

Table 12.1 (continued)

Protein name	Category	Changes under knockout and/or knockdown (KO/KD)	Interaction proteins	References
Neurofascin-155 (NF155)	Adhesion			Chang et al. (2000)
Na^+-K^+-$2Cl^-$ cotransporter-1 (NKCC1)	Ion cotransporter			Alvarez-Leefmans et al. (2001)
Par3	Tight junction		Deacetylated by Sirt2	Poliak et al. (2002) and Beirowski et al. (2011)
P2X7 purinoceptor	ATP-gated cation channels (Increased in the injured nerve)			Luo et al. (2013)
p120-catenin	Adherens junction	Hypomyelination	E-cadherin	Tricaud et al. (2005) and Perrin-Tricaud et al. (2007)
Protein 4.1G	4.1-ezrin-radixin-moesin (FERM) family	Focal hypermyelination; Aberrant structure of paranodes	Actin; Spectrin; CADM4; MPP6	Ohno et al. (2006), Ivanovic et al. (2012) and Saitoh et al. (2017)
RAS-related C3 botulinus toxin substrate-1 (Rac1)	Rho GTPase family (increased during Wallerian degeneration)	Decrease ovoid formation		Jung et al. (2011)
Sir-two-homolog-2 (Sirt2)	NAD^+-dependent deacetylase	Transient delay of myelination: Deacetylates Par3		Beirowski et al. (2011)
Src	Src family kinase; signaling molecule (increased Y416 phosphorylation in $4.1G^{-/-}$)		MPP6	Terada et al. (2013)
Spectrin	Membrane skeleton		Actin; Protein 4.1	Trapp et al. (1989)
Tricellulin	Tight junction			Kikuchi et al. (2010)
Zonula occludens-1, 2 (ZO1, ZO2)	Tight junction			Poliak et al. (2002)

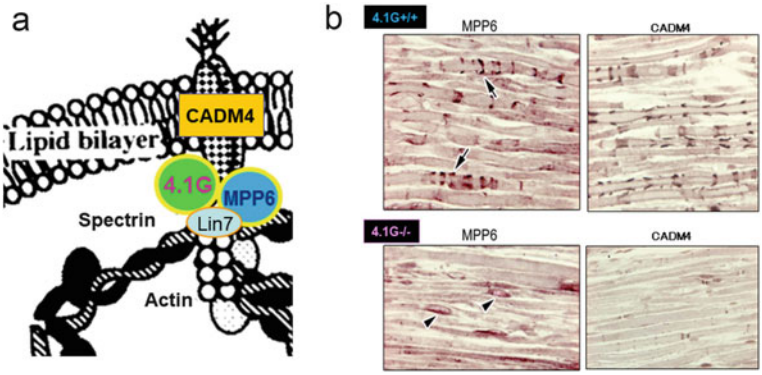

Fig. 12.3 Molecular complex of 4.1G–MPP6–Lin7–CADM4 in SLI. (**a**) Model of the membrane skeletal protein complex under SLI lipid bilayer. Actual spectrin–actin filamentous structure has not been demonstrated in Schwann cells. (**b**) The disappearance of MPP6 and CADM4 in SLI of the 4.1G-deficient nerve demonstrates the 4.1G function as transportation of MPP6 and CADM4. Some images are adapted from previous paper (Terada et al. 2012), with permission

12.2 Membrane Skeletal Proteins in SLI

12.2.1 Protein 4.1G

A fine meshwork structure under the erythrocyte membrane is termed "membrane skeleton," and it has been well known to function for the membrane stability. Spectrin–actin networks interact with ankyrin and 4.1R, to connect intramembranous proteins band 3 (anion exchanger) and glycophorin C. Development of hemolytic anemia due to defects of the membrane skeletal components indicates that the membrane skeleton plays an important role in resistance against external mechanical forces in blood vessels (Da Costa et al. 2013).

Other than erythrocytes, the membrane skeletal structure is also incorporated in various tissues and organs, and it has specific functions as a scaffold of various intramembranous proteins (Baines 2010a). The 4.1R has three 4.1-family members; 4.1B, 4.1G, and 4.1N (Peters et al. 1998; Parra et al. 1998; Walensky et al. 1998), which has three domains; an ezrin-radixin-moesin (ERM) domain, spectrin–actin-binding domain, and C-terminal domain. The ERM domain was reported to play a role in interactions with several intramembranous proteins, including ion exchangers (Baines 2010b).

12.2.2 MAGUK Family Proteins

12.2.2.1 MPP Family

MAGUK proteins possess PDZ [postsynaptic density (PSD)-95/Drosophila disks large (Dlg)/ZO1], guanylate kinase (GK), and src-homology-3 (SH3) domains, which are localized at specific domains in cell membranes (de Mendoza et al. 2010; Funke et al. 2005). Among MAGUK families, MPP family proteins are characterized by having HOOK domain and Lin-2/7 binding L27 domain in addition to the PDZ, SH3, and GK domains, and 7 members have been identified to date; MPP1-7 (te Velthuis et al. 2007). In erythrocytes, one of the MAGUK family proteins, MPP1 was identified as a molecule that interacts with 4.1R to strengthen the interaction of the spectrin–actin networks (Nunomura et al. 2000).

In peripheral nerve fibers, we identified MPP6 (Fig. 12.3b) (Terada et al. 2012). Src is incorporated in SLI, which is partially related to MPP6 (Terada et al. 2013). In the 4.1G-deficient $^{(-/-)}$ mouse sciatic nerve fibers, MPP6 rarely localized in SLI and was abnormally detected in the cytoplasm near the Schwann cell nuclei (Fig. 12.3b) (Terada et al. 2012). Although some MAGUK proteins are considered to transport cargo and interact with motor molecules on microtubules (Verhey and Rapoport 2001), 4.1G itself functions as a transporter for MPP6 in Schwann cells. An interaction between 4.1G and microtubules, demonstrated in a recent study on the mouse retina (Sanuki et al. 2015), implies the existence of a similar molecular mechanism in Schwann cells.

12.2.2.2 Other MAGUKs

Other MAGUKs, Dlg1 (Bolis et al. 2009) and MPP5 (Pals1) (Ozcelik et al. 2010), were reported to function in Schwann cells. They were thought to affect myelination by disrupting the metabolism of phosphatidylinositol performed by myotubularin-related-2 (MTMR2) and phosphatase and tensin homolog (PTEN). Dlg1 was shown to be a regulatory factor for myelination, and overmyelination was demonstrated after Dlg1 depletion in a lentivirus shRNA-expression experiment (Cotter et al. 2010). The molecules relating to overmyelination will be described in the following sections. Some structural alterations in nerve fibers were recently demonstrated in the MPP5-deficient mouse (Zollinger et al. 2015). As MAGUK family members, such as Dlg1 and MPP7, interact with themselves in epithelial cells (Bohl et al. 2007), elucidation of their appearance and relationships between MPP6, MPP5, and/or Dlg1 in SLIs would be interesting.

12.2.3 Intramembranous CADM Family

CADM family members, CADM1-4, are Ca^{++}-independent immunoglobulin-like cell adhesion molecules that homo- and heterophilically interact with themselves or with other protein families (Ogita and Takai 2006). Axons strongly express CADM1 and CADM3, whereas Schwann cells express CADM4 and lower amounts of CADM1, and they have been shown to play a role in the Schwann cell–axon

interaction (Golan et al. 2013; Maurel et al. 2007; Spiegel et al. 2007). We also demonstrated the disrupted localization of CADM4 in Schwann cells including SLIs in 4.1G$^{-/-}$ peripheral nerve fibers (Fig. 12.3c) (Ivanovic et al. 2012). Morphological and physiological impairments in peripheral nerves have been reported in the conditional deficiency of CADM4 in Schwann cells, which is a similar pathological state to that of Charcot–Marie–Tooth neuropathy (Golan et al. 2013).

12.2.4 Lin7 Family Proteins

Lin7 family has L27 and PDZ domain, and there are three family members, Lin7a (Veli1; MALS1), Lin7b (Veli2; MALS2), and Lin7c (Veli3; MALS3) (Jo et al. 1999). We recently reported the incorporation of Lin7 in SLI (Saitoh et al. 2017). It broadened potential molecular complex in PNS, because Lin7 family proteins have been reported to interact with many proteins as a scaffold; such as (1) ErbB2 (HER2) in cultured Madin-Darby canine kidney (MDCK) cells (Shelly et al. 2003), (2) inward rectifier potassium (Kir) channels in the rat brain and heart (Leonoudakis et al. 2004), (3) 5-hydroxytryptamine (5-HT) receptors in the mouse brain (Becamel et al. 2004), nectins in cultured EL cells (Yamamoto et al. 2002), (4) β-catenin in the rat brain (Perego et al. 2000), (5) calmodulin sensitive kinase (CASK; Lin2)–munc 18-interacting protein 1 (Mint1)–liprin in the rat brain (Butz et al. 1998; Olsen et al. 2005), (6) PSD95–*N*-methyl-D-aspartic acid (NMDA)-type glutamate receptor in the rat brain (Jo et al. 1999), (7) MPP4 in mouse retinas (Stohr et al. 2005), (8) MPP7 in MDCK cells (Bohl et al. 2007), and (9) Pals families in various cell lines and tissues (Kamberov et al. 2000). Deletion of Lin7a was reported to disrupt the development of mouse cerebral cortex (Matsumoto et al. 2014). Lin7c was also reported to regulate polarity and early neurogenesis in the developing mouse cerebral cortex (Srinivasan et al. 2008), and Lin7c$^{-/-}$ mice demonstrated cystic and fibrotic renal disease (Olsen et al. 2007).

Lin7 family proteins were originally identified as binding proteins for both MPP5 and MPP6 in epithelial cells (Kamberov et al. 2000) and were reported to be associated with MPP4 and MPP5 in the retina (Stohr et al. 2005). Although Lin7 proteins have been thought to stabilize tight junctions, including MPP5 and ZO1 in some tissues (Straight et al. 2006), it seems that Lin7 is dependent on MPP6, but not on MPP5, for transportation in Schwann cells. Further study is needed to elucidate the molecular complex of the MPP6–Lin7 in PNS, because some MAGUK proteins interact with each other via their L27 domains (Feng et al. 2004).

12.2.5 Myelin Abnormalities in 4.1G$^{-/-}$ Nerve Fibers in PNS

In 4.1G$^{-/-}$ sciatic nerves, an abnormal myelin structure including hypermyelination was observed (Saitoh et al. 2017), which resembled the pathological features observed in some types of human Charcot–Marie–Tooth disease, as demonstrated in Fig. 12.4a. So far, six types of genetically engineered mice were reported to

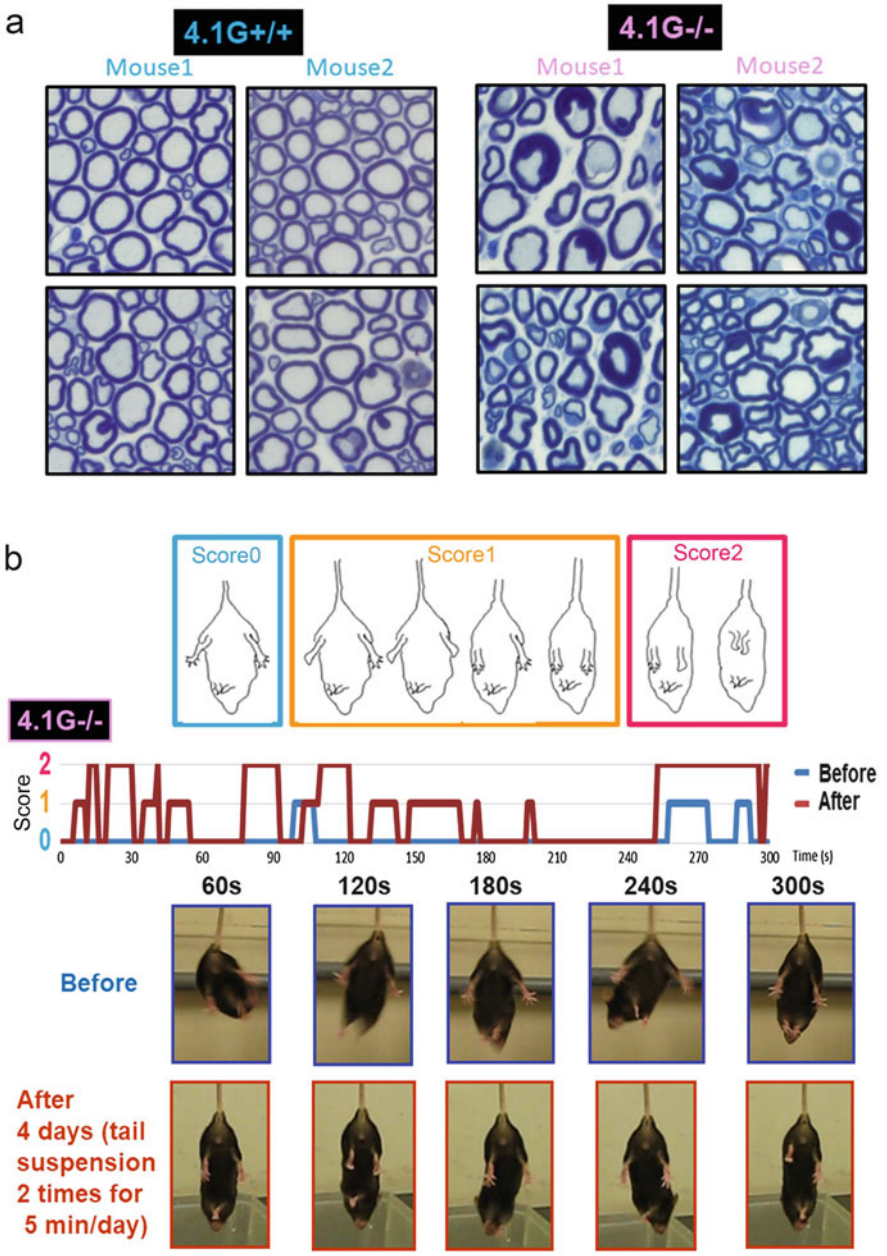

Fig. 12.4 Structural and functional abnormalities in 4.1G-deficient peripheral nerves. (**a**) Light microscopic images of transversely sectioned sciatic nerves are different between $4.1G^{+/+}$ and $4.1G^{-/-}$ nerves. (**b**) Tail-suspension test, especially after daily suspension reveals abnormal behavior in $4.1G^{-/-}$ mice. The top images demonstrate the schematic representation of postures for giving scores. Some images are adapted from previous paper (Saitoh et al. 2017), with permission

exhibit induced hypermyelination in PNS: (1) neuregulin overexpression in axons (Michailov et al. 2004), (2) phosphatase and tensin homolog deleted from chromosome 10 (PTEN) deficiency (Goebbels et al. 2010; Goebbels et al. 2012), (3) Dlg1 silencing (Cotter et al. 2010) or deficiency (Noseda et al. 2013), (4) CADM4 deficiency (Golan et al. 2013), (5) cytohesin overexpression (Torii et al. 2013), and (6) Akt (protein kinase B) overexpression (Domenech-Estevez et al. 2016). In particular, the aforementioned CADM4 is directly related to 4.1G because localization of CADM4 in SLIs is deleted in $4.1G^{-/-}$ nerves (Ivanovic et al. 2012).

The reason for active myelination was attributed to the change in the PTEN–Akt/mammalian target of the rapamycin (mTOR) system (Dowling et al. 2010; Foster and Fingar 2010; Norrmen et al. 2014; Norrmen and Suter 2013; Sherman et al. 2012). It is thought that the system leads to the phosphoinositide 3 (PI3)-kinase pathway for myelination (Heller et al. 2014) because PTEN dephosphorylates phosphatidylinositol (3, 4, 5)-trisphosphate (PIP3) as well as inhibits Akt/mTOR activity (Goebbels et al. 2012). Dlg1 was reported to interact and decrease the activity of PTEN (Adey et al. 2000). Dlg1 was also demonstrated to interact with MTMR2, and disruption of MTMR2 produced Charcot–Marie–Tooth 4B1-like neuropathy (Bolino et al. 2004; Bolis et al. 2009). Abnormal myelination in $4.1G^{-/-}$ nerves is probably related to such signal molecules. A Charcot–Marie–Tooth neuropathy originated from the peripheral myelin protein-22 (PMP22) deficiency was reported to be related to the impairment of some junctional protein functions in SLI (Guo et al. 2014; Hu et al. 2016). Examination of interactions between such junctional proteins and the 4.1G-including molecular complex would be interesting to understand relationships of signal transductions in SLI.

Because 4.1G is localized not only in SLI but also in paranodes, abnormal attachment pattern of 4.1G-deficient Schwann cell processes to axons in paranodes is also interesting. Another membrane skeletal ankyrin family protein, ankyrin B, was reported to interact with neurofascin (NF)-155 in paranodes (Chang et al. 2014). As membrane skeletons stabilize nodes of Ranvier and paranodes (Susuki et al. 2016), it is necessary to evaluate precise molecular interactions between these membrane skeletal proteins.

12.3 Overwork Weakness in Peripheral Neuropathy from Viewpoint of SLI

We have reported that muscle strength of all $4.1G^{-/-}$ mice weakened after daily exercise, as demonstrated in Fig. 12.4d. In human clinical research in Charcot–Marie–Tooth neuropathy, weakness of muscle strength in the dominant side compared to the non-dominant suggested overwork weakness (van Pomeren et al. 2009; Vinci et al. 2009). As demonstrated in the aforementioned section, when peripheral nerves were extended, SLI functioned as a suspension structure for the mechanical extension. We assume that cell membrane collapse easier compared to normal conditions under disruption of the membrane skeleton, such as in the case of 4.1G deficiency, resulting in impairment of the nerve fibers. In this respect, for some types

of Charcot–Marie–Tooth neuropathy, overwork weakness is likely related to over-extension of peripheral nerves. Therefore, we think that the rehabilitation protocol has to be considered from the viewpoint of molecular disease.

12.4 Conclusion

In this review, we described molecular components in SLI, especially from viewpoint of the membrane skeletal protein complex 4.1G–MPP6–CADM4 in addition to a dynamic morphofunctional perspective; thereby, providing an insight into pathological conditions in peripheral nerves such as Charcot–Marie–Tooth neuropathy.

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References

Adey NB, Huang L, Ormonde PA, Baumgard ML, Pero R, Byreddy DV, Tavtigian SV, Bartel PL (2000) Threonine phosphorylation of the MMAC1/PTEN PDZ binding domain both inhibits and stimulates PDZ binding. Cancer Res 60:35–37

Alanne MH, Pummi K, Heape AM, Grenman R, Peltonen J, Peltonen S (2009) Tight junction proteins in human Schwann cell autotypic junctions. J Histochem Cytochem 57:523–529

Alvarez-Leefmans FJ, Leon-Olea M, Mendoza-Sotelo J, Alvarez FJ, Anton B, Garduno R (2001) Immunolocalization of the Na^+-K^+-$2Cl^-$ cotransporter in peripheral nervous tissue of vertebrates. Neuroscience 104:569–582

Baines AJ (2010a) Evolution of the spectrin-based membrane skeleton. Transfusion Clinique Biol 17:95–103

Baines AJ (2010b) The spectrin-ankyrin-4.1-adducin membrane skeleton: adapting eukaryotic cells to the demands of animal life. Protoplasma 244:99–131

Becamel C, Gavarini S, Chanrion B, Alonso G, Galeotti N, Dumuis A, Bockaert J, Marin P (2004) The serotonin 5-HT2A and 5-HT2C receptors interact with specific sets of PDZ proteins. J Biol Chem 279:20257–20266

Beirowski B, Gustin J, Armour SM, Yamamoto H, Viader A, North BJ, Michan S, Baloh RH, Golden JP, Schmidt RE, Sinclair DA, Auwerx J, Milbrandt J (2011) Sir-two-homolog 2 (Sirt2) modulates peripheral myelination through polarity protein Par-3/atypical protein kinase C (aPKC) signaling. Proc Natl Acad Sci U S A 108:952–961

Bohl J, Brimer N, Lyons C, Vande Pol SB (2007) The stardust family protein MPP7 forms a tripartite complex with LIN7 and DLG1 that regulates the stability and localization of DLG1 to cell junctions. J Biol Chem 282:9392–9400

Bolino A, Bolis A, Previtali SC, Dina G, Bussini S, Dati G, Amadio S, Del Carro U, Mruk DD, Feltri ML, Cheng CY, Quattrini A, Wrabetz L (2004) Disruption of Mtmr2 produces CMT4B1-like neuropathy with myelin outfolding and impaired spermatogenesis. J Cell Biol 167:711–721

Bolis A, Coviello S, Visigalli I, Taveggia C, Bachi A, Chishti AH, Hanada T, Quattrini A, Previtali SC, Biffi A, Bolino A (2009) Dlg1, Sec8, and Mtmr2 regulate membrane homeostasis in Schwann cell myelination. J Neurosci 29:8858–8870

Butz S, Okamoto M, Sudhof TC (1998) A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. Cell 94:773–782

Chang BJ, Cho IJ, Brophy PJ (2000) A study on the immunocytochemical localization of neurofascin in rat sciatic nerve. J Veterin Sci 1:67–71

Chang KJ, Zollinger DR, Susuki K, Sherman DL, Makara MA, Brophy PJ, Cooper EC, Bennett V, Mohler PJ, Rasband MN (2014) Glial ankyrins facilitate paranodal axoglial junction assembly. Nature Neurosci 17:1673–1681

Cotter L, Ozcelik M, Jacob C, Pereira JA, Locher V, Baumann R, Relvas JB, Suter U, Tricaud N (2010) Dlg1-PTEN interaction regulates myelin thickness to prevent damaging peripheral nerve overmyelination. Science 328:1415–1418

Da Costa L, Galimand J, Fenneteau O, Mohandas N (2013) Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev 27:167–178

de Mendoza A, Suga H, Ruiz-Trillo I (2010) Evolution of the MAGUK protein gene family in premetazoan lineages. BMC Evol Biol 10:93

Domenech-Estevez E, Baloui H, Meng X, Zhang Y, Deinhardt K, Dupree JL, Einheber S, Chrast R, Salzer JL (2016) Akt regulates axon wrapping and myelin sheath thickness in the PNS. J Neurosci 36:4506–4521

Dowling RJ, Topisirovic I, Fonseca BD, Sonenberg N (2010) Dissecting the role of mTOR: lessons from mTOR inhibitors. Biochim Biophys Acta 1804:433–439

Duffy HS, Iacobas I, Hotchkiss K, Hirst-Jensen BJ, Bosco A, Dandachi N, Dermietzel R, Sorgen PL, Spray DC (2007) The gap junction protein connexin32 interacts with the Src homology 3/hook domain of discs large homolog 1. J Biol Chem 282:9789–9796

Fannon AM, Sherman DL, Ilyina-Gragerova G, Brophy PJ, Friedrich VL Jr, Colman DR (1995) Novel E-cadherin-mediated adhesion in peripheral nerve: Schwann cell architecture is stabilized by autotypic adherens junctions. J Cell Biol 129:189–202

Feng W, Long JF, Fan JS, Suetake T, Zhang M (2004) The tetrameric L27 domain complex as an organization platform for supramolecular assemblies. Nature Struct Mol Biol 11:475–480

Foster KG, Fingar DC (2010) Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. J Biol Chem 285:14071–14077

Freidin M, Asche S, Bargiello TA, Bennett MV, Abrams CK (2009) Connexin 32 increases the proliferative response of Schwann cells to neuregulin-1 (Nrg1). Proc Natl Acad Sci U S A 106:3567–3572

Funke L, Dakoji S, Bredt DS (2005) Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions. Annu Rev Biochem 74:219–245

Goebbels S, Oltrogge JH, Kemper R, Heilmann I, Bormuth I, Wolfer S, Wichert SP, Mobius W, Liu X, Lappe-Siefke C, Rossner MJ, Groszer M, Suter U, Frahm J, Boretius S, Nave KA (2010) Elevated phosphatidylinositol 3,4,5-trisphosphate in glia triggers cell-autonomous membrane wrapping and myelination. J Neurosci 30:8953–8964

Goebbels S, Oltrogge JH, Wolfer S, Wieser GL, Nientiedt T, Pieper A, Ruhwedel T, Groszer M, Sereda MW, Nave KA (2012) Genetic disruption of Pten in a novel mouse model of tomaculous neuropathy. EMBO Mol Med 4:486–499

Golan N, Kartvelishvily E, Spiegel I, Salomon D, Sabanay H, Rechav K, Vainshtein A, Frechter S, Maik-Rachline G, Eshed-Eisenbach Y, Momoi T, Peles E (2013) Genetic deletion of Cadm4 results in myelin abnormalities resembling Charcot-Marie-Tooth neuropathy. J Neurosci 33:10950–10961

Guo L, Moon C, Niehaus K, Zheng Y, Ratner N (2012) Rac1 controls Schwann cell myelination through cAMP and NF2/merlin. J Neurosci 32:17251–17261

Guo J, Whan L, Zhang Y, Wu J, Arpag S, Hu B, Imhof BA, Tian X, Carter BD, Suter U, Li J (2014) Abnormal junctions and permeability of myelin in PMP22-deficient nerves. Ann Neurol 75:255–265

Hayashi A, Nakashima K, Yamagishi K, Hoshi T, Suzuki A, Baba H (2007) Localization of annexin II in the paranodal regions and Schmidt-Lanterman incisures in the peripheral nervous system. Glia 55:1044–1052

Heffernan C, Jain MR, Liu T, Kim H, Barretto K, Li H, Maurel P (2017) Nectin-like 4 complexes with choline transporter-like protein-1 and regulates Schwann cell choline homeostasis and lipid biogenesis in vitro. J Biol Chem 292:4484–4498

Heller BA, Ghidinelli M, Voelkl J, Einheber S, Smith R, Grund E, Morahan G, Chandler D, Kalaydjieva L, Giancotti F, King RH, Fejes-Toth AN, Fejes-Toth G, Feltri ML, Lang F, Salzer JL (2014) Functionally distinct PI3-kinase pathways regulate myelination in the peripheral nervous system. J Cell Biol 204:1219–1236

Hu B, Arpag S, Zhang X, Mobius W, Werner H, Sosinsky G, Ellisman M, Zhang Y, Hamilton A, Chernoff J, Li J (2016) Tuning PAK activity to rescue abnormal myelin permeability in HNPP. PLoS Genet 12:e1006290

Ivanovic A, Horresh I, Golan N, Spiegel I, Sabanay H, Frechter S, Ohno S, Terada N, Mobius W, Rosenbluth J, Brose N, Peles E (2012) The cytoskeletal adapter protein 4.1G organizes the internodes in peripheral myelinated nerves. J Cell Biol 196:337–344

Jo K, Derin R, Li M, Bredt DS (1999) Characterization of MALS/Velis-1, −2, and −3: a family of mammalian LIN-7 homologs enriched at brain synapses in association with the postsynaptic density-95/NMDA receptor postsynaptic complex. J Neurosci 19:4189–4199

Jung J, Cai W, Lee HK, Pellegatta M, Shin YK, Jang SY, Suh DJ, Wrabetz L, Feltri ML, Park HT (2011) Actin polymerization is essential for myelin sheath fragmentation during Wallerian degeneration. J Neurosci 31:2009–2015

Kamberov E, Makarova O, Roh M, Liu A, Karnak D, Straight S, Margolis B (2000) Molecular cloning and characterization of Pals, proteins associated with mLin-7. J Biol Chem 275:11425–11431

Kamijo A, Saitoh Y, Ohno N, Ohno S, Terada N (2014) Immunohistochemical study of mouse sciatic nerves under various stretching conditions with "in vivo cryotechnique". J Neurosci Method 227:181–188

Kikuchi S, Ninomiya T, Tatsumi H, Sawada N, Kojima T (2010) Tricellulin is expressed in autotypic tight junctions of peripheral myelinating Schwann cells. J Histochem Cytochem 58:1067–1073

Leonoudakis D, Conti LR, Anderson S, Radeke CM, McGuire LM, Adams ME, Froehner SC, Yates JR 3rd, Vandenberg CA (2004) Protein trafficking and anchoring complexes revealed by proteomic analysis of inward rectifier potassium channel (Kir2.x)-associated proteins. J Biol Chem 279:22331–22346

Luo J, Lee S, Wu D, Yeh J, Ellamushi H, Wheeler AP, Warnes G, Zhang Y, Bo X (2013) P2X7 purinoceptors contribute to the death of Schwann cells transplanted into the spinal cord. Cell Death Dis 4:e829

Matsumoto A, Mizuno M, Hamada N, Nozaki Y, Jimbo EF, Momoi MY, Nagata K, Yamagata T (2014) LIN7A depletion disrupts cerebral cortex development, contributing to intellectual disability in 12q21-deletion syndrome. PLoS One 9:e92695

Maurel P, Einheber S, Galinska J, Thaker P, Lam I, Rubin MB, Scherer SS, Murakami Y, Gutmann DH, Salzer JL (2007) Nectin-like proteins mediate axon Schwann cell interactions along the internode and are essential for myelination. J Cell Biol 178:861–874

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave KA (2004) Axonal neuregulin-1 regulates myelin sheath thickness. Science 304:700–703

Mindos T, Dun XP, North K, Doddrell RD, Schulz A, Edwards P, Russell J, Gray B, Roberts SL, Shivane A, Mortimer G, Pirie M, Zhang N, Pan D, Morrison H, Parkinson DB (2017) Merlin controls the repair capacity of Schwann cells after injury by regulating Hippo/YAP activity. J Cell Biol 216:495–510

Norrmen C, Suter U (2013) Akt/mTOR signalling in myelination. Biochem Soc Transaction 41:944–950

Norrmen C, Figlia G, Lebrun-Julien F, Pereira JA, Trotzmuller M, Kofeler HC, Rantanen V, Wessig C, van Deijk AL, Smit AB, Verheijen MH, Ruegg MA, Hall MN, Suter U (2014) mTORC1 controls PNS myelination along the mTORC1-RXRgamma-SREBP-lipid biosynthesis axis in Schwann cells. Cell Rep 9:646–660

Noseda R, Belin S, Piguet F, Vaccari I, Scarlino S, Brambilla P, Martinelli Boneschi F, Feltri ML, Wrabetz L, Quattrini A, Feinstein E, Huganir RL, Bolino A (2013) DDIT4/REDD1/RTP801 is a novel negative regulator of Schwann cell myelination. J Neurosci 33:15295–15305

Nunomura W, Takakuwa Y, Parra M, Conboy J, Mohandas N (2000) Regulation of protein 4.1R, p55, and glycophorin C ternary complex in human erythrocyte membrane. J Biol Chem 275:24540–24546

Ogita H, Takai Y (2006) Nectins and nectin-like molecules: roles in cell adhesion, polarization, movement, and proliferation. IUBMB Life 58:334–343

Ohno N, Terada N, Yamakawa H, Komada M, Ohara O, Trapp BD, Ohno S (2006) Expression of protein 4.1G in Schwann cells of the peripheral nervous system. J Neurosci Res 84:568–577

Ohno S, Saitoh Y, Ohno N, Terada N (2017) Renaissance of morphological studies: the examination of functional structures in living animal organs using the in vivo cryotechnique. Anat Sci Int 92:55–78

Olsen O, Moore KA, Fukata M, Kazuta T, Trinidad JC, Kauer FW, Streuli M, Misawa H, Burlingame AL, Nicoll RA, Bredt DS (2005) Neurotransmitter release regulated by a MALS-liprin-alpha presynaptic complex. J Cell Biol 170:1127–1134

Olsen O, Funke L, Long JF, Fukata M, Kazuta T, Trinidad JC, Moore KA, Misawa H, Welling PA, Burlingame AL, Zhang M, Bredt DS (2007) Renal defects associated with improper polarization of the CRB and DLG polarity complexes in MALS-3 knockout mice. J Cell Biol 179:151–164

Ozcelik M, Cotter L, Jacob C, Pereira JA, Relvas JB, Suter U, Tricaud N (2010) Pals1 is a major regulator of the epithelial-like polarization and the extension of the myelin sheath in peripheral nerves. J Neurosci 30:4120–4131

Parra M, Gascard P, Walensky LD, Snyder SH, Mohandas N, Conboy JG (1998) Cloning and characterization of 4.1G (EPB41L2), a new member of the skeletal protein 4.1 (EPB41) gene family. Genomics 49:298–306

Perego C, Vanoni C, Massari S, Longhi R, Pietrini G (2000) Mammalian LIN-7 PDZ proteins associate with beta-catenin at the cell-cell junctions of epithelia and neurons. EMBO J 19:3978–3989

Perrin-Tricaud C, Rutishauser U, Tricaud N (2007) P120 catenin is required for thickening of Schwann cell myelin. Mol Cell Neurosci 35:120–129

Peters LL, Weier HU, Walensky LD, Snyder SH, Parra M, Mohandas N, Conboy JG (1998) Four paralogous protein 4.1 genes map to distinct chromosomes in mouse and human. Genomics 54:348–350

Poliak S, Matlis S, Ullmer C, Scherer SS, Peles E (2002) Distinct claudins and associated PDZ proteins form different autotypic tight junctions in myelinating Schwann cells. J Cell Biol 159:361–372

Rangwala R, Banine F, Borg JP, Sherman LS (2005) Erbin regulates mitogen-activated protein (MAP) kinase activation and MAP kinase-dependent interactions between Merlin and adherens junction protein complexes in Schwann cells. J Biol Chem 280:11790–11797

Rash JE, Vanderpool KG, Yasumura T, Hickman J, Beatty JT, Nagy JI (2016) KV1 channels identified in rodent myelinated axons, linked to Cx29 in innermost myelin: support for electrically active myelin in mammalian saltatory conduction. J Neurophysiol 115:1836–1859

Saitoh Y, Ohno N, Yamauchi J, Sakamoto T, Terada N (2017) Deficiency of a membrane skeletal protein, 4.1G, results in myelin abnormalities in the peripheral nervous system. Histochem Cell Biol 148:597–606

Sanuki R, Watanabe S, Sugita Y, Irie S, Kozuka T, Shimada M, Ueno S, Usukura J, Furukawa T (2015) Protein-4.1G-mediated membrane trafficking is essential for correct rod synaptic location in the retina and for normal visual function. Cell Rep 10:796–808

Scheiermann C, Meda P, Aurrand-Lions M, Madani R, Yiangou Y, Coffey P, Salt TE, Ducrest-Gay D, Caille D, Howell O, Reynolds R, Lobrinus A, Adams RH, Yu AS, Anand P, Imhof BA, Nourshargh S (2007) Expression and function of junctional adhesion molecule-C in myelinated peripheral nerves. Science 318:1472–1475

Scherer SS, Xu YT, Nelles E, Fischbeck K, Willecke K, Bone LJ (1998) Connexin32-null mice develop demyelinating peripheral neuropathy. Glia 24:8–20

Segura-Anaya E, Martinez-Gomez A, Dent MA (2015) Localization of aquaporin 1 water channel in the Schmidt-Lanterman incisures and the paranodal regions of the rat sciatic nerve. Neurosci 285:119–127

Seo PS, Quinn BJ, Khan AA, Zeng L, Takoudis CG, Hanada T, Bolis A, Bolino A, Chishti AH (2009) Identification of erythrocyte p55/MPP1 as a binding partner of NF2 tumor suppressor protein/Merlin. Exp Biol Med 234:255–262

Shelly M, Mosesson Y, Citri A, Lavi S, Zwang Y, Melamed-Book N, Aroeti B, Yarden Y (2003) Polar expression of ErbB-2/HER2 in epithelia. Bimodal regulation by Lin-7. Dev Cell 5:475–486

Sherman DL, Krols M, Wu LM, Grove M, Nave KA, Gangloff YG, Brophy PJ (2012) Arrest of myelination and reduced axon growth when Schwann cells lack mTOR. J Neurosci 32:1817–1825

Spiegel I, Adamsky K, Eshed Y, Milo R, Sabanay H, Sarig-Nadir O, Horresh I, Scherer SS, Rasband MN, Peles E (2007) A central role for Necl4 (SynCAM4) in Schwann cell-axon interaction and myelination. Nature Neurosci 10:861–869

Srinivasan K, Roosa J, Olsen O, Lee SH, Bredt DS, McConnell SK (2008) MALS-3 regulates polarity and early neurogenesis in the developing cerebral cortex. Development 135:1781–1790

Stohr H, Molday LL, Molday RS, Weber BH, Biedermann B, Reichenbach A, Kramer F (2005) Membrane-associated guanylate kinase proteins MPP4 and MPP5 associate with Veli3 at distinct intercellular junctions of the neurosensory retina. J Comp Neurol 481:31–41

Straight SW, Pieczynski JN, Whiteman EL, Liu CJ, Margolis B (2006) Mammalian lin-7 stabilizes polarity protein complexes. J Biol Chem 281:37738–37747

Susuki K, Raphael AR, Ogawa Y, Stankewich MC, Peles E, Talbot WS, Rasband MN (2011) Schwann cell spectrins modulate peripheral nerve myelination. Proc Natl Acad Sci U S A 108:8009–8014

Susuki K, Otani Y, Rasband MN (2016) Submembranous cytoskeletons stabilize nodes of Ranvier. Exp Neurol 283:446–451

Tang W, Zhang Y, Chang Q, Ahmad S, Dahlke I, Yi H, Chen P, Paul DL, Lin X (2006) Connexin29 is highly expressed in cochlear Schwann cells, and it is required for the normal development and function of the auditory nerve of mice. J Neurosci 26:1991–1999

Tao Y, Dai P, Liu Y, Marchetto S, Xiong WC, Borg JP, Mei L (2009) Erbin regulates NRG1 signaling and myelination. Proc Natl Acad Sci U S A 106:9477–9482

te Velthuis AJ, Admiraal JF, Bagowski CP (2007) Molecular evolution of the MAGUK family in metazoan genomes. BMC Evol Biol 7:129

Terada N, Saitoh Y, Ohno N, Komada M, Saitoh S, Peles E, Ohno S (2012) Essential function of protein 4.1G in targeting of membrane protein palmitoylated 6 into Schmidt-Lanterman incisures in myelinated nerves. Mol Cell Biol 32:199–205

Terada N, Saitoh Y, Ohno N, Komada M, Yamauchi J, Ohno S (2013) Involvement of Src in the membrane skeletal complex, MPP6-4.1G, in Schmidt-Lanterman incisures of mouse myelinated nerve fibers in PNS. Histochem Cell Biol 140:213–222

Terada N, Saitoh Y, Kamijo A, Ohno S, Ohno N (2016) Involvement of membrane skeletal molecules in the Schmidt-Lanterman incisure in Schwann cells. Med Mol Morphol 49:5–10

Torii T, Miyamoto Y, Onami N, Tsumura H, Nemoto N, Kawahara K, Kato M, Kotera J, Nakamura K, Tanoue A, Yamauchi J (2013) In vivo expression of the Arf6 Guanine-nucleotide exchange factor cytohesin-1 in mice exhibits enhanced myelin thickness in nerves. J Mol Neurosci 51:522–531

Trapp BD, Andrews SB, Wong A, O'Connell M, Griffin JW (1989) Co-localization of the myelin-associated glycoprotein and the microfilament components, F-actin and spectrin, in Schwann cells of myelinated nerve fibres. J Neurocytol 18:47–60

Tricaud N, Perrin-Tricaud C, Bruses JL, Rutishauser U (2005) Adherens junctions in myelinating Schwann cells stabilize Schmidt-Lanterman incisures via recruitment of p120 catenin to E-cadherin. J Neurosci 25:3259–3269

van Pomeren M, Selles RW, van Ginneken BT, Schreuders TA, Janssen WG, Stam HJ (2009) The hypothesis of overwork weakness in Charcot-Marie-Tooth: a critical evaluation. J Rehabilitation Med 41:32–34

Verhey KJ, Rapoport TA (2001) Kinesin carries the signal. Trend Biochem Sci 26:545–550

Vinci P, Perelli SL, Gargiulo P (2009) About the hypothesis of overwork weakness in Charcot-Marie-Tooth disease. J Rehabilitation Med 41:778

Walensky LD, Gascard P, Fields ME, Blackshaw S, Conboy JG, Mohandas N, Snyder SH (1998) The 13-kD FK506 binding protein, FKBP13, interacts with a novel homologue of the erythrocyte membrane cytoskeletal protein 4.1. J Cell Biol 141:143–153

Yamamoto Y, Mandai K, Okabe N, Hoshino T, Nakanishi H, Takai Y (2002) Localization of mLin-7 at nectin-based cell-cell junctions. Oncogene 21:2545–2554

Yin X, Crawford TO, Griffin JW, Tu P, Lee VM, Li C, Roder J, Trapp BD (1998) Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. J Neurosci 18:1953–1962

Zhao S, Fort A, Spray DC (1999) Characteristics of gap junction channels in Schwann cells from wild-type and connexin-null mice. Ann N Y Acad Sci 883:533–537

Zhou L, Messing A, Chiu SY (1999) Determinants of excitability at transition zones in Kv1.1-deficient myelinated nerves. J Neurosci 19:5768–5781

Zollinger DR, Chang KJ, Baalman K, Kim S, Rasband MN (2015) The polarity protein Pals1 regulates radial sorting of axons. J Neurosci 35:10474–10484

Part III

Myelin Pathology in the Central Nervous System

Pelizaeus-Merzbacher Disease: Molecular and Cellular Pathologies and Associated Phenotypes

13

Ken Inoue

Abstract

Pelizaeus-Merzbacher disease (PMD) represents a group of disorders known as hypomyelinating leukodystrophies, which are characterized by abnormal development and maintenance of myelin in the central nervous system. PMD is caused by different types of mutations in the proteolipid protein 1 (*PLP1*) gene, which encodes a major myelin membrane lipoprotein. These mutations in the *PLP1* gene result in distinct cellular and molecular pathologies and a spectrum of clinical phenotypes. In this chapter, I discuss the historical aspects and current understanding of the mechanisms underlying how different *PLP1* mutations disrupt the normal process of myelination and result in PMD and other disorders.

Keywords

Hypomyelinating leukodystrophies · Pelizaeus-Merzbacher disease · Molecular mechanism · *PLP1* mutations · Genotype–phenotype correlation

13.1 Leukodystrophies

Leukodystrophies consist of a broad range of genetic disorders that primarily affect the white matter of the brain. Usually, disorders with infectious or immunological causes are not included in this disease category. Most leukodystrophies become apparent in childhood and sometimes in infancy, and they can have devastating neurological and cognitive outcomes. Pathologically, the loss of myelin is the most common feature of leukodystrophies, and based on how the myelin is lost, leukodystrophies can be classified into demyelinating or hypomyelinating

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leukodystrophies (Vanderver et al. 2014). Demyelinating leukodystrophies are characterized by degeneration of the white matter in the central nervous system (CNS), which is typically progressive and mainly affects, but is not restricted to, myelin. Demyelinating leukodystrophies include several well-known white matter diseases, such as adrenoleukodystrophy, metachromatic leukodystrophy, and Alexander disease, while hypomyelinating leukodystrophies are characterized by myelination failure or abnormal myelin development. These leukodystrophies are nondegenerative and often clinically static. Thus, the name *hypomyelinating leukodystrophies* does not accurately convey the characteristics of this group of diseases because *dystrophy*, which means *wasting*, is not a major feature of these diseases. Nevertheless, the term *leukodystrophy* is widely recognized and used to describe heritable disorders that affect the CNS white matter, and the renaming of these disorders is not the goal of this chapter. The most common hypomyelinating leukodystrophy is Pelizaeus-Merzbacher disease (PMD). In this chapter, I will describe the history and current understanding of the clinical, genetic, cellular, and molecular characteristics of PMD and related diseases.

13.2 Phenotypes Resulting from CNS Hypomyelination Due to Dysfunctional Myelin Development

13.2.1 Hypomyelinating Leukodystrophies

Among the wide range of etiologies underlying leukodystrophies, hypomyelinating leukodystrophies are caused by deficits in permanent myelin deposition. Myelin sheaths are not formed properly, which alters myelin physiology, which was discussed in other chapters. This group of disorders includes more than 10 distinct diseases (Table 13.1), and additional diseases may be delineated in the future with rapid progress being made in the clinical applications of comprehensive genomic analyses, especially whole-exome sequencing. The causative genes and clinical characteristics that distinguish among hypomyelinating leukodystrophies, as well as the variations within them, have been identified (Vanderver et al. 2015). Despite these differences and variations, certain clinical features are common to all patients with hypomyelinating leukodystrophies. All patients show global developmental delays, which tend to be more obvious in motor functions than in cognitive functions, and neurological symptoms, including hypotonia, spasticity, dystonia, and ataxia. T2-weighted brain magnetic resonance imaging (MRI) has revealed diffuse hyperintensities in the white matter (Fig. 13.1). Thus, the characteristics of these diseases indicate that the genes responsible for hypomyelinating leukodystrophies may be involved in the development, structure, function, and/or maintenance of myelinating oligodendrocytes.

Table 13.1 Known hypomyelinating leukodystrophies and disease genes

Disease	Abbreviation	Gene locus	Gene
Group 1 (no peripheral neuropathy)			
Pelizaeus-Merzbacher disease	PMD	Xq22	*PLP1*
Pelizaeus-Merzbacher-like disease 1	PMLD	1q42.13	*GJC2*
Hypomyelinatioin with atrophy of basal ganglia and corpus callous	HABC	19p13.3	*TUBB4A*
18q(−) syndrome	18qDEL	18q22-qter	*MBP*
MCT8 deficiency (Allan-Herndon-Dudley syndrome)	AHDS	Xq13.2	*SLC16A2*
Mitochondrial Hsp60 chaperonopathy	MitChap60	2q33.1	*HSPD1*
Salla disease	SD	6q13	*SLC17A5*
POLR-related hypomyelinating leukodystrophies (hypomyelinating leukodystrophy with hypodontia and hypogonadotrophic hypogonadism)	4H	10q22.3	*POLR3A*
		12q23.3	*POLR3B*
		6p21.1	*POLR1C*
Group 2 (with peripheral neuropathy)			
Hypomyelination and congenital cataract	HCC	7p15.3	*FAM126A*
Peripheral demyelinating neuropathy, central dysmyenating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease	PCWH	22q13	*SOX10*

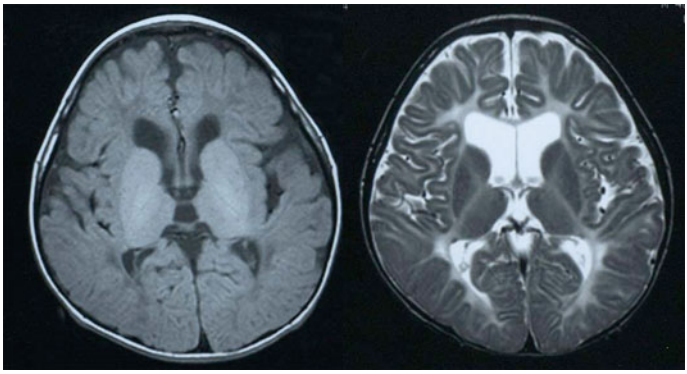

Fig. 13.1 Brain MRI of a PMD patient. Left, a T1-weighted image; right, a T2-weighted image. Note that the white matter show diffuse hypointensities in T1 and hyperintensities in T2-weighted images. These images are provided by courtesy of Dr. Jun-ichi Takanashi (Tokyo Women's Medical University, Japan)

13.2.2 PMD, a Hypomyelinating Leukodystrophy

PMD is the most common hypomyelinating leukodystrophy. The incidence of PMD is estimated to be between 1.45 and 1.9 per 100,000 live male births (Bonkowsky et al. 2010; Numata et al. 2014). PMD was first described in 1885 by Friedrich Pelizaeus, a German physician who noted a family with multiple male individuals

having nystagmus, spastic quadriplegia, ataxia, and developmental delay (Pelizaeus 1885). In 1910, Ludwig Merzbacher, a German pathologist, reported that the family's disease was transmitted by a process now known as X-linked recessive inheritance (Merzbacher 1910). In addition, he reported that a patient's autopsied brain showed profound myelin loss in the white matter. Most subsequent studies in the literature have described the pathologies of new cases, which has broadened the disease entity. Seitelberger has comprehensively studied the clinicopathological findings of PMD and proposed a classification system for the disease (Seitelberger 1970, 1995). However, this classification system also appeared to include diseases showing patterns of inheritance other than the X-linked recessive form and that are now categorized as hypomyelinating leukodystrophies other than PMD.

13.2.3 Discovery of *PLP1* Mutations in Patients with PMD

Because PMD is transmitted by an X-linked process, a major breakthrough in the genetic understanding of PMD occurred with the mapping of the proteolipid protein 1 (*PLP1*) gene on the X chromosome (Willard and Riordan 1985). As expected, mutations in the protein coding regions of the *PLP1* gene have been identified in animals with myelin deficiencies (Dautigny et al. 1986; Nave et al. 1989; Schneider et al. 1992; Gencic and Hudson 1990; Boison and Stoffel 1994; Nadon et al. 1990) and patients with PMD (Trofatter et al. 1989; Hudson et al. 1989).

Despite the excitement resulting from the identification of *PLP1* coding mutations as the genetic cause of the disease, mutations were identified in less than half of patients with PMD, which was disappointing. Several explanations have been suggested. First, PMD is not genetically homogenous but rather genetically heterogeneous. Thus, more PMD-associated genes need to be identified, as has been found in other genetic diseases. Second, other types of mutations might occur in the *PLP1* gene. In contrast to our expectations, the latter was reported more likely by a genetic linkage study that was performed in a strictly defined and typical PMD cohort (Boespflug-Tanguy et al. 1994). However, the first explanation is also correct for PMD-like diseases, and more than 10 genes have been identified as causes of hypomyelinating leukodystrophies that have now been clinically and genetically differentiated from PMD (Pouwels et al. 2014).

The following findings have suggested that increased copy numbers of *PLP1* also cause PMD. First, a patient with a large chromosomal duplication involving *PLP1* exhibited a PMD-like phenotype (Cremers et al. 1987). Second, Southern blot analysis revealed increased gene dosages in two patients with no *PLP1* point mutations (Ellis and Malcolm 1994). Third, mice overexpressing *PLP1* show drastic myelin deficiencies in the CNS (Kagawa et al. 1994; Readhead et al. 1994). Based on these reports, we identified *PLP1* duplication as a major cause of PMD in patients without coding mutations, and we have suggested that a *PLP1* gene dosage effect is the mechanism underlying this hypomyelinating disease (Inoue et al. 1996a). A study with a larger cohort confirmed that *PLP1* duplication is the most common cause of PMD (Mimault et al. 1999).

PLP1 deletion has also been found in patients with PMD (Raskind et al. 1991; Inoue et al. 2002). Interestingly, *PLP1* deletions are rare compared to duplications. The reason for this rareness is unknown, but larger deletions that involve multiple neighboring genes are thought to result in embryonic lethality and only those with smaller deletion events survive (Inoue et al. 2002). Accordingly, the genomic intervals of *PLP1* deletions are clearly smaller than those of most duplications.

In humans, *PLP1* is ~17-kb long and located on chromosome Xq22.1. *PLP1* has seven coding exons that encode the 276-amino acid PLP1 protein and an alternative splicing variant, DM20, which is 35 amino acids shorter (Nave et al. 1987). PLP1 and DM20 are differentiated by the inclusion or exclusion of the latter half of exon3 (exon3B), which is 105-bp long. PLP1/DM20 is tetraspan membrane protein (Weimbs and Stoffel 1992) that is most expressed in the myelin sheaths generated by oligodendrocytes in the CNS. Both PLP1 and DM20 are predominantly expressed in oligodendrocyte lineage cells, but low levels have been detected in other types of cells, including Schwann cells (Griffiths et al. 1995) and restricted neurons (Miller et al. 2003). Low levels of DM20, but not PLP1, are expressed during development when oligodendrocyte precursor cells dominate the population (Ikenaka et al. 1992). When mature oligodendrocytes begin to form large quantities of myelin sheaths and myelination is initiated, the expression of PLP1 greatly increases to the point that PLP1/DM20 together constitute ~50% of the total protein in myelin. Despite their major expression levels and well-defined structural features, the exact physiological functions of PLP1/DM20 in both the developing and the myelinating stages remain unclear, but a role in compact myelin sheath stabilization is verified (Boison et al. 1995).

13.2.4 Genotype–Phenotype Correlations in PMD and Related Disorders

Recent genetic, imaging, and clinical dissection studies have shown that PMD consists of a spectrum of disorders involving various *PLP1* mutations that are therefore called *PLP1*-related disorders (Inoue 2005). Based on clinical and neuropathological findings, the most severe and common forms were characterized as the connatal and classic forms, respectively, by Seitelberger (Seitelberger 1970). Genetic studies have expanded the spectrum to involve spastic paraplegia type 2 (SPG2), which is an X-linked hereditary spastic paraplegia that was initially considered distinct from PMD (Saugier-Veber et al. 1994). The identification of *PLP1* mutations in patients with SPG2 has indicated that PMD and SPG2 are allelic disorders on the same spectrum (Saugier-Veber et al. 1994; Osaka et al. 1995). In practice, many patients have clinical presentations that transition between the connatal and classic forms or the classic form and SPG2. Therefore, a more precise classification based on clinical severity and developmental dysfunction was required. Boespflug-Tanguy and her colleagues have proposed a five-grade classification of the patients' best motor achievement, with the most severe class indicated by form 0 and the mildest class indicated by form 4 (Cailloux et al. 2000).

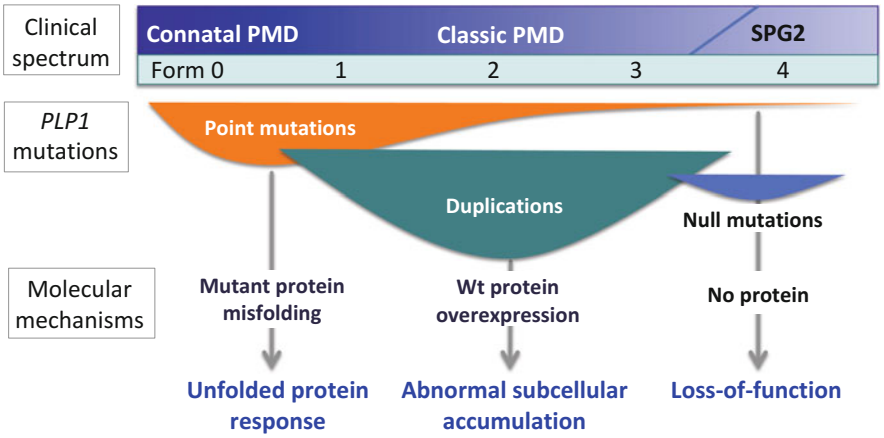

Fig. 13.2 Genotype-phenotype correlations and associated cellular and molecular mechanisms. Different *PLP1* mutations result in distinct molecular mechanisms underlying a wide variety of clinical phenotypes

The accumulation of mutational data and clinical information has resulted in the establishment of comprehensive genotype–phenotype correlations in PMD and related disorders (Fig. 13.2) (Inoue 2005). The spectrum of *PLP1*-related disorders is caused by various degrees of point mutations, duplications, and null (meaning, none) mutations, including deletions. Point mutations, which often result in amino acid substitutions, result in diseases that fall throughout the entire spectrum from the most severe form (0) to the mildest form (4). The most severe form is exclusively caused by point mutations. Of the different point mutations, changes in evolutionally conserved residues that encode both the PLP1 and DM20 isoforms are thought to cause the severe phenotypes (Cailloux et al. 2000), while changes in less conserved residues or in the PLP1-specific region (i.e., exon3B) often result in the milder phenotypes (forms 3 and 4). Duplications commonly cause forms 1 and 2. Null mutations, including deletions and early nonsense/frameshift mutations, that result in premature terminations often cause diseases on the mild end of the spectrum with forms 3 and 4.

Intronic mutations that do not change the putative amino acid sequences in the exons but disrupt the splicing of the *PLP1* gene also cause various degrees of severity of PMD (Hobson et al. 2000). Mutations are found not only in the donor/acceptor regions but also in the deep introns, which can be overlooked by conventional mutation screening methods (Taube et al. 2014). In fact, considerable numbers of patients with intronic mutations have been reported (Lassuthova et al. 2013; Hobson et al. 2002; Hubner et al. 2005). The phenotypic presentations of intronic mutations are variable and difficult to predict, probably because the outcome of altered splicing is complex; some changes simply cause the removal of consecutive exons, while others result in multiple splicing variants with altered protein sequences.

13.3 Distinct Molecular Mechanisms Underlying Different *PLP1* Mutations Associated with PMD

13.3.1 Cellular Pathology of Point Mutations Resulting in Amino Acid Substitutions

PLP1 is a membrane protein that consists of four transmembrane domains and cytoplasmic N- and C- termini. Following translation in the endoplasmic reticulum (ER), PLP1 is properly folded, and disulfide bonds are formed before it is transported to the Golgi apparatus for further posttranslational modification. However, the vast majority of *PLP1* point mutations result in amino acid substitutions and misfolding of the mutant PLP1 (Jung et al. 1996). Misfolded mutant PLP1 does not transport to the Golgi apparatus but instead accumulates in the ER (Gow and Lazzarini 1996). The accumulation of mutant PLP1 results in not only a lack of normally functioning PLP1 that should be properly located in the myelin sheath but also toxicity to oligodendrocytes (Swanton et al. 2005). This cytotoxic theory helps explain why many patients with point mutations exhibit more severe phenotypes compared with those with null mutations (Cailloux et al. 2000) and why the severe phenotype exhibited by PMD model mice with a *Plp1* point mutation cannot be rescued by supplementing *Plp1* with wild-type *Plp1* expression by an autosomal transgene (Schneider et al. 1995).

The unfolded protein response (UPR) hypothesis was proposed to explain the major molecular pathology underlying the cellular toxicity resulting from *PLP1* point mutations (Southwood et al. 2002; Clayton and Popko 2016; Inoue 2017). The amino acid substitutions resulting from the mutation eventually disturb normal folding and lead to the misfolding and accumulation of PLP1 in the ER, which then interrupts PLP1 trafficking from the ER to the Golgi apparatus. The accumulation of such misfolded proteins increases ER stress, which then needs to be eliminated to maintain cellular homeostasis, and the UPR is the cellular mechanism responsible for protecting cells from ER stress caused by misfolded protein accumulation. The UPR consists of two major functions: the adaptive and maladaptive responses (Lin et al. 2007). To adapt to the ER stress, the UPR reduces translation of proteins entering ER, increases the retrotranslocation and degradation of ER-localized misfolded proteins, and bolsters the protein-folding capacity of the ER (adaptive response). However, when the ER stress exceeds the capacity of this cell protective response, the UPR removes the cells by inducing apoptosis through upregulation of the proapoptotic branch of the UPR (maladaptive response) (Lin et al. 2007). When postnatal myelin synthesis increases, oligodendrocytes produce massive amounts of PLP1, which may lead to an overwhelming amount of misfolded PLP1 that cannot be handled by the adaptive pathway. The apoptotic pathway is then stimulated to result in the apoptotic cell death of the oligodendrocytes.

The UPR consists of three signaling pathways that interactively respond to the ER stress evoked by the accumulation of misfolded proteins in the ER. Each pathway is initiated by the activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and protein kinase R-like ER kinase (PERK) stress sensors (Szegezdi et al.

2006). In unstressed conditions, the ER chaperone glucose-regulated protein 78 [GRP78, also known as binding immunoglobulin protein (BiP)] negatively regulates the UPR signaling pathways by binding to the ER stress sensors ATF6, IRE1, and PERK (Schroder and Kaufman 2005). When unfolded/misfolded proteins accumulate in the ER, GRP78 binds to the unfolded/misfolded proteins, which results in dissociation from the ER stress sensors, thereby initiating the UPR. GRP78 expression is continuously upregulated in in vitro cells and in vivo oligodendrocytes in PMD model mice expressing mutant PLP1, and all three pathways are activated (Numata et al. 2013; Southwood et al. 2002). Of these three pathways, the PERK pathway is responsible for the UPR's proapoptotic branch, which is thought to play a central role in the cellular pathogenesis underlying the massive oligodendrocyte cell death in PMD. Modification of this response pathway may have the potential for mitigating the cellular pathology of PMD. The molecular and cellular mechanisms other than UPR have also been proposed to be involved in the pathogenesis of PLP1 point mutations, as recently reviewed elsewhere (Inoue 2017).

13.3.2 Genomic and Cellular Pathology Underlying *PLP1* Duplications

Duplication of the variously sized genomic segments that all encompass the *PLP1* gene is the most common cause of PMD, accounting for 60–70% of all patients with PMD. The smallest duplication is less than 50 kb, while the largest spans more than 1 Mb (Woodward et al. 1998; Inoue et al. 1999). Duplications are often tandem in nature. Because the clinical severities of the patients are not directly associated with the size or range of the duplicated genomic intervals, except for those with changes over 1 Mb in size, *PLP1* is likely the only gene responsible for the neurological phenotypes resulting from the duplications. The most common size of duplication is around 200–500 kb (Lee et al. 2006).

PLP1 duplications appear to increase the levels of expression of PLP1 transcripts and protein. However, the mechanisms underlying how the increased amount of PLP1 detrimentally affects myelinating oligodendrocytes are largely undetermined. Rare patients with PMD who carry triplications of the *PLP1* gene exhibit more severe phenotypes than patients with duplications (Wolf et al. 2005; Beck et al. 2015). Similarly, in *Plp1* transgenic (Tg) mice that have been utilized as a model of *PLP1* overexpression, mice with higher copy numbers of the transgene exhibit more severe phenotypes (Readhead et al. 1994; Kagawa et al. 1994; Inoue et al. 1996b). These findings suggest that the cellular toxicity resulting from an increased amount of PLP1 is dosage dependent. Interestingly, Tg mice with lower copy numbers exhibit milder dysmyelinating phenotypes but do show axonal degeneration, which suggests abnormal amounts of PLP1 also affect axon maintenance in mice (Anderson et al. 1998). Similar axonal degenerative changes that are combined with slowly progressive demyelination, and not typical hypomyelination, have been found in mice with *Plp1* duplications generated by chromosomal engineering

technology that serves as the complete genocopy of human patients with PMD (Clark et al. 2013). Because humans with *PLP1* duplications show prominent hypomyelination in the white matter, the threshold for sensitivity to PLP1 gene dosage may be lower in humans than it is in mice.

Excessive PLP1 appears to accumulate in the oligodendrocyte cell body instead of in the myelin sheath. However, it is not clear how this abnormal accumulation of wild-type PLP1 occurs and if this accumulation directly causes the failure in myelination and the premature death of oligodendrocytes. The accumulated PLP1 colocalizes with cholesterol in late endosomes/lysosomes and the ER (Simons et al. 2002). The mislocalization of cholesterol has been hypothesized to cause the mistrafficking of myelin raft components, which in turn perturbs the process of myelination and leads to the premature death of oligodendrocytes.

Conceptually, PMD resulting from *PLP1* duplications and deletions is considered as a genomic disorder (Inoue 2005), which is a group of diseases that result from genomic dosage alterations that lead to quantitative changes in the expression of the involved gene rather than base-pair changes in the DNA that leads to qualitative changes in the gene products (Lupski 1998). Many different mechanisms underlie disease-causing genomic rearrangements, and these are comprehensively reviewed elsewhere (Carvalho and Lupski 2016). The genomic rearrangement involving *PLP1* is classified as a nonrecurrent rearrangement in which the rearranged genomic segments have unique sizes and genomic contents in unrelated patients but all encompass *PLP1* (Inoue et al. 1999). One unique feature of the genomic rearrangements that are associated with PMD is that, although each rearrangement is unique in size and location, the locations of the distal ends of the rearranged genomic segments often cluster at or near the local low copy repeats (LCRs), which are large (>10 kb) genomic segments with high sequence homology (>90%) to each other (Lee et al. 2006). The LCRs that are located distal to the *PLP1* gene show multiple segments that face each other in a head-to-head nature; such genomic structures may stimulate genomic rearrangements (Fig. 13.3).

Investigations of high-resolution copy number variations using custom array comparative genomic hybridization and the sequencing of the junction fragments of the genomic rearrangements involving *PLP1* have uncovered the molecular mechanisms underlying how *PLP1* duplications and deletions can be generated (Lee et al. 2007). In genomic disorders mainly caused by recurrent genomic rearrangements, such as Charcot–Marie–Tooth disease type 1A and DiGeorge

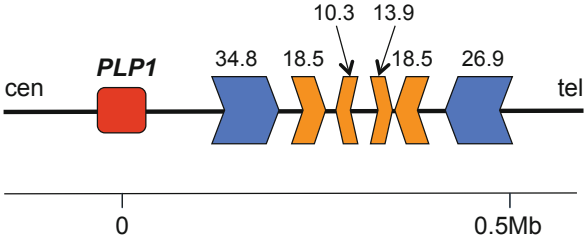

Fig. 13.3 Genomic architecture of LCRs located distal to *PLP1*. Units with same color indicate homologous pairs. Numbers above each LCR indicate their size in kb

syndrome, the involvement of a non-allelic homologous recombination (NAHR) between a pair of tandem LCRs flanking the genomic segments that are commonly deleted or duplicated is observed (Inoue and Lupski 2002; Lupski 1998). However, NAHR does not seem to apply to PMD. Analyses of the breakpoint junctions of *PLP1* duplications and deletions have indicated the involvement of distinct mechanisms, such as nonhomologous end joining and replication-based mechanisms (RBMs), including break-induced replication, microhomology-mediated break-induced replication, and fork stalling and template switching (Lee et al. 2007). The detailed rearrangement processes of these RBMs are reviewed elsewhere (Liu et al. 2012; Hastings et al. 2009). The resultant genomic rearrangements at the *PLP1* locus are characterized by complex genomic rearrangements consisting of more than two breakpoint junctions and multiple de novo copy number-amplified segments that are interspersed and stitched together in single mutation events. From a clinical point of view, such RBMs can also cause triplication of *PLP1*, which causes more severe phenotypes than duplications do. In fact, analyses of the genomic rearrangements associated with PMD have largely contributed to the discovery that RBMs underlie the generation of non-recurrent genomic rearrangements that eventually cause a number of other genomic disorders (Carvalho and Lupski 2016).

13.3.3 *PLP1* Null Mutations

Entire and partial deletions of *PLP1* are rare causes of PMD (Inoue et al. 2002; Inoue 2005). Point mutations that lead to disruption of the initiation codon (i.e., the first methionine) or the generation of early premature termination codons (PTCs, which are nonsense and frameshift mutations in upstream exons) also result in a lack of PLP1 production. PTC-causing mutations presumably trigger the nonsense-mediated mRNA decay pathway, which serves as a cellular surveillance system for specifically detecting mRNA with PTCs in upstream exons to degrade them before they are translated (Khajavi et al. 2006). All patients with *PLP1* null mutations appear to show unique clinical phenotypes (Garbern et al. 1997; Inoue et al. 2002). In general, the neurological manifestations are much milder than those of the other types of mutations. These patients usually retain the ability to walk, and their cognitive impairments are mild. Some are diagnosed with SPG2, which is an adolescent-onset motor disease now known to be allelic to PMD (Saugier-Veber et al. 1994). The hyperintensities in the white matter revealed by T2-weighted MRI are also milder. Despite their mild phenotypes, these patients often show a slowly progressive deterioration of their motor functions later in life. In addition, patients with null mutations often exhibit mild peripheral demyelinating neuropathy, which is not observed in patients with other type of mutations (Garbern et al. 1997). Studies using immunogold electron microscopy have reported that a small amount of PLP1 is localized in the peripheral myelin and that the absence of PLP1, and not alterations in PLP1 or an increased dosage of PLP1, causes demyelination of the peripheral nerves (Garbern et al. 1997). Why only null mutations show this additional phenotype is unclear. Findings in patients with PMD and peripheral neuropathy have

suggested that PLP1-specific residues in exon3 (i.e., exon3B) are required for normal peripheral nerve function and that the mutations that eliminate this 35 amino acid domain cause this unique phenotype (Shy et al. 2003).

These mild phenotypes observed in human patients despite the absence of *PLP1* are consistent with the phenotypic and histological findings in mice lacking the *Plp1* gene. *Plp1* null mice are fully viable, and almost normal myelin forms in their CNS (Klugmann et al. 1997). However, the myelin lacking Plp1 appears to be physically fragile (Jurevics et al. 2003), which results in incomplete compaction (Rosenbluth et al. 2006). Surprisingly, these mice undergo axonal swelling and degeneration that is slowly progressive and becomes apparent in old age (Griffiths et al. 1998). Similar axonal degeneration occurring in the absence of demyelination and inflammation has been identified in the CNS of patients lacking *PLP1* (Garbern et al. 2002).

13.3.4 Hypomyelination of Early Myelinating Structures: An Alternative Phenotype of *PLP1* Mutations

Particular *PLP1* mutations cause a unique phenotype that was recently characterized as the hypomyelination of early myelinating structures (HEMS) disorder (Steenweg et al. 2012). Clinically, most patients with HEMS show mild functional disabilities that are indistinguishable from SPG2. However, HEMS is characterized by the MRI findings of hypomyelination of particular brain regions that usually myelinate early. These patients exhibit mild hyperintensities in T2-weighted images of the medulla oblongata, caudal pons, dentate nucleus hilus, peridentate white matter, subcortical cerebellar white matter, optic radiation, and frontoparietal periventricular white matter (Steenweg et al. 2012). Initial genetic screenings of *PLP1* have failed to identify mutations in the patients' DNA, but extensive comprehensive genetic screenings have identified mutations in specific regions of *PLP1*: the alternatively spliced exon3B that is eliminated in the DM20 isoform and intron3 (Kevelam et al. 2015). Remarkably, these mutations result in subtle changes, such as deep intronic alterations outside the donor/acceptor sites, silent mutations with no amino acid changes and missense mutations with the contradictory prediction of its pathogenicity in exon3B. Many of these mutations are thought to affect the alternative splicing of PLP1/DM20, which suggests that regulation of these alternative splicing variants is important for early myelination.

13.4 Conclusions

In this chapter, I have reviewed the historical aspects; clinical features; and molecular, cellular, and genetic bases of the inherited hypomyelinating disease, PMD and its related disorders. Different mutations in the causative gene *PLP1* result in distinct cellular and molecular pathologies, which lead to various levels of hypomyelination in the CNS. Although PMD is incurable at this point, several studies have suggested potential therapies for PMD using various model systems that are based on known

molecular and cellular pathologies. Rapidly developing biotechnologies, including induced pluripotent stem cell biology and regenerative medicine (Osorio et al. 2017), high-throughput screening of chemical compounds (Nevin et al. 2017), practical application of adeno-associated virus-mediated gene therapy (Li et al. 2019), RNA interference therapy, and genome editing technology, will help in the development of potential therapies for PMD and other inherited intractable diseases.

References

Anderson TJ, Schneider A, Barrie JA, Klugmann M, Mcculloch MC, Kirkham D, Kyriakides E, Nave KA, Griffiths IR (1998) Late-onset neurodegeneration in mice with increased dosage of the proteolipid protein gene. J Comp Neurol 394:506–519

Beck CR, Carvalho CMB, Banser L, Gambin T, Stubbolo D, Yuan B, Sperle K, Mccahan SM, Henneke M, Seeman P, Garbern JY, Hobson GM, Lupski JR (2015) Complex genomic rearrangements at the PLP1 locus include triplication and quadruplication. PLoS Genet 11: e1005050

Boespflug-Tanguy O, Mimault C, Melki J, Cavagna A, Giraud G, Pham Dinh D, Dastugue B, Dautigny A, Group TPC (1994) Genetic homogeneity of Pelizaeus-Merzbacher disease: tight linkage to the proteolipoprotein locus in 16 affected families. Am J Hum Genet 55:461–467

Boison D, Stoffel W (1994) Disruption of the compacted myelin sheath of axons of the central nervous system in proteolipid protein-deficient mice. Proc Natl Acad Sci U S A 91:11709–11713

Boison D, Bussow H, D'urso D, Müller HW, Stoffel W (1995) Adhesive properties of proteolipid protein are responsible for the compaction of CNS myelin sheaths. J Neurosci 15:5502–5513

Bonkowsky JL, Nelson C, Kingston JL, Filloux FM, Mundorff MB, Srivastava R (2010) The burden of inherited leukodystrophies in children. Neurology 75:718–725

Cailloux F, Gauthier-Barichard F, Mimault C, Isabelle V, Courtois V, Giraud G, Dastugue B, Boespflug-Tanguy O, Clinical Europian Network on Brain Dysmyelinating Disease (2000) Genotype-phenotype correlation in inherited brain myelination defects due to proteolipid protein gene mutations. Eur J Hum Genet 8:837–845

Carvalho CMB, Lupski JR (2016) Mechanisms underlying structural variant formation in genomic disorders. Nat Rev Genet 17:224–238

Clark K, Sakowski L, Sperle K, Banser L, Landel CP, Bessert DA, Skoff RP, Hobson GM (2013) Gait abnormalities and progressive myelin degeneration in a new murine model of Pelizaeus-Merzbacher disease with tandem genomic duplication. J Neurosci 33:11788–11799

Clayton BL, Popko B (2016) Endoplasmic reticulum stress and the unfolded protein response in disorders of myelinating glia. Brain Res 1648:594–602

Cremers FPM, Pfeiffer RA, Van De Pol TJR, Hofker MH, Kruse TA, Wieringa B, Ropers HH (1987) An interstitial duplication of the X chromosome in a male allows physical fine mapping of probes from the Xq13-q22 region. Hum Genet 77:23–27

Dautigny A, Mattei M-G, Morello D, Alliel PM, Pham-Dinh D, Amar L, Arnaud D, Simon D, Mattei J-F, Guenet J-L, Jollès P, Avner P (1986) The structural gene coding for myelin-associated proteolipid protein is mutated in jimpy mice. Nature 321:867–869

Ellis D, Malcolm S (1994) Proteolipid protein gene dosage effect in Pelizaeus-Merzbacher disease. Nat Genet 6:333–334

Garbern JY, Cambi F, Tang X-M, Sima AA, Vallat JM, Bosch EP, Lewis R, Shy M, Sohi J, Kraft G, Chen KL, Joshi I, Leonard DG, Johnson W, Raskind W, Dlouhy SR, Pratt V, Hodes ME, Bird T, Kamholz J (1997) Proteolipid protein is necessary in peripheral as well as central myelin. Neuron 19:205–218

Garbern JY, Yool DA, Moore GJ, Wilds IB, Faulk MW, Klugmann M, Nave KA, Sistermans EA, Van Der Knaap MS, Bird TD, Shy ME, Kamholz JA, Griffiths IR (2002) Patients lacking the

major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation. Brain 125:551–561

Gencic S, Hudson LD (1990) Conservative amino acid substitution in the myelin proteolipid protein of jimpy^{msd} mice. J Neurosci 10:117–124

Gow A, Lazzarini RA (1996) A cellular mechanism governing the severity of Pelizaeus-Merzbacher disease. Nat Genet 13:422–428

Griffiths IR, Dickinson P, Montague P (1995) Expression of the proteolipid protein gene in glial cells of the post-natal peripheral nervous system of rodents. Neuropathol Appl Neurobiol 21:97–110

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, Mcculloch M, Nadon N, Nave KA (1998) Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science 280:1610–1613

Hastings PJ, Ira G, Lupski JR (2009) A microhomology-mediated break-induced replication model for the origin of human copy number variation. PLoS Genet 5:e1000327

Hobson GM, Davis AP, Stowell NC, Kolodny EH, Sistermans EA, De Coo IF, Funanage VL, Marks HG (2000) Mutations in noncoding regions of the proteolipid protein gene in Pelizaeus-Merzbacher disease. Neurology 55:1089–1096

Hobson GM, Huang Z, Sperle K, Stabley DL, Marks HG, Cambi F (2002) A PLP splicing abnormality is associated with an unusual presentation of PMD. Ann Neurol 52:477–488

Hubner CA, Orth U, Senning A, Steglich C, Kohlschutter A, Korinthenberg R, Gal A (2005) Seventeen novel PLP1 mutations in patients with Pelizaeus-Merzbacher disease. Hum Mutat 25:321–322

Hudson LD, Puckett C, Berndt J, Chan J, Gencic S (1989) Mutation of the proteolipid protein gene PLP in a human X chromosome-linked myelin disorder. Proc Natl Acad Sci U S A 86:8128–8131

Ikenaka K, Kagawa T, Mikoshiba K (1992) Selective expression of DM-20, an alternatively spliced myelin proteolipid protein gene product, in developing nervous system and in nonglial cells. J Neurochem 58:2248–2253

Inoue K (2005) PLP1-related inherited dysmyelinating disorders: Pelizaeus-Merzbacher disease and spastic paraplegia type 2. Neurogenetics 6:1–16

Inoue K (2017) Cellular pathology of Pelizaeus-Merzbacher disease involving chaperones associated with endoplasmic reticulum stress. Front Mol Biosci 4:7

Inoue K, Lupski JR (2002) Molecular mechanisms for genomic disorders. Annu Rev Genomics Hum Genet 3:199–242

Inoue K, Osaka H, Sugiyama N, Kawanishi C, Onishi H, Nezu A, Kimura K, Kimura S, Yamada Y, Kosaka K (1996a) A duplicated PLP gene causing Pelizaeus-Merzbacher disease detected by comparative multiplex PCR. Am J Hum Genet 59:32–39

Inoue Y, Kagawa T, Matsumura Y, Ikenaka K, Mikoshiba K (1996b) Cell death of oligodendrocytes or demyelination induced by overexpression of proteolipid protein depending on expressed gene dosage. Neurosci Res 25:161–172

Inoue K, Osaka H, Imaizumi K, Nezu A, Takanashi J, Arii J, Murayama K, Ono J, Kikawa Y, Mito T, Shaffer LG, Lupski JR (1999) Proteolipid protein gene duplications causing Pelizaeus-Merzbacher disease: molecular mechanism and phenotypic manifestations. Ann Neurol 45:624–632

Inoue K, Osaka H, Thurston VC, Clarke JT, Yoneyama A, Rosenbarker L, Bird TD, Hodes ME, Shaffer LG, Lupski JR (2002) Genomic rearrangements resulting in PLP1 deletion occur by nonhomologous end joining and cause different dysmyelinating phenotypes in males and females. Am J Hum Genet 71:838–853

Jung M, Sommer I, Schachner M, Nave KA (1996) Monoclonal antibody O10 defines a conformationally sensitive cell-surface epitope of proteolipid protein (PLP): evidence that PLP misfolding underlies dysmyelination in mutant mice. J Neurosci 16:7920–7929

Jurevics H, Hostettler J, Sammond DW, Nave KA, Toews AD, Morell P (2003) Normal metabolism but different physical properties of myelin from mice deficient in proteolipid protein. J Neurosci Res 71:826–834

Kagawa T, Ikenaka K, Inoue Y, Kuriyama S, Tsujii T, Nakao J, Nakajima K, Aruga J, Okano H, Mikoshiba K (1994) Glial cell degeneration and hypomyelination caused by overexpression of myelin proteolipid protein gene. Neuron 13:427–442

Kevelam SH, Taube JR, Van Spaendonk RML, Bertini E, Sperle K, Tarnopolsky M, Tonduti D, Valente EM, Travaglini L, Sistermans EA, Bernard G, Catsman-Berrevoets CE, Van Karnebeek CDM, Ostergaard JR, Friederich RL, Elsaid MF, Schieving JH, Tarailo-Graovac M, Orcesi S, Steenweg ME, Van Berkel CGM, Waisfisz Q, Abbink TEM, Van Der Knaap MS, Hobson GM, Wolf NI (2015) Altered PLP1 splicing causes hypomyelination of early myelinating structures. Ann Clin Transl Neurol 2:648–661

Khajavi M, Inoue K, Lupski JR (2006) Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. Eur J Hum Genet 14:1074–1081

Klugmann M, Schwab MH, Puhlhofer A, Schneider A, Zimmermann F, Griffiths IR, Nave KA (1997) Assembly of CNS myelin in the absence of proteolipid protein. Neuron 18:59–70

Lassuthova P, Zaliova M, Inoue K, Haberlova J, Sixtova K, Sakmaryova I, Paderova K, Mazanec R, Zamecnik J, Siskova D, Garbern J, Seeman P (2013) Three new PLP1 splicing mutations demonstrate pathogenic and phenotypic diversity of Pelizaeus-Merzbacher disease. J Child Neurol 29(7):924–931

Lee JA, Inoue K, Cheung SW, Shaw CA, Stankiewicz P, Lupski JR (2006) Role of genomic architecture in PLP1 duplication causing Pelizaeus-Merzbacher disease. Hum Mol Genet 15:2250–2265

Lee JA, Carvalho CM, Lupski JR (2007) A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. Cell 131:1235–1247

Li H, Okada H, Suzuki S, Sakai K, Izumi H, Matsushima Y, Ichinohe N, Goto YI, Okada T, Inoue K (2019) Gene suppressing therapy for Pelizaeus-Merzbacher disease using artificial microRNA. JCI Insight 4(10). pii:125052. https://doi.org/10.1172/jci.insight.125052

Lin JH, Li H, Yasumura D, Cohen HR, Zhang C, Panning B, Shokat KM, Lavail MM, Walter P (2007) IRE1 signaling affects cell fate during the unfolded protein response. Science 318:944–949

Liu PF, Carvalho CMB, Hastings PJ, Lupski JR (2012) Mechanisms for recurrent and complex human genomic rearrangements. Curr Opin Genet Dev 22:211–220

Lupski JR (1998) Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. Trends Genet 14:417–422

Merzbacher L (1910) Eine eigenartige familiäre-hereditäre Erkrankungsform (Aplasia axialis extracorticalis congenita). Z Ges Neurol Psychiatr 3:1–138

Miller MJ, Haxhiu MA, Georgiadis P, Gudz TI, Kangas CD, Macklin WB (2003) Proteolipid protein gene mutation induces altered ventilatory response to hypoxia in the myelin-deficient rat. J Neurosci 23:2265–2273

Mimault C, Giraud G, Courtois V, Cailloux F, Boire JY, Dastugue B, Boespflug-Tanguy O, The Clinical European Network on Brain Dysmyelinating Disease (1999) Proteolipoprotein gene analysis in 82 patients with sporadic Pelizaeus-Merzbacher disease: duplications, the major cause of the disease, originate more frequently in male germ cells, but point mutations do not. Am J Hum Genet 65:360–369

Nadon NL, Duncan ID, Hudson LD (1990) A point mutation in the proteolipid protein gene of the 'shaking pup' interrupts oligodendrocyte development. Development 110:529–537

Nave KA, Lai C, Bloom FE, Milner RJ (1987) Splice site selection in the proteolipid protein (PLP) gene transcript and primary structure of the DM-20 protein of central nervous system myelin. Proc Natl Acad Sci U S A 84:5665–5669

Nave KA, Lai C, Bloom F, Milner RJ (1989) Jimpy mutant mouse: a 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in mRNA splicing. Proc Natl Acad Sci U S A 83:9264–9268

Nevin ZS, Factor DC, Karl RT, Douvaras P, Laukka J, Windrem MS, Goldman SA, Fossati V, Hobson GM, Tesar PJ (2017) Modeling the mutational and phenotypic landscapes of Pelizaeus-Merzbacher disease with human iPSC-derived oligodendrocytes. Am J Hum Genet 100:617–634

Numata Y, Morimura T, Nakamura S, Hirano E, Kure S, Goto YI, Inoue K (2013) Depletion of molecular chaperones from the endoplasmic reticulum and fragmentation of the Golgi apparatus associated with pathogenesis in Pelizaeus-Merzbacher disease. J Biol Chem 288:7451–7466

Numata Y, Gotoh L, Iwaki A, Kurosawa K, Takanashi J, Deguchi K, Yamamoto T, Osaka H, Inoue K (2014) Epidemiological, clinical, and genetic landscapes of hypomyelinating leukodystrophies. J Neurol 261:752–758

Osaka H, Kawanishi C, Inoue K, Uesugi H, Hiroshi K, Nishiyama K, Yamada Y, Suzuki K, Kimura S, Kosaka K (1995) Novel nonsense proteolipid protein gene mutation as a cause of X-linked spastic paraplegia in twin males. Biochem Biophys Res Commun 215:835–841

Osorio MJ, Rowitch DH, Tesar P, Wernig M, Windrem MS, Goldman SA (2017) Concise review: stem cell-based treatment of Pelizaeus-Merzbacher disease. Stem Cells 35:311–315

Pelizaeus F (1885) Über eine eigentümliche Form spastischer Lähmung mit cerebral Erscheinungen auf hereditärer Grundlage (multiple Sklerose). Arch Psychiatr Nervenkr 16:698–710

Pouwels PJW, Vanderver A, Bernard G, Wolf NI, Dreha-Kulczewksi SF, Deoni SCL, Bertini E, Kohlschutter A, Richardson W, Ffrench-Constant C, Kohler W, Rowitch D, Barkovich AJ (2014) Hypomyelinating leukodystrophies: translational research progress and prospects. Ann Neurol 76:5–19

Raskind WH, Williams CA, Hudson LD, Bird TD (1991) Complete deletion of the proteolipid protein gene (PLP) in a family with X-linked Pelizaeus-Merzbacher disease. Am J Hum Genet 49:1355–1360

Readhead C, Schneider A, Griffiths I, Nave KA (1994) Premature arrest of myelin formation in transgenic mice with increased proteolipid protein gene dosage. Neuron 12:583–595

Rosenbluth J, Nave KA, Mierzwa A, Schiff R (2006) Subtle myelin defects in PLP-null mice. Glia 54:172–182

Saugier-Veber P, Munnich A, Bonneau D, Rozet JM, Le Merrer M, Gil R, Boespflug-Tanguy O (1994) X-linked spastic paraplegia and Pelizaeus-Merzbacher disease are allelic disorders at the proteolipid protein locus. Nat Genet 6:257–262

Schneider A, Montague P, Griffiths I, Fanarraga M, Kennedy P, Brophy P, Nave K-A (1992) Uncoupling of hypomyelination and glial cell death by a mutation in the proteolipid protein gene. Nature 358:758–761

Schneider AM, Griffiths IR, Readhead C, Nave KA (1995) Dominant-negative action of the jimpy mutation in mice complemented with an autosomal transgene for myelin proteolipid protein. Proc Natl Acad Sci U S A 92:4447–4451

Schroder M, Kaufman RJ (2005) The mammalian unfolded protein response. Annu Rev Biochem 74:739–789

Seitelberger F (1970) Pelizaeus-Merzbacher disease. In: Vinken P, Bruyn G (eds) Handbook of clinical neurology. Leucodystrophies and poliodystrophies. North-Holland, Amsterdam

Seitelberger F (1995) Neuropathology and genetics of Pelizaeus-Merzbacher disease. Brain Pathol 5:267–273

Shy ME, Hobson G, Jain M, Boespflug-Tanguy O, Garbern J, Sperle K, Li W, Gow A, Rodriguez D, Bertini E, Mancias P, Krajewski K, Lewis R, Kamholz J (2003) Schwann cell expression of PLP1 but not DM20 is necessary to prevent neuropathy. Ann Neurol 53:354–365

Simons M, Kramer EM, Macchi P, Rathke-Hartlieb S, Trotter J, Nave KA, Schulz JB (2002) Overexpression of the myelin proteolipid protein leads to accumulation of cholesterol and proteolipid protein in endosomes/lysosomes: implications for Pelizaeus-Merzbacher disease. J Cell Biol 157:327–336

Southwood CM, Garbern J, Jiang W, Gow A (2002) The unfolded protein response modulates disease severity in Pelizaeus-Merzbacher disease. Neuron 36:585–596

Steenweg ME, Wolf NI, Schieving JH, Elsaid MF, Friederich RL, Ostergaard JR, Barkhof F, Pouwels PJW, Van Der Knaap MS (2012) Novel hypomyelinating leukoencephalopathy affecting early myelinating structures. Arch Neurol 69:125–128

Swanton E, Holland A, High S, Woodman P (2005) Disease-associated mutations cause premature oligomerization of myelin proteolipid protein in the endoplasmic reticulum. Proc Natl Acad Sci U S A 102:4342–4347

Szegezdi E, Logue SE, Gorman AM, Samali A (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 7:880–885

Taube JR, Sperle K, Banser L, Seeman P, Cavan BC, Garbern JY, Hobson GM (2014) PMD patient mutations reveal a long-distance intronic interaction that regulates PLP1/DM20 alternative splicing. Hum Mol Genet 23:5464–5478

Trofatter JA, Dlouhy SR, Demyer W, Conneally PM, Hodes ME (1989) Pelizaeus-Merzbacher disease: tight linkage to proteolipid protein gene exon variant. Proc Natl Acad Sci U S A 86:9427–9430

Vanderver A, Tonduti D, Schiffmann R, Schmidt J, Van Der Knaap MS (2014) Leukodystrophy overview. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A (eds) GeneReviews((R)). University of Washington, Seattle, WA

Vanderver A, Prust M, Tonduti D, Mochel F, Hussey HM, Helman G, Garbern J, Eichler F, Labauge P, Aubourg P, Rodriguez D, Patterson MC, Van Hove JL, Schmidt J, Wolf NI, Boespflug-Tanguy O, Schiffmann R, Van Der Knaap MS, Consortium G (2015) Case definition and classification of leukodystrophies and leukoencephalopathies. Mol Genet Metab 114:494–500

Weimbs T, Stoffel W (1992) Proteolipid protein (PLP) of CNS myelin: positions of free, disulfide-bonded, and fatty acid thioester-linked cysteine residues and implications for the membrane topology of PLP. Biochemistry 31:12289–12296

Willard HF, Riordan JR (1985) Assignment of the gene for myelin proteolipid protein to the X chromosome: implications for X-linked myelin disorders. Science 230:940–942

Wolf NI, Sistermans EA, Cundall M, Hobson GM, Davis-Williams AP, Palmer R, Stubbs P, Davies S, Endziniene M, Wu Y, Chong WK, Malcolm S, Surtees R, Garbern JY, Woodward KJ (2005) Three or more copies of the proteolipid protein gene PLP1 cause severe Pelizaeus-Merzbacher disease. Brain 128:743–751

Woodward K, Kendall E, Vetrie D, Malcolm S (1998) Pelizaeus-Merzbacher disease: identification of Xq22 proteolipid-protein duplications and characterization of breakpoints by interphase FISH. Am J Hum Genet 63:207–217

Multiple Sclerosis

14

Ryo Yamasaki and Jun-ichi Kira

Abstract

Multiple sclerosis (MS) is an inflammatory demyelinating disorder. Although all MS patients initially show a relapsing-remitting course, 20–50% subsequently enter a chronic progressive course at 10–20 years after onset that greatly influences their activities of daily living. There are 2.5 million MS patients worldwide with large regional and racial differences. In particular, there are many MS patients among Caucasians living in Europe, while the disease is relatively rare in Asians and Africans.

Although MS is regarded as an autoimmune disease, many factors such as genetic background, environmental factors, and sex are involved in its pathogenesis. While the immunological mechanisms remain to be fully elucidated, invasion of autoreactive T cells into the central nervous system (CNS) tissue is considered the first step of the disease. These T cells react with myelin antigens and initiate demyelination of the CNS by activating cytotoxic T cells, macrophages, and B cells through the release of inflammatory cytokines. As a treatment option, disease-modifying therapies have recently been developed to prevent the recurrence of MS in addition to conventional treatment with corticosteroids for acute relapse. However, there are still few effective treatments for the chronic progressive phase, and it is thus imperative to decipher the mechanism for chronic progression.

Keywords

Multiple sclerosis · HLA · Common features · Histology · Immune pathogenesis · Disease-modifying therapy

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14.1 Introduction

Multiple sclerosis (MS) is a representative inflammatory demyelinating disease of the central nervous system (CNS) with unknown etiology. MS more frequently affects people of northern European descent than Asians and Africans, although its prevalence is markedly increasing even in Asian populations. As indicated by the disease name, the main feature is the dissemination of various neurological symptoms over time and space caused by multiple demyelinating lesions in the CNS. MS initially shows a relapsing-remitting course, which turns into a secondary progressive course at 10–20 years after onset. Disease-modifying therapies (DMTs) have been developed as a treatment modality in addition to conventional corticosteroid therapies for acute relapse, resulting in dramatic improvements in outcome. Despite these great achievements in treatment for relapse prevention in the relapse-remitting phase with DMTs, huge unmet medical needs still exist in treatment for the secondary progressive phase. In this review, we introduce the basic and clinical aspects of MS, focusing on recent advances.

14.2 Basic Aspects

14.2.1 Epidemiology

The prevalence of MS differs according to residential areas and races. The estimated prevalence in Western countries such as Europe, Australia, and North America is 30–150/100,000, while that in Asia and Africa is 1–10/100,000, suggesting a contribution of genetic factors to MS susceptibility (Browne et al. 2014). In addition, migration studies revealed an increased risk in immigrants who moved from lower latitude areas to higher latitude areas before puberty, indicating that environmental factors also play an important role in determining MS susceptibility (Dean and Elian 1997; Detels et al. 1978; Ebers 2008; Hammond et al. 2000; Kurtzke et al. 1971). Therefore, MS is considered to be caused by a complex interplay between genetic and environmental factors, with genetic factors accounting for approximately 30% and environmental factors accounting for approximately 70% of the MS risk (Ebers 2008).

The incidence and prevalence of MS have increased worldwide, especially in women (Barnett et al. 2003; Noonan et al. 2002; Wallin et al. 2004). The prevalence of MS in Japan has increased from 1.4/100,000 to 7.7/100,000 over the past 30 years, despite its lower prevalence compared with Western countries (Osoegawa et al. 2009). At the same time, the peak age at onset shifted from the early 30s in 1989 to the early 20s in 2003, and the female-to-male ratio increased from 1.7:1 in 1972 to 2.9:1 in 2003. The worldwide increase in the number of female MS patients, as well as the younger age at onset in some countries, cannot be fully explained by improved diagnostic criteria or case ascertainment (Kira 2012). These observations indicate that MS susceptibility has markedly increased among younger women who have grown up in a Westernized environment, resulting in earlier age at onset. It is

possible that women are more likely to be exposed to changes in potential MS environmental factors.

14.2.2 Environmental Aspects

Environmental factors affect susceptibility to MS at all stages of life. For example, the MS concordance rate is higher in dizygotic twins (30.8%) than in full siblings (4.7%), indicating that the intrauterine environment may confer a risk (Sadovnick et al. 1993). Month-of-birth effects also support the importance of the intrauterine environment. In the Northern Hemisphere, more MS patients are born in April and May, while fewer are born in October and November. This month-of-birth effect is significant in high-latitude areas, but not in low-latitude areas, indicating that exposure to sunlight during the maternal period is an important factor (Dobson et al. 2013). Because vitamin D, a known protective factor against MS, is mainly produced in the skin upon sunlight exposure, low serum vitamin D levels in expectant mothers during the winter period may be related to the month-of-birth effect. In childhood before puberty, migration from a low-prevalence area to a high-prevalence area increases the MS risk, indicating that the childhood environment has significant effects on MS susceptibility (Kennedy et al. 2006). Irrespective of the life stage, low serum vitamin D levels, Epstein–Barr virus (EBV) infection, and smoking are the main risk factors for MS (Ascherio and Munger 2007; Ebers 2008; Ramagopalan et al. 2010). Vitamin D downregulates pathogenic type 1 T helper (Th1) cells but potentiates anti-inflammatory Th2 cells and regulatory T cells (Tregs) (O'Gorman et al. 2012). Cigarette smoking not only increases the risk for MS but also accelerates the transition from relapsing-remitting MS (RRMS) to secondary progressive MS (SPMS) (Ascherio and Munger 2007; Niino et al. 2015). This can be partly explained by the observation that airway inflammation facilitates the recruitment of autoreactive T cells to the CNS tissue via pulmonary lymph nodes (Odoardi et al. 2012). While EBV is a known causal virus for infectious mononucleosis (kissing disease) and malignant lymphoma occurring after infection at puberty, EBV infection also confers an MS risk (Levin et al. 2010). EB nuclear antigen-1 (EBNA-1), a nuclear protein in EBV, shows close molecular mimicry with myelin basic protein (MBP), a major constituent of the myelin sheath that induces experimental autoimmune encephalomyelitis (EAE) as an animal model of MS (Gabibov et al. 2011). EAE is the most utilized animal model of MS, and was first described by Kotritschoner and Schweinburg in 1925. They induced EAE in rabbits by inoculation with human spinal cord homogenate. Since then, EAE has been induced in many different species, including rodents and primates, and it has become clear that EAE can reproduce many of the clinical, neuropathological, and immunological aspects of MS. Recently, rats and mice have been the most commonly used rodents for the induction of EAE (Gold et al. 2006).

14.2.3 Genetic Aspects

In Caucasian populations, the *DR15* haplotype (*DRB1∗15:01-DQA1∗01:02-DQB1∗06:02*), especially *HLA-DRB1∗15:01*, is most strongly associated with MS susceptibility (Nischwitz et al. 2011). After *HLA-DRB1∗15:01*, the alleles conferring MS susceptibility are *HLA-DRB1∗03:01*, *HLA-DRB1∗13:01*, and *HLA-DRB1∗08:01* in the major histocompatibility complex (MHC) class II region, while alleles such as *HLA-A∗02:01*, *HLA-B∗44:02*, *HLA-B∗38:01*, and *HLA-B∗55:01* in the MHC class I region were reported to be protective (Moutsianas et al. 2015). Patients with *HLA-DRB1∗15:01* tend to show classical MS, including younger age at onset, increased brain lesion loads, reduced brain parenchymal volume, and cognitive impairment (Okuda et al. 2009b). In the Japanese population, *HLA-DRB1∗15:01* and *DRB1∗04:05* are two major risk alleles for MS (odds ratio: 1.97 and 1.93, respectively) (Nakamura et al. 2016). Among Japanese MS patients, 40% harbor *DRB1∗04:05*, while 30% harbor *HLA-DRB1∗15:01*. Patients with *DRB1∗04:05* are characterized by an earlier age at onset, milder disability, lower frequencies of brain MRI lesion loads, and lower frequencies of oligoclonal IgG bands (OCBs) (Yoshimura et al. 2012). *DRB1∗04:05* is common in general population residing in some isolated island countries, such as Japan, Sardinia, and Papua New Guinea, and interestingly *DRB1∗04:05* confers an MS risk in Sardinians (Marrosu et al. 2002). *HLA-DRB1∗09:01*, which is more frequently observed in Japanese and Chinese populations than in Europeans (30% of Japanese vs. 1% of Caucasians), is protective against MS (Fujisao et al. 1996; Qiu et al. 2011; Yoshimura et al. 2012). These data suggest that the lower prevalence of MS in Asian populations may be partly caused by the different frequencies of the *HLA-DRB1∗09:01* alleles among races.

Besides the *HLA*-related locus, it is known that genetic abnormalities of CD25 constituting the α chain of the interleukin (IL)2 receptor and CD127 constituting the α chain of the IL7 receptor increase the risk of MS development (Pandit et al. 2011; Fang et al. 2011; Ainiding et al. 2014). CD25 and CD127 are both expressed in Tregs that suppress immune responses. These genetic abnormalities may well contribute to the dysfunction of Tregs that has repeatedly been shown in MS (Gregory et al. 2007; Lundmark et al. 2007). MS is more often transmitted to the next generation by mothers than by fathers. This parent-of-origin effect suggests that epigenetic mechanisms, such as DNA methylation and histone deacetylation, may be operative in MS development (Ebers et al. 2004).

14.2.4 Pathology

14.2.4.1 White Matter Involvement

MS lesions manifest themselves as sharp-boundary plaques with relative sparing of axons. The following histological classification is a simple classification of MS lesions based on the presence or absence and distribution (inflammatory activity) of macrophages/microglia and the presence or absence of ongoing demyelination

(demyelination activity) (Lassmann et al. 1998). *Active lesions* are characterized by macrophages/microglia throughout the lesion area, while *mixed active/inactive lesions* have a hypocellular lesion center with macrophages/microglia limited to the lesion boundaries. *Inactive lesions* are almost completely devoid of macrophages/microglia. Active and active/inactive mixed lesions are further subdivided into *demyelinating lesions*, where myelin destruction is still in progress (macrophages contain myelin degradation products), and *post-demyelinating lesions*, where macrophages are still present but the destruction of myelin has ceased (macrophages do not contain myelin degradation products) (Fig. 14.1). Active demyelinating lesions are accompanied by perivascular cell cuffing that mainly consists of $CD4^+$ T cells, while clonally expanded $CD8^+$ T cells substantially invade the CNS parenchyma (Babbe et al. 2000).

Remission arises from a resolution of acute inflammation, partial remyelination, and redistribution of ion channels along demyelinated axons. Even in normal-appearing white matter, it is obvious that mild global inflammation characterized by microglial activation and diffuse low T cell infiltration is more pronounced in SPMS and primary progressive (PPMS) than in RRMS. In chronic MS plaques, there is no leakage from the blood−brain barrier (BBB), corresponding to the lack of gadolinium-enhanced lesions in PPMS and SPMS.

Demyelination in the MS brain and spinal cord can be followed by variable degrees of remyelination. Remyelination is more pronounced at early stages of the disease, while chronic lesions have little or no remyelination. Invasion of fewer inflammatory cells and more remyelination is observed in PPMS compared with SPMS (Reynolds et al. 2011). Oligodendrocytes are sensitive to oxidative stress because they contain a large pool of iron but have a low capacity antioxidant system. This is why oligodendrocytes are vulnerable to glutamate toxicity (Benarroch 2009). However, oligodendrocyte progenitor cells (OPCs) are also present in chronic MS lesions (Chang et al. 2002). Therefore, failure of remyelination is not due to a lack of OPCs, but rather to inhibition of OPC differentiation to myelinating oligodendrocytes. OPC differentiation to myelinated oligodendrocytes can be inhibited by LINGO 1 in astrocytes and macrophages (Satoh et al. 2007), and PSA-NCAM abnormally expressed in demyelinating axons (Charles et al. 2002), myelin debris (Kotter et al. 2006), and aggregated fibronectin (Stoffels et al. 2013). Acute injury can be detected by the presence of accumulated amyloid precursor protein (APP)-positive spheroids, which reflect axonal transport impairment (Ferguson et al. 1997). APP-positive spheroids are most extensive in the first year after disease onset and decrease with increasing disease duration (Kuhlmann et al. 2002). The extent of axonal loss is well correlated with the number of closely existing $CD8^+$ T cells and macrophages/activated microglia (Kuhlmann et al. 2002). Many $CD8^+$ T cells infiltrating the CNS parenchyma may be able to cut axons through MHC class I-mediated self-antigen recognition (Trapp et al. 1998). Furthermore, reactive oxygen and nitrogen species and proinflammatory cytokines secreted from these cells suppress axonal function and may cause mitochondrial damage (Dutta et al. 2006).

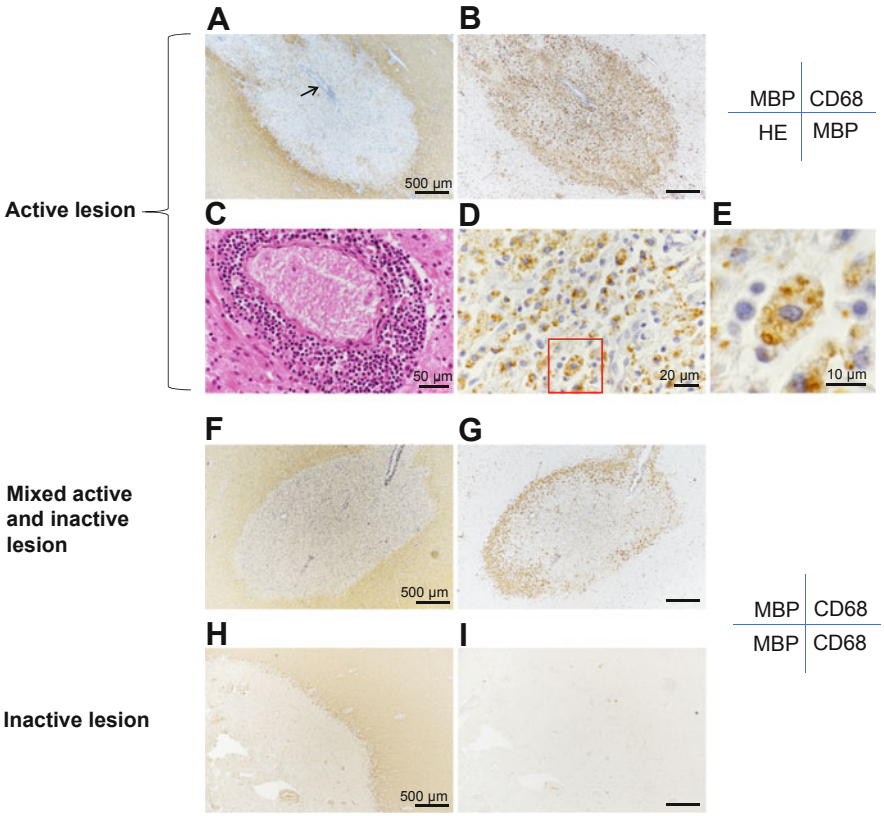

Fig. 14.1 Representative histology for each stage of multiple sclerosis lesions. (**a**–**e**) Active lesion. (**f**, **g**) Mixed active and inactive lesion. (**h**, **i**): Inactive lesion. Antibodies used: (**a**), (**b**), (**e**), (**f**), (**h**), anti-myelin basic protein (MBP); (**b**), (**g**), (**i**): anti-CD68. All images were counterstained with hematoxylin. (**c**): Hematoxylin & eosin (HE) staining. The arrow in (**a**) shows a central vein in the center of a demyelinating lesion. (**b**) shows CD68-positive phagocytes packed in a demyelinating lesion, indicating an active lesion. (**c**) shows an enlarged image of the central vein with a perivascular cuff between the vascular basement membrane and glia limitans. (**d**) shows an enlarged image of CD68-positive phagocytes. (**e**) shows a further enlarged image of the single phagocyte indicated by the red square in (**d**), which harbors myelin debris. (**f**) and (**g**) show decreased macrophages that remain in the marginal ridge of a demyelinating lesion, indicating a mixed active and inactive lesion. (**h**) and (**i**) show almost no macrophages in a demyelinating lesion, indicating an inactive lesion. Bars: (**a**, **b**) 500 μm, (**c**) 50 μm, (**d**) 20 μm, (**e**) 10 μm, (**f**–**i**) 500 μm

14.2.4.2 Gray Matter Involvement

Although the cortical lesion burden demonstrated on MRI by double-inversion recovery imaging, together with cortical and spinal atrophy is significantly associated with disability progression and cognitive impairment, white matter atrophy is not correlated with disability (Shinoda et al. 2018) (Fig. 14.2g, h). Thus, cortical lesions may play a major role in the development of both physical and cognitive impairment (Calabrese et al. 2010b). Pathologically, demyelination is

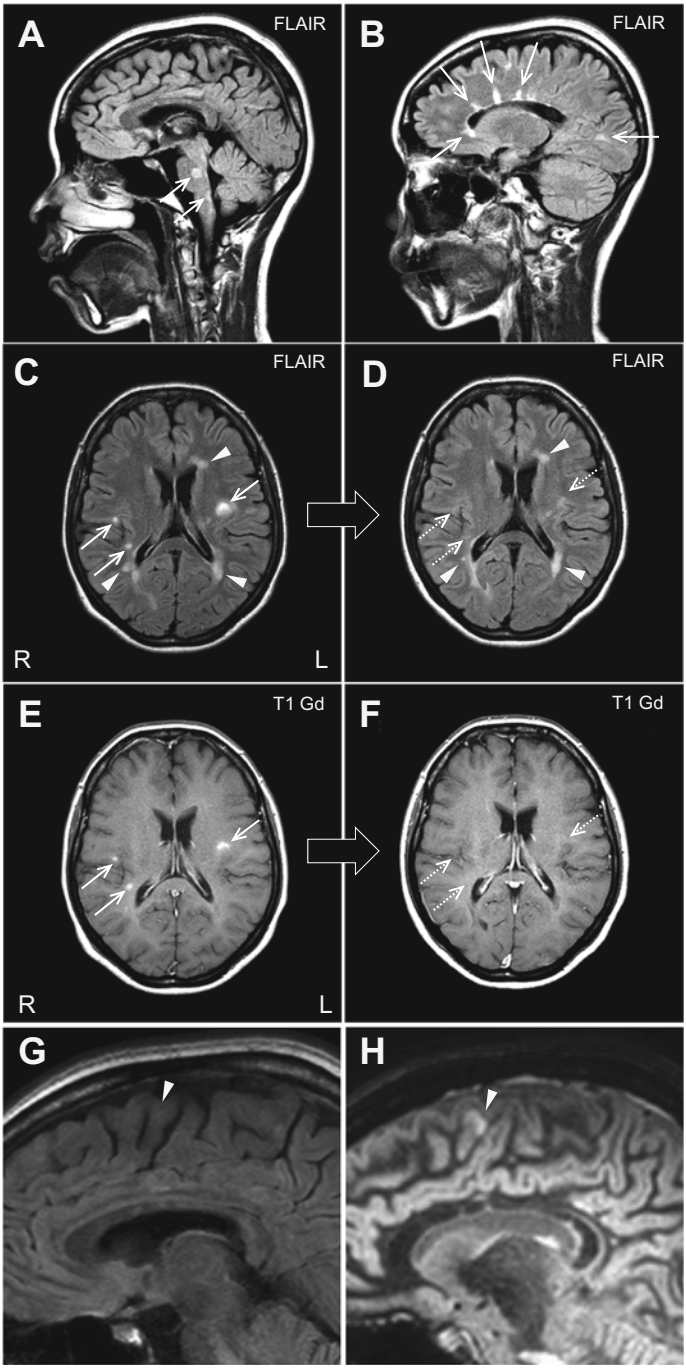

Fig. 14.2 Brain MRI lesions in a representative RRMS patient. The patient was a 32-year-old woman. She experienced a sensory disturbance on the right face in year X–6 that disappeared within 1 month. A sensory disturbance on the right upper limb occurred in year X–2 and disappeared

present to varying degrees in the cerebral and cerebellar cortex, deep gray matter including the thalamus, basal ganglia and hypothalamus, and spinal cord central gray matter (Peterson et al. 2001). The frontal and temporal cortex, cingulate gyrus, and hippocampus are most frequently affected, which may explain the correlation between cognitive impairment and cortical pathology (Reynolds et al. 2011). The localization of cortical demyelination lesions is not correlated with the severity of underlying white matter lesions, indicating that an independent mechanism is involved (Bo et al. 2003). Cortical lesions show increased levels of activated microglia without apparent inflammatory infiltration or significant leakage of plasma proteins, indicating a preserved BBB (Reynolds et al. 2011). Meningeal ectopic lymph follicles consisting of $CD20^+$ B cells and $CD35^+$ dendritic cells are present in about 40% of necropsy MS cases and are mainly located in the deep cortical grooves of the temporal, frontal, cingulate gyri, and islet cortex (Howell et al. 2011). In extensive subpial demyelinating lesions adjacent to the pia mater, increased numbers of activated microglia, increased axonal injury, and neuronal loss are maximal near the pial surface (Magliozzi et al. 2010).

Expression of proinflammatory cytokines/chemokines, such as interferon (IFN)γ, tumor necrosis factor, and CXCL13 (B lymphocyte chemoattractant), was upregulated in autopsied meninges from MS patients (Magliozzi et al. 2018). A similar increase in cytokines/chemokines was detected in the cerebrospinal fluid (CSF) of MS patients with high levels of cortical gray matter lesions at the time of diagnosis (Magliozzi et al. 2018). In summary, secretion of proinflammatory cytokines from lymphocytes in the meningeal follicles to the CSF can cause cortical demyelinating lesions. Diffuse loss of cortical neurons and axons was also observed, even in normal-appearing gray matter (Magliozzi et al. 2010). Neuronal apoptosis and damage to mitochondria are thought to be responsible for the loss of neurons (Reynolds et al. 2011; Dutta et al. 2006; Mahad and Ransohoff 2003), but demyelination and loss of neurons may not be directly correlated with one another in gray matter lesions (Reynolds et al. 2011). In other words, the mechanisms for cortical damage, including glial activation, meningeal ectopic lymph follicles, and high level of cytokine-induced pial damage, irrespective of the presence of myelin are different from those in white matter demyelinating lesions.

Fig. 14.2 (continued) within 3 months. At year X (time of MRI scan), she noted paresthesia on her right face, upper limb, and lower limb and was referred to our clinic. She was diagnosed as RRMS and treated with corticosteroids and fingolimod. (**a–c**, **e**): MRI before treatment. (**d**, **f**) MRI after 3 months of treatment. (**a–d**) FLAIR images. (**e**, **f**) Gadolinium-enhanced T1-weighted images (T1 Gd). (**a**) Demyelinating lesions are visible in the pons and medulla oblongata (arrow). (**b**) Ovoid lesions (Dawson's fingers) are recognized around the ventricle (arrow). (**c**) Axial image of (**a**). (**d**): Axial image of (**c**). When comparing images before (**c**) and after (**d**) treatment, the hyperintense lesions indicated by the arrows are resolving, while the hyperintense lesions indicated by the arrowheads do not change. (**e**) Multiple gadolinium-enhanced lesions (arrows) are seen before treatment. (**f**) After corticosteroid therapy, contrast-enhanced lesions have almost disappeared. (**g**, **h**) Brain MRI sagittal sections taken at year X + 3. (**g**) T2 FLAIR image. (**h**) Double-inversion recovery (DIR) image. The arrowhead shows no obvious lesion in (**g**), while a cortical lesion becomes apparent on the DIR image in (**h**)

14.2.4.3 Glial Inflammation

In the white matter, active and active/inactive lesions are accompanied by macrophages and activated microglia. In the cortical gray matter, disseminated microglial activation exists without visible inflammatory infiltrates. In addition to damage and repair of CNS tissue, macrophages and resident microglia are thought to play a major role in demyelinating lesion formation by restimulating T cells within the CNS. In the CNS, perivascular and meningeal macrophages act as major antigen-presenting cells for restimulation of T cells. Without restimulation by their relevant antigens, T cells will not invade the CNS parenchyma by destroying the glia limitans perivascularis. Recruitment of monocytes/macrophages is mediated by CCL2-CCR2 signaling. Hypertrophic astrocytes in active MS lesions produce CCL2, while its receptor CCR2 is expressed on monocytes/macrophages (Mahad and Ransohoff 2003). Thus, macrophages play a major role in antigen presentation and tissue destruction. Activated microglia produce numerous proinflammatory cytokines/chemokines, growth factors, and active oxygen and nitrogen species via oxidative bursts and inducible nitric oxide synthase (iNOS), thereby causing tissue damage. Alternatively, microglia can exert neuroprotective effects by engulfing tissue pieces and producing neurotrophic substances.

Acute MS lesions contain a number of hypertrophic astrocytes with increased expression of glial fibrillary acidic protein (GFAP), vimentin, and nestin (Fig. 14.3). Such activated astroglia secretes many proinflammatory cytokines, including IL1, IL6, IL12, IL15, IL23, IL27, IL33, CCL5 (RANTES), CXCL8 (IL8), CXCL10 (IP10), and CXCL12 (SDF1). In addition, astroglia produces iNOS, leading to the production of superoxide anions and peroxynitrite that can damage oligodendrocytes with low antioxidant levels (Antony et al. 2004). Astroglia can also produce various growth factors that promote oligodendrocytes to form myelin by influencing OPCs (Moore et al. 2011). IL6 and transforming growth factor (TGF)-β produced by activated astrocytes can promote neuroprotection (Allaman et al. 2011). In chronic MS lesions, astrocyte scars are formed, which can interfere with axonal growth and tissue repair. However, ablation of proliferative astroglia worsens EAE and is associated with the mass invasion of macrophages and T cells, indicating the importance of astroglia in preventing the spreading of inflammation (Voskuhl et al. 2009). These findings indicate that astroglia can play proinflammatory as well as neuroprotective roles in MS.

14.2.5 Pathogenesis: Immune Mechanism of MS

T cell involvement in MS is supported by the following observations: (1) increase in frequency of autoreactive T cells exhibiting intermolecular and intramolecular epitope spreading against myelin proteins; (2) elevated levels of IFNγ, IL17, and downstream proinflammatory cytokines/chemokines in the CSF at relapse; (3) increase in frequency of Th1 cells secreting IFNγ and Th17 cells secreting IL17 at relapse; and (4) induction of relapse after administration of IFNγ, a representative Th1 cytokine (Durelli et al. 2009). Myelin antigen-specific Th1 and Th17

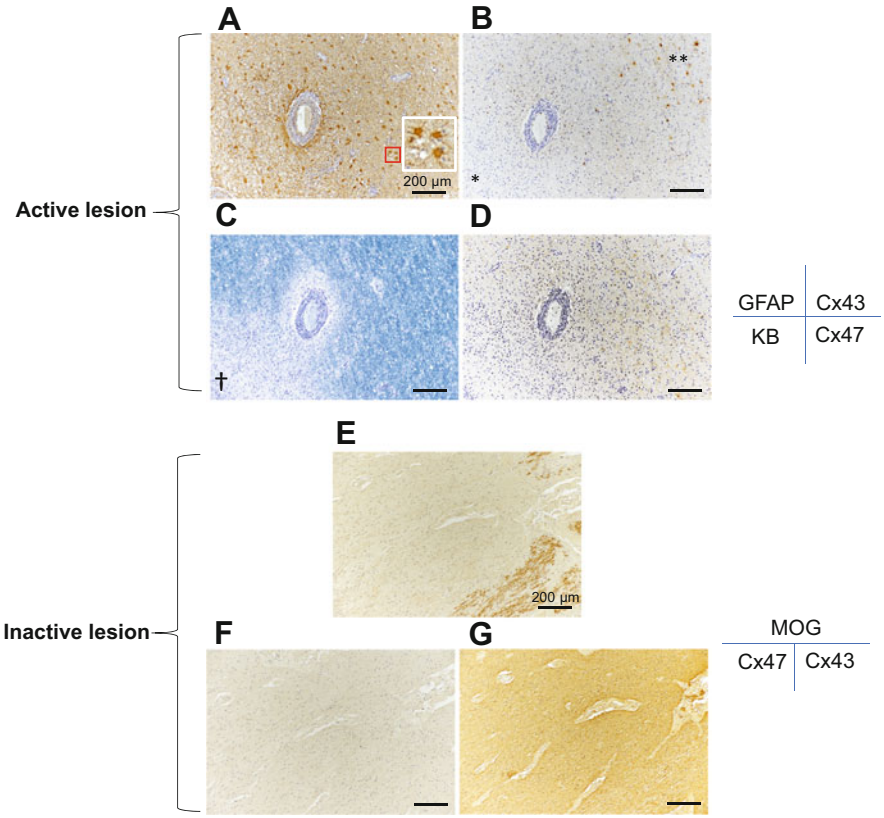

Fig. 14.3 Astrogliosis and expression of connexins at different stages of MS brain lesions. (**a**–**d**) Active lesion. (**e**–**g**) Inactive lesion. Antibodies used: (**a**) Anti-glial fibrillary acidic protein (GFAP), (**b**, **g**) anti-Cx43; (**d**, **f**) anti-Cx47; (**e**) anti-myelin oligodendrocyte glycoprotein (MOG). (**c**) Klüver-Barrera (KB) staining for myelin. All images were counterstained with hematoxylin. (**a**) Shows astrogliosis in an active lesion with atypical astrocytes (inset), covering both demyelinated and normal-appearing white matter. (**b**) Cx43 is lost in a demyelinated lesion [left lower corner (single asterisk)] compared with the right upper corner (double asterisk). (**c**) Demyelinated lesions are indicated by loss of KB staining around the vein and left lower corner (dagger). (**d**) Cx47 expression is also decreased in the demyelinated area. In the inactive (chronic) lesion, the demyelinated area without MOG staining (**e**) also shows loss of Cx47, while Cx43 expression is upregulated, indicating glial activation in the lesion. Bars: 200 μm

cells can transfer EAE into naive animals. Thus, it is hypothesized that naive T cells are initially sensitized by myelin antigens in peripheral lymph nodes, such as deep cervical and hilar lymph nodes, and differentiate into myelin antigen-specific Th1 or Th17 cells in MS. At acute relapse, these peripherally activated Th1 or Th17 cells express increased amounts of adhesion molecules that allow them to cross the BBB. Activated T cells can firmly attach to the surface of endothelial cells by interactions between α4β1 integrin expressed on T cells and vascular cell adhesion molecule (VCAM)-1 expressed on endothelial cells. The attached T cells then enter

transcellularly or paracellularly from the postcapillary venules (high endothelial venules) to reside in the perivascular space defined by the endothelial and glial basement membranes (Virchow-Robins space) (Ransohoff and Engelhardt 2012). Antigen presentation to autoreactive T cells by perivascular macrophages is essential for these T cells to penetrate further the CNS parenchyma beyond the perivascular glial border. This presentation promotes the secretion of matrix metalloproteinase-2 and -9, which destroy the basement membrane and induce destabilization of the astrocyte end-feet anchored to the parenchymal basement membrane (Ransohoff and Engelhardt 2012). Perivascular macrophages that have continuously repopulated from the peripheral blood flow can engulf CNS antigens in the perivascular space where myelin antigens are transported from the CNS parenchyma via the "glymphatic system" and the CSF flow pathway to the subarachnoid space. Upon entering the CNS parenchyma, T cells secrete proinflammatory cytokines/chemokines, which further recruit effector cells like macrophages, activated microglia, and neutrophils to destroy the parenchymal tissue. However, there is a report describing oligodendrocyte apoptosis without lymphocyte infiltration in autopsied cases with very early MS (Barnett and Prineas 2004). The issue of whether T cell invasion is the primary event or a secondary event to oligodendrocyte apoptosis and subsequent microglial activation has not been elucidated.

Although B cells are rare in the CNS parenchyma, they are present in the perivascular regions and leptomeningeal membrane during all stages (Magliozzi et al. 2007). Plasma cells are rare in the early stages of MS but become increasingly prominent in the CNS over time. As a result, the frequency of CSF OCBs also increases as the disease progresses (Meinl et al. 2006). The importance of B cells in MS is directly indicated by the fact that anti-CD20 monoclonal antibodies targeting B cells, such as rituximab, ocrelizumab, and ofatumumab, but not those targeting plasma cells are highly effective in MS (Hauser et al. 2008; Montalban et al. 2017; Sorensen et al. 2014). The number of B cells, but not the total antibody level, decreases in parallel with the decrease in relapse number. It is plausible that B cell–T cell interactions, such as antigen presentation and proinflammatory cytokine secretion by B cells, are crucial for MS mechanisms. Immunoglobulin and complement deposits are also seen in lesions from approximately 50% of autopsied MS patients, suggesting that antibody and complement-mediated myelin phagocytosis can be a major mechanism in established MS lesions (Breij et al. 2008; Stadelmann et al. 2011). The importance of antiglycolipid antibodies and recently described autoantibodies against KIR 4.1, an ATP-sensitive inward rectifying potassium channel expressed in astroglial end-feet and oligodendroglia (Srivastava et al. 2012), requires further confirmation in large-scale independent cohorts.

Nevertheless, acute relapse has only minor effects on disability progression (Confavreux et al. 2000). In both patients with relapse onset (SPMS) and those with insidious onset (PPMS), the progressive phase was shown to occur retrospectively at approximately 40 years of age and then proceed at a similar rate independent of the initial disease course (Kremenchutzky et al. 2006). During the progressive phase of MS, none of the recently developed DMTs, except for anti-CD20 monoclonal antibody ocrelizumab, are effective, even if they show high

efficacy for decreasing both annualized relapse rates and new MRI lesions. Therefore, the mechanism for chronic progression of disability may be different from the mechanism for acute relapse, which is closely related to BBB disruption by peripheral immune cells. The lack of noticeable peripheral immune cell-mediated inflammation, as shown by contrast-enhanced MRI and neuropathology, suggests that compartmentalized glial inflammation behind the BBB and neurodegeneration may play important roles in chronic progressive MS.

Recently, we found that iguratimod, a disease-modifying anti-rheumatic drug (DMARD), is effective for treating not only acute but also chronic EAE, a mouse model of SPMS. Although iguratimod is known to attenuate peripheral immune responses by suppressing T cell and macrophage activation, we revealed that it can also alleviate CNS inflammation in EAE by inhibiting inflammatory cytokine release by macrophages and activated microglia in the CNS parenchyma. In vitro, iguratimod also attenuates nuclear translocation of NF-kB p65 in both macrophages and microglia. These results indicate that iguratimod can be a new therapeutic option for the treatment of SPMS (Li et al. 2018).

Regarding the mechanisms of chronic progressive MS, the expression levels of connexin proteins that form gap junction channels between glial cells were shown to be altered in MS lesions in human postmortem samples (Markoullis et al. 2012; Masaki et al. 2013). In the CNS, astrocytes mainly express Cx30 and Cx43, while oligodendroglia express Cx32 and Cx47 (Rouach et al. 2002). These connexins form both homotypic gap junctions between the same cells and heterotypic gap junctions between different cells. Cx30 and Cx43 are upregulated in chronic MS lesions, while Cx47 is downregulated in both acute and chronic lesions (Masaki et al. 2013) (Fig. 14.3). We reported that Cx30 whole-body knockout mice showed milder signs of EAE without affecting peripheral immunity, suggesting that the altered CNS milieu induced by deficiency of Cx30 can cause deviation of microglial activation toward an anti-inflammatory phenotype (Fang et al. 2018). We also induced EAE in genetically modified mice with oligodendroglia-specific inducible ablation of Cx47, because Cx47 whole-body-deficient mice showed lethality, and found that the mice exhibited exacerbation of acute and chronic EAE with high relapse rates at the chronic phase (submitted for publication). These data collectively indicate the importance of glial connexins in regulating neuroinflammation upon receipt of demyelinating insults in EAE and MS.

14.3 Clinical Aspects

14.3.1 Clinical Course

The onset of MS is usually acute or subacute, although some patients exhibit an insidious onset. Sensory impairment, paresthesia, limb weakness, visual impairment, and double vision are common initial manifestations. According to its clinical course, MS is classified into RRMS and PPMS. RRMS occupies 80–90% of cases. MS patients who have only one symptomatic episode and do not fulfill the current

MS diagnostic criteria (Table 14.1) are diagnosed as a clinically isolated syndrome (CIS). About half of these RRMS patients experience gradually worsening disability without relapse for 15–20 years after onset. This is known as SPMS (Fig. 14.4) (Tremlett et al. 2008). At this stage, patients suffer from progressive deterioration of neural function that is not related to relapse. It is estimated that the rate of conversion from RRMS to SPMS has decreased to about 20% in the era of DMTs (Scalfari et al. 2014). Approximately 10–20% of MS patients follow a progressive course from onset, called PPMS. Progressive MS (SPMS and PPMS) preferentially involves the distal parts of the pyramidal tract and the cerebellum, thereby leading to a sustained deterioration of spastic paraparesis and/or cerebellar ataxia.

14.3.2 Clinical Symptoms and Signs

The posterior column and spinothalamic tracts of the spinal cord are frequently involved, presenting as decreased position sense and vibratory sense, hypesthesia, hypalgesia, thermal hypesthesia, and paresthesia below a horizontal line on the body (sensory level). Posterior column lesions induce Romberg's sign, which manifests as unstable standing with the eyes closed. Cervical posterior column lesions cause Lhermitte's sign, which manifests as an electric shock-like sensation on the back and lower limbs on neck flexion. An unpleasant girdle sensation of the torso and limbs sometimes afflicts MS patients. Cerebral hemispheric or thalamic demyelinating lesions often cause sensory impairment of the hemicorpus including the face.

For pyramidal tract involvement, spastic hemiparesis, paraparesis, and quadriparesis are frequent manifestations, accompanied by hyperreflexia, pathological reflexes, and ankle and patellar clonus. MS frequently presents visual impairment, blurred vision, reduced color perception, and field defects. In fundus examinations, normal (retrobulbar neuritis) or hyperemic (papillitis), disc swelling is seen, followed by temporal pallor due to papillomacular bundle damage or pallor (optic atrophy) throughout the optic disk. Pain on eye movements is often associated with optic neuritis. Limb and truncal ataxia, gaze-evoked nystagmus, and scanning speech are common symptoms of cerebellar demyelinating lesions. Involvement of the brainstem is often accompanied by double vision, internuclear ophthalmoplegia, trigeminal neuralgia, facial muscle weakness, facial myokymia, taste impairment, vertigo, tinnitus, hearing impairment, and dysarthria.

Bladder dysfunction is common and hinders daily activities. Detrusor muscle hyperreflexia causes urgency, nocturia, and uncontrollable bladder emptying. Detrusor sphincter dyssynergia appears as difficulty initiating urination, discontinuation of urination, urinary retention, and overflow incontinence. Most patients show a combination of both types of bladder dysfunction. Both can cause complications including recurrent urinary tract infections, skin ulceration and infections, nephrolithiasis, and rarely renal failure. While bowel dysfunction often manifests as constipation, impetuous urgency, or incontinence may also occur. Sexual dysfunction, loss of libido, genital sensory disorder, decreased vaginal lubrication, and sexual dysfunction such as male impotence are also frequent. Cerebral damage,

Table 14.1 The 2017 McDonald criteria for MS diagnosis

For patients with an attack at the onset		
Number of attacks	Number of lesions with objective clinical evidence	Additional data needed for diagnosis of MS
≥2 clinical attacks	≥2	None[a]
≥2 clinical attacks	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location)	None[a]
≥2 clinical attacks	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site *OR* MRI[b]
1 clinical attack	≥2	Dissemination in time demonstrated by an additional clinical attack *OR* by MRI or demonstration of CSF-specific OCBs[c]
1 clinical attack	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI *AND* Dissemination in time demonstrated by an additional clinical attack or by MRI OR demonstration of CSF-specific OCBs
For patients with insidious onset		
Clinical course	Additional data needed for a diagnosis of progressive MS	
1 year of disability progression	• One or more T2-hyperintense lesions characteristic of MS in one or more of the following brain regions: Periventricular, cortical or juxtacortical, or infratentorial • Two or more T2-hyperintense lesion in the spinal cord • Presence of CSF-specific oligoclonal bands	

MRI magnetic resonance imaging, *CSF* cerebrospinal fluid, *OCB* oligoclonal band

[a]No additional tests are required to demonstrate dissemination in space and time. However, unless MRI is not possible, brain MRI should be obtained for all patients in whom the diagnosis of MS is being considered. In addition, spinal cord MRI or CSF examination should be considered in patients with insufficient clinical and MRI evidence supporting MS, with a presentation other than a typical clinically isolated syndrome, or with atypical features. If imaging or other tests (e.g., CSF) are undertaken and are negative, caution is required before making a diagnosis of MS, and alternative diagnoses should be considered

[b]Dissemination in space can be demonstrated by one or more T2-hyperintense lesions that are characteristic of MS in two or more of four areas of the CNS: periventricular, cortical or juxtacortical, and infratentorial brain regions, and the spinal cord

[c]Dissemination in time can be demonstrated by the simultaneous presence of gadolinium-enhanced and non-enhanced lesions at any time or by a new T2-hyperintense or gadolinium-enhanced lesion on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI

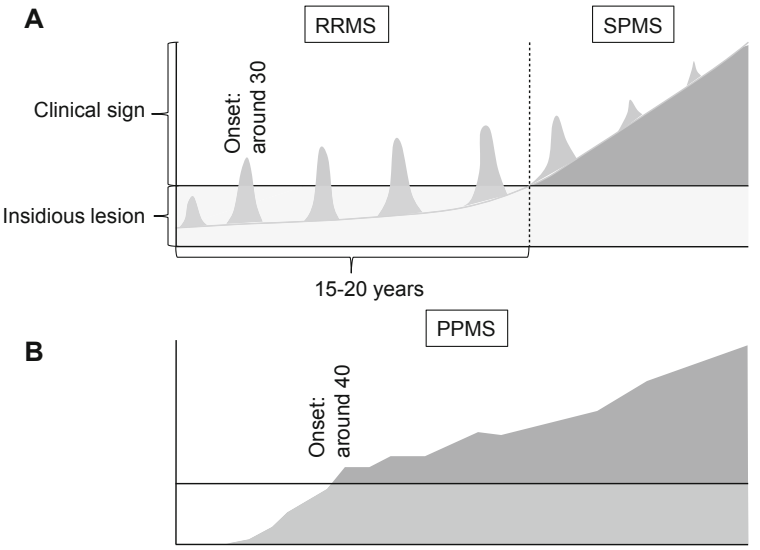

Fig. 14.4 Representative clinical course of MS. (**a**) RRMS patients may harbor latent lesions for several years before clinical onset, which usually occurs around 30 years of age. RRMS patients experience relapse and remission of clinical symptoms at the beginning. At 15–20 years after the clinical onset of MS, clinical disability accumulates irrespective of relapse, resulting in secondary progressive MS (SPMS). (**b**) Primary progressive MS (PPMS) usually shows clinical onset around 40 years of age, and the disability progression in PPMS is faster than that in relapse-onset MS

including damage to the hippocampus, causes cognitive dysfunction such as attention failure, performance impairment, slow information processing, and memory impairment in up to 30% to 50% of MS patients. Mood disorders such as depression and euphoria are found in more than half of MS patients.

Fatigue is very common in MS and is often disabling. MS patients frequently show heat sensitivity. As the body temperature rises, existing symptoms, such as visual blur and limb weakness, are exacerbated (Uhthoff's sign). The paroxysmal symptoms seen in MS patients, other than trigeminal neuralgia, are glossopharyngeal neuralgia, hemifacial spasm, and painful tonic spasm (tonic spasm of one or two limb muscles about 1 min in duration without consciousness disturbance due to spinal cord lesions). Symptoms and signs derived from the involvement of the gray matter, such as severe cortical dementia, parkinsonism, severe muscle atrophy, and epileptic seizures, are rare in MS.

14.3.3 Laboratory Findings

14.3.3.1 Magnetic Resonance Imaging Findings

Demyelinating lesions in MS appear as high signal intensity on T2-weighted, fluid-attenuated inversion recovery (FLAIR), and proton density weighted images, and as

low signal intensity on T1-weighted images. Clinical relapse is often preceded by the emergence of gadolinium-enhanced lesions on T1-weighted images, indicating a breach of the BBB. Recent 7-T MRI studies clearly showed the presence of blood vessels at the center of MS lesions, consistent with the pathological finding that perivascular lymphocytic infiltration is common in active MS lesions (Mistry et al. 2013). Therefore, it is likely that clinical relapse is caused by peripheral blood-borne inflammation around the blood vessels. MS lesions in the brain are frequently oriented perpendicular to the lateral ventricular surface (ovoid lesion or Dawson's fingers) (Fig. 14.2b). This configuration of typical MS lesions is explained by the fact that postcapillary venules, from which T cells migrate into the CNS parenchyma, radiate vertically from the lateral ventricles. As gadolinium enhancement of acute lesions disappears within 2–3 months, the coexistence of gadolinium-enhanced (new) and -unenhanced (old) lesions on the same MRI scans indicates dissemination in time (relapsing-remitting course) (Fig. 14.2) (Thompson et al. 2018a). While MS lesions rarely expand to more than 3 cm in diameter, such large lesions resembling brain tumors are called tumefactive demyelinating lesions or tumefactive MS (Marburg mutant MS). A low signal intensity region (T1 black hole) on T1-weighted images reflects demyelination and edema in the acute phase and irreversible axonal loss in the chronic phase. Therefore, the accumulation of chronic T1 black holes is related to disability progression. Most brain MRI lesions are asymptomatic. Therefore, MS-like lesions are coincidentally discovered in asymptomatic individuals, and such cases are called radiologically isolated symptoms (RIS) (Okuda et al. 2009a).

The recent development and application of double inversion recovery imaging allow cortical lesions to be detected much more frequently in MS, consistent with the demonstration of abundant cortical demyelinating lesions in autopsied MS brains (Calabrese et al. 2010a) (Fig. 14.2g, h). The presence of such cortical lesions is associated with disability progression and poor prognosis. As the disease progresses, brain volume is continuously lost (>0.4% per year), resulting in brain atrophy and enlargement of ventricles (De Stefano et al. 2016).

MS lesions in the spinal cord can also be detected using MRI. A typical spinal cord MS lesion involves the peripheral white matter of the spinal cord and occupies less than half of the transverse spinal cord area. Although the underlying mechanism is undetermined, MS preferentially affects the posterior column of the cervical spinal cord. The length of an MS lesion in the sagittal plane is typically less than two vertebral segments. Extensive spinal cord lesions in the longitudinal direction spanning more than three vertebral segments indicate neuromyelitis optica spectrum disorders or other inflammatory disorders.

14.3.3.2 Other Laboratory Findings

Evoked potentials (EPs) are useful for detecting demyelinating lesions in certain pathways of the CNS. EPs include visual evoked potentials (VEPs), somatosensory evoked potentials (SEPs), brainstem auditory evoked potentials (BAEPS), and motor evoked potentials (MEPs), which reflect visual, posterior column sensory, auditory, and pyramidal pathways, respectively. A marked delay in the latency of a specific EP

without a marked decrease in its amplitude is suggestive of demyelination in the relevant pathway. EP abnormalities are relatively supportive findings to underscore clinical symptoms and other neuroimaging data. Exceptionally, VEPs are useful for detecting asymptomatic optic tract lesions.

At acute relapse, the CSF shows mild mononuclear pleocytosis (5–50 cells/μl) and normal or mild elevation of protein levels (40–100 mg/dl). As the disease progresses, more plasma cells and B cells infiltrate intrathecally and CSF IgG levels increase. The IgG index, which expresses the ratio of IgG to albumin in the CSF divided by the same ratio in the serum, indicates the intrathecal production of IgG. The cutoff value for the IgG index is 0.73. Isoelectric focusing of CSF can detect OCBs in the gamma-globulin region in more than 90% of Caucasian MS patients and about 60% of Asian MS patients (Ebers 2008). OCBs may not be present at the onset but may appear later and their number may increase with time. MBP levels are elevated at acute relapse, reflecting myelin destruction. However, increased MBP levels are not specific for MS. For example, acute destruction of CNS tissue as a result of a stroke can also increase the MBP level in the CSF.

Peripheral blood tests are necessary to exclude other diseases through autoantibody testing of peripheral blood. In particular, anti-aquaporin 4 (AQP4) antibodies for neuromyelitis optica spectrum disorders and anti-myelin oligodendrocyte glycoprotein (MOG) antibodies for anti-MOG antibody disease should be carefully excluded. Furthermore, collagen vascular disease mimicking MS should be distinguished by autoantibody tests. Recently, an ultrasensitive immunoassay for small molecules, designated the single-molecule array (SIMOA) assay, has enabled sensitive and specific measurements of serum GFAP and neurofilament light chain (NfL) levels. For example, serum NfL levels have a strong positive correlation with CSF NfL levels and show positive correlations with disease severity and activity (Disanto et al. 2017; Chitnis et al. 2018). Thus, the serum levels of these molecules measured by the SIMOA assay may be new surrogate markers for MS.

14.4 Diagnosis

The diagnosis of MS is still based on both spatial and temporal evidence of multiplicity, and exclusion of other diseases. According to the latest diagnostic criteria (2017 McDonald criteria; Table 14.1) (Lassmann et al. 1998), *cases with* $\geq$ *2 clinical attacks and* $\geq$ *2 lesions* shown by neurological examination can be diagnosed as MS when other diseases are excluded. *Cases with* $\geq$ *2 clinical attacks but 1 lesion* require an additional attack that implicates a different CNS site or MRI evidence of dissemination in space (presence of typical MS lesions in $\geq$2 regions among four CNS sites, including periventricular, cortical or subcortical, infratentorial, and spinal cord lesions). Cases with only one clinical attack usually require waiting for an additional clinical attack; however, to achieve MS diagnosis as early as possible such that DMTs can be administered, the 2017 McDonald criteria allow the second episode to be substituted by MRI evidence of dissemination in time or even by the presence of CSF-specific OCBs. *Cases with 1 clinical attack but* $\geq$ *2*

lesions require dissemination in time shown by an additional clinical attack or by MRI (emergence of new T2 lesion or gadolinium-enhanced lesion, or even coexistence of gadolinium-enhanced and -unenhanced lesions) *or* CSF OCBs. *Cases with one clinical attack and one lesion* require dissemination in space shown by an additional clinical attack implicating a different CNS site or by MRI *and* dissemination in time shown by an additional clinical attack or by MRI *or* CSF OCBs.

When applying the new diagnostic criteria, various conditions that mimic MS (Table 14.2) must first be carefully excluded by disease-specific tests. As described above, anti-AQP4 antibodies and anti-MOG antibodies should be examined in atypical MS patients, characterized by longitudinally extensive spinal cord lesions, bilateral optic neuritis, horizontal visual field defects, refractory hiccups, prominent CSF pleocytosis, and neutrophilia, especially in Asians. In patients with a single clinical attack, acute disseminated encephalomyelitis (ADEM) should be carefully excluded because not all lesions always show uniform enhancement. The latest criteria should not be used for suspected pediatric cases of ADEM. Other differential diagnoses include neuromyelitis optica spectrum disorders, anti-MOG antibody disease, cerebral small vessel diseases, spinal cord infarction, CNS lymphoma, collagen-vascular diseases (systemic lupus erythematosus, Sjögren's syndrome, others), neurosarcoidosis, neuro-Behçet disease, cerebral abscess, HTLV-1-associated myelopathy, progressive multifocal leukoencephalopathy, and hereditary leukodystrophies.

14.5 Prognosis

Because there is no curative treatment, once a patient suffers from MS, the disease persists throughout life. The average life expectancy is not remarkably shortened and maybe about 10 years shorter than the average life expectancy of unaffected people (Lunde et al. 2017). In general, patients with RRMS require assisted gait by 20–25 years after onset and become wheelchair-bound by about 35 years after onset (Confavreux and Vukusic 2006). PPMS shows a more rapid progression than relapse-onset MS. Although MS is essentially a progressive disease, some long-term follow-up studies reported that 22% of cases were non-progressive after 40 years and 14% were non-progressive after 50 years (Skoog et al. 2012). The Expanded Disability Status Scale (EDSS) is the most popular clinical rating scale (Kurtzke 1983). An MS patient with an EDSS score of 2–3 or less after 10 years can be regarded as having a benign MS course. A benign course may be predicted by female sex, young age at onset, no motor symptoms at onset, less than 2 relapses in the first few years of the disease, no OCBs, minimal physical disability at 5 years after onset, and low brain MRI lesion loads at 5 years after onset (Thompson et al. 2018b). By contrast, a poor prognosis is suggested by male sex, age at onset, presence of motor symptoms at onset, presence of cerebellar ataxia, presence of sphincter disturbance, short relapse intervals, frequent relapses in the early course of the disease, residual symptoms from the beginning, involvement of multifunctional systems, high disability at 5 years after onset, progressive course, high brain MRI

Table 14.2 Major differential diagnoses of MS

Differential diagnosis	Key features for differentiating each disease from MS
Acute disseminated encephalomyelitis (ADEM)	Monophasic (multiphasic ADEM has encephalopathy), encephalopathy, bilateral white matter lesions (could be asymmetric), deep gray matter involvement, uniform gadolinium enhancement of all lesions (the same disease stage)
Anti-myelin oligodendrocyte glycoprotein (MOG) antibody disease	Anti-myelin oligodendrocyte glycoprotein antibodies, longitudinally extensive spinal cord lesions, sacral spinal cord lesions, and focal cortical encephalitis
Atopic myelitis	Sensory disturbance and sometimes pyramidal symptoms with cervical cord lesions in patients with atopic diathesis
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)	Migraine, stroke, dementia, depression, parkinsonism, lacunar infarct, microbleeds, temporal pole and external capsule lesions, widespread confluent white matter lesions, *NOTCH3* gene mutation, and absence of CSF OCBs
Cerebral small vessel disease	Stroke, cognitive impairment, focal neurological signs, lacunar infarct, microbleeds, sparing U fibers, diabetes mellitus, and other vascular risk factors, absence of CSF OCBs
Chronic lymphocytic inflammation with pontine periventricular enhancement responsive to steroids (CLIPPERS)	Brainstem and cerebellar symptoms, multiple punctate gadolinium-enhanced lesions in the pons and cerebellum
CNS lymphoma	Headache, raised intracranial pressure, cognitive and consciousness impairment, psychomotor slowing, mass effect, dense enhancement by gadolinium, lymph node swelling, skin rash, ^{18}F-fluorodeoxyglucose (FDG) PET
Combined central and peripheral demyelination (CCPD)	MS-like lesions in the CNS with CIDP-like peripheral polyneuropathy sometimes coincided with serum anti-NF155 antibody
Congenital leukodystrophy (adrenoleukodystrophy, metachromatic leukodystrophy, hereditary diffuse leukoencephalopathy with spheroid)	Chronic progressive course, cognitive impairment, peripheral nerve involvement, widespread confluent white matter lesions, CSF protein increase without pleocytosis, absence of CSF OCBs, relevant gene mutation
Connective tissue diseases (e.g., Sjögren syndrome, systemic lupus erythematosus)	Neuropsychiatric symptoms, seizure, ischemic stroke, serum autoantibodies (including anti-nuclear antibody, anti-SS-A/B antibody), systemic organ manifestations, absent CSF OCBs, brain infarct and hemorrhage, and peripheral neuropathy

(continued)

Table 14.2 (continued)

Differential diagnosis	Key features for differentiating each disease from MS
Facial onset sensory and motor neuronopathy (FOSMN)	Facial onset sensory abnormalities spreading to the scalp, upper trunk and extremities, and is followed by lower motor neuron deficits
Hashimoto's encephalopathy	Loss of consciousness, seizure, dementia, involuntary movement, ataxia, abnormal brain MRI, and anti-N terminal alpha-enolase antibody
HTLV-1-associated myelopathy (HAM)/ tropical spastic paraparesis (TSP)	Chronic progressive spastic paraparesis, anti-HTLV-1 antibodies in serum and CSF, thoracic spinal cord atrophy on MRI
Neuro-Behçet's disease	Brainstem symptoms, cognitive impairment, meningoencephalitis, basal ganglia lesions, predominant brainstem lesions, oral and genital ulcers, uveitis, HLA-B51, CSF IL6, CSF pleocytosis, cerebral venous sinus thrombosis
Neuro-sweet disease	Meningoencephalitis, headache, consciousness disturbance, seizure, cognitive impairment, painful erythematous plaques, dermal infiltration of neutrophils on skin biopsy, HLA-B54, HLA-Cw1, CSF pleocytosis, and CSF IL6
Neuromyelitis optica spectrum disorders (NMOSD)	Anti-aquaporin 4 antibodies, longitudinally extensive spinal cord lesions, optic chiasma lesions, area postrema lesions, bilateral hypothalamic lesions, cloud-like enhancement, bright spotty lesions in the spinal cord, absence of MS-like brain lesions
Neurosarcoidosis	Cranial nerve involvement, headache, seizure, meningeal enhancement, raised intracranial pressure, peripheral neuropathy, serum and CSF angiotensin-converting enzyme and lysozyme, bilateral hilar lymphadenopathy, and CD4/CD8 ratio in bronchoalveolar lavage
Primary and secondary CNS vasculitis	Headache, seizure, confusion, stroke-like episodes, microbleeds, intracranial hemorrhage, ischemic lesions, vessel stenosis on angiography, and anti-neutrophil cytoplasmic antibodies
Susac's syndrome	Headache, encephalopathy, visual loss, sensorineural hearing loss, snowball lesions in the corpus callosum, leptomeningeal enhancement

CSF cerebrospinal fluid, *HTLV-1* human T cell lymphotropic virus type-1, *MRI* magnetic resonance imaging, *OCBs* oligoclonal IgG bands, *PET* positron emission tomography

lesion load at 5 years after onset, presence of brain atrophy and cortical lesions, and presence of spinal cord atrophy (Thompson et al. 2018b). Relapse rates decrease during pregnancy but increase in the puerperium period (within 3 months after delivery) (Confavreux et al. 1998). However, as a whole, the course of the disease is not affected by pregnancy (Poser and Poser 1983).

14.6 Treatment

Therapy for MS has three purposes (1) to decrease the severity of acute relapse and accelerate recovery from acute relapse, (2) to reduce relapse frequency and prevent disability progression with DMTs, and (3) to alleviate residual symptoms.

14.6.1 Treatment of Acute Relapse

Acute relapse that limits the activities of daily living is treated with corticosteroids. Generally, intravenous high-dose (1000 mg/day) methylprednisolone (IVMP) for 3 consecutive days is administered. Short-term oral corticosteroids (about 1 mg/kg/day) with gradual tapering usually follow IVMP. However, because corticosteroids are ineffective for preventing relapse and disability progression, oral corticosteroids should not be maintained for a long time. If recovery from acute relapse is inadequate, IVMP may be repeated once or twice. In the case of corticosteroid-resistant relapse, especially when the patient has a large demyelinating lesion, plasmapheresis may be effective.

14.6.2 Prevention of Relapse and Disability Progression

The different DMTs approved for RRMS can effectively reduce relapses, but primarily target the peripheral immune system and have little benefit for the chronic progression in SPMS and PPMS (Table 14.3) (Ontaneda et al. 2015).

14.6.2.1 DMTs for RRMS

The first-choice DMTs for RRMS include interferon-beta (IFNβ)-1a and -1b, glatiramer acetate (GA), and dimethyl fumarate (DMF) (Rae-Grant et al. 2018). IFNβ and GA have similar efficacy: approximately 30% reduction in relapse rate and 50% reduction in new expanding lesions on MRI (Durelli et al. 2002; Group 1993; Jacobs et al. 1996; Johnson et al. 1995; Paty and Li 1993). However, approximately 30% of RRMS patients are refractory to these injectable drugs. These drugs correct the Th1/Th17 shift, suppress antigen presentation and T cell proliferation, and restore immune regulatory function. Both drugs have been used for a long time in RRMS without serious adverse events other than rare liver toxicity. Both IFNβ and GA frequently show injection site reactions, while IFNβ is generally associated with influenza-like symptoms at the time of injection. Oral DMF reduces the relapse rate

Table 14.3 Widely used disease-modifying therapies for RRMS

Generic name	Route, dose and schedule	Efficacy on relapse rate	Side effects (rare but serious)	Refs.
Interferon β-1b	250 μg, SC, every other day	−34%	Influenza-like symptoms, injection site reactions, increased liver enzymes, (liver toxicity)	Group (1993)
Interferon β-1a	30 μg, IM, once a week	−32%	Influenza-like symptoms, injection site reactions, increased liver enzymes, (liver toxicity)	Jacobs et al. (1996)
Glatiramer acetate	20 mg, SC, every day	−29%	Injection site reactions, post-injection general reaction, lipoatrophy	Johnson et al. (1995)
Dimethyl fumarate	240 mg, PO, twice a day	−51%	Flushing, diarrhea, abdominal pain, lymphopenia, (PML)	Gold et al. (2012) Fox et al. (2012)
Fingolimod	0.5 mg, PO, once a day	−52%	Bradycardia and heart conduction block at first dose, lymphopenia, increased liver enzymes, (macular edema, generalized herpes zoster infection, herpes simplex encephalitis, PML)	Kappos et al. (2010) Cohen et al. (2016)
Natalizumab	300 mg, IV, once every 4 weeks	−68%	Hypersensitivity reactions, (PML)	Polman et al. (2006)
Teriflunomide	7 or 14 g/tab, once/daily PO	−31%	Hepatotoxicity Risk of teratogenicity	O'Connor et al. (2011)
Alemtuzumab	One IV infusion (12 mg) a day on 5 consecutive days + one IV infusion a day on 3 consecutive days	−70%	Thyroid disorders Idiopathic thrombocytopenic purpura (ITP)	Investigators et al. (2008)

SC subcutaneous, *IM* intramuscular, *IV* intravenous, *PML* progressive multifocal leukoencephalopathy, *PO* per oral

by 50% and new and expanding MRI lesions by up to 80% (Fox et al. 2012; Gold et al. 2012). DMF reduces proinflammatory cytokine production, corrects the Th1/Th17 shift, suppresses T cell infiltration into the CNS via activation of the hydroxycarboxylic acid receptor 2 (HCAR2) pathway, and activates the nuclear factor-like (erythrocyte-derived 2)-2 (Nrf-2) pathway showing an antioxidant action. DMF frequently causes gastrointestinal symptoms such as hot flushes, diarrhea, and abdominal pain, especially within 1 month of initiation of the medication. DMF occasionally causes severe lymphopenia (<500 /μl). Persistent lymphopenia is a risk factor for the rare, but serious, complication of progressive multifocal leukoencephalopathy (PML) by John-Cunningham virus (JCV) (incidence of about 1:50,000) (Berger 2017). For MS patients with mild disease activity, IFNβ, GA, or DMF is a reasonable choice. Patients with CIS or RRMS who have not relapsed in the past 2 years and have new lesions that are not active on recent MRI may be followed up by serial MRI at least annually for the first 5 years, rather than initiating DMTs (Rae-Grant et al. 2018). If an MS patient experiences one or more relapses, more than one new MRI lesion, or increased disability in a 1-year period during the use of first-line DMTs, they are considered non-responsive. In this case, direct switching to another first-line DMT is one option.

If the first-line DMTs are ineffective or the disease activity is high, second-line DMTs are considered. Commonly used second- and third-line DMTs include fingolimod and natalizumab, while teriflunomide, cladribine, and alemtuzumab are less commonly used (Giovannoni et al. 2010; Investigators et al. 2008; O'Connor et al. 2011; Thompson et al. 2018b). Fingolimod, an oral DMT for RRMS, is an antagonist of sphingosine-1-phosphate receptor 1 ($S1P_1$) and has a unique mechanism of action. It downregulates $S1P_1$, which is essential for lymphocyte egress from lymph nodes. Thus, fingolimod captures central memory T cells that home to lymph nodes, thereby preventing autoreactive T cells from circulating in the bloodstream. Fingolimod effectively reduces the relapse rate by 60% and newly expanding brain MRI lesions by 80% (Kappos et al. 2010). The initial administration of fingolimod often causes bradycardia, and occasionally causes conduction block through $S1P_1$. Lymphopenia is also common and a drug holiday period is recommended if the lymphocyte count is reduced to <200/μl. Macular edema and hepatic dysfunction are occasionally encountered in patients taking fingolimod. Fingolimod increases the risk of infection and patients may develop a generalized varicella-zoster infection, herpes encephalitis, or PML (1:12,000) (Berger 2017). Suppression of brain atrophy progression has been shown for fingolimod, but not for first-line DMTs. Because fingolimod only traps autoreactive T cells within lymph nodes, and does not deplete them, drug withdrawal may induce a rebound phenomenon (flare-up of disease activity).

Natalizumab, an anti-α4β1 integrin antibody, effectively blocks the interaction between VCAM-1 on vascular endothelial cells and very late antigen 4 (VLA4) (consisting of α4β1 integrin) on lymphocytes. As a result, T cell adhesion on vessel walls is suppressed and T cell migration into the CNS tissue is efficiently inhibited (Coisne et al. 2009). Natalizumab reduces the relapse rate by 70%, and suppresses new and expanding brain MRI lesions by 90% (Polman et al. 2006). The effect of

natalizumab significantly suppress relapse supports the importance of T cell inflammation in the CNS at the time of relapse. However, because natalizumab almost completely blocks T cell migration to the CNS tissue, surveillance of the CNS by T cells is significantly compromised, thus allowing the occurrence of JCV-mediated PML (4.19/1000) (Berger 2017). Risk factors for PML under natalizumab treatment are long-term use (>2 years), use of previous immunosuppressants, and high anti-JCV antibody index (>1.5) (Plavina et al. 2014). When natalizumab-PML emerges, rapid withdrawal of natalizumab by plasmapheresis may cause massive infiltration of lymphocytes into the CNS (immune reconstitution inflammatory syndrome; IRIS), resulting in severe tissue destruction and residual disability. If anti-JCV antibodies are negative and the disease activity is high, natalizumab is suitable.

14.6.2.2 DMTs for Progressive MS

For SPMS patients, two novel $S1P_1$ antagonists, siponimod and ozanimod, were recently found to be effective in preventing disability progression (Cohen et al. 2016; Kappos et al. 2018). In addition to their inhibitory effect on lymphocyte egress from secondary lymphoid organs, these drugs may act directly on glial cells expressing $S1P_1$ such as microglia and astrocytes. For PPMS, ocrelizumab (anti-CD20 humanized monoclonal antibody) significantly decreases disability progression (24% reduction in clinical disability progression) (Montalban et al. 2018). Ocrelizumab also reduces the annual relapse rate by approximately 50% and prevents new MRI lesions by 95% in RRMS. Ocrelizumab depletes circulating B cells but does not deplete plasma cells that do not express CD20. Therefore, interruption of B cell–T cell interactions, including antigen presentation and suppression of proinflammatory cytokine secretion from B cells, is presumed to be a mechanism of action for ocrelizumab, rather than a decrease in autoantibody production.

14.6.3 Symptomatic Therapy

Spasticity is best managed by a combination of anticonvulsant administration and physical therapy. Although some spasticity can often be useful for patient standing or walking, excessive intake of antispasmogenic drugs may result in worsening of motor capacity. Baclofen, tizanidine, and gabapentin are used as first-line drugs. Dantrolene is a second-line drug, especially for outpatients with spasticity. Benzodiazepines are particularly useful as a nighttime dose. Repeated local injection of botulinum toxin may help to relieve localized spasticity. An intrathecal baclofen pump can be tried in oral anticonvulsant-resistant cases.

Prolonged anticholinergic drugs or tricyclic antidepressants are useful for urinary urgency in the absence of excessive urinary retention. Nasal desmopressin spray is effective against nocturia. Alpha-adrenergic blockers relieve difficult urination or urinary retention. Intermittent self-catheterization or indwelling or suprapubic catheter can be considered when urinary retention reaches a residual urine volume of >100–150 ml after urination. In some cases, intravesical botulinum toxin injection

or sacral electrical stimulation may be useful. Laxatives are used for constipation, but fecal incontinence is difficult to treat. For impotence, a phosphodiesterase type 5 inhibitor, penile suppository, or prostaglandin injection is useful for men.

Commonly seen pain symptoms, such as trigeminal and glossopharyngeal neuralgia and other neuropathic pain, can be treated with anticonvulsants including carbamazepine, pregabalin, gabapentin, and topiramate. Painful tonic spasms are effectively treated with low doses of carbamazepine. It is difficult to treat girdle sensation and sustained numbness. Tremor and ataxia are also difficult to treat and drugs for essential tremor such as beta-adrenergic blockers and clonazepam are of limited efficacy. Use of wrist weights may reduce action tremor in the hand or arm in some patients. Amantadine, modafinil, and fampridine help fatigue. Dalfampridine, a sustained-release form of 4-aminopyridine, may improve power, endurance, and ambulation in some patients. Depression is usually treated with antidepressants and counseling. Cognitive disorders are very difficult to treat. Cognitive rehabilitation and occupational therapy are also worth undertaking.

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References

Ainiding G, Kawano Y, Sato S, Isobe N, Matsushita T, Yoshimura S, Yonekawa T, Yamasaki R, Murai H, Kira J-i et al (2014) Interleukin 2 receptor alpha chain gene polymorphisms and risks of multiple sclerosis and neuromyelitis optica in southern Japanese. J Neurol Sci 337:147–150

Allaman I, Belanger M, Magistretti PJ (2011) Astrocyte-neuron metabolic relationships: for better and for worse. Trends Neurosci 34:76–87

Antony JM, van Marle G, Opii W, Butterfield DA, Mallet F, Yong VW, Wallace JL, Deacon RM, Warren K, Power C (2004) Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. Nat Neurosci 7:1088–1095

Ascherio A, Munger KL (2007) Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. Ann Neurol 61:504–513

Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, Friese M, Schroder R, Deckert M, Schmidt S et al (2000) Clonal expansions of CD8(+) T cells dominate the T cell

infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J Exp Med 192:393–404
Barnett MH, Prineas JW (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 55:458–468
Barnett MH, Williams DB, Day S, Macaskill P, McLeod JG (2003) Progressive increase in incidence and prevalence of multiple sclerosis in Newcastle, Australia: a 35-year study. J Neurol Sci 213:1–6
Benarroch EE (2009) Oligodendrocytes: susceptibility to injury and involvement in neurologic disease. Neurology 72:1779–1785
Berger JR (2017) Classifying PML risk with disease modifying therapies. Mult Scler Relat Disord 12:59–63
Bo L, Vedeler CA, Nyland H, Trapp BD, Mork SJ (2003) Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. Mult Scler (Houndmills, Basingstoke, England) 9:323–331
Breij ECW, Brink BP, Veerhuis R, van den Berg C, Vloet R, Yan R, Dijkstra CD, van der Valk P, Bo L (2008) Homogeneity of active demyelinating lesions in established multiple sclerosis. Ann Neurol 63:16–25
Browne P, Chandraratna D, Angood C, Tremlett H, Baker C, Taylor BV, Thompson AJ (2014) Atlas of multiple sclerosis 2013: a growing global problem with widespread inequity. Neurology 83:1022–1024
Calabrese M, Battaglini M, Giorgio A, Atzori M, Bernardi V, Mattisi I, Gallo P, De Stefano N (2010a) Imaging distribution and frequency of cortical lesions in patients with multiple sclerosis. Neurology 75:1234–1240
Calabrese M, Filippi M, Gallo P (2010b) Cortical lesions in multiple sclerosis. Nat Rev Neurol 6:438–444
Chang A, Tourtellotte WW, Rudick R, Trapp BD (2002) Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 346:165–173
Charles P, Reynolds R, Seilhean D, Rougon G, Aigrot MS, Niezgoda A, Zalc B, Lubetzki C (2002) Re-expression of PSA-NCAM by demyelinated axons: an inhibitor of remyelination in multiple sclerosis? Brain 125:1972–1979
Chitnis T, Gonzalez C, Healy BC, Saxena S, Rosso M, Barro C, Michalak Z, Paul A, Kivisakk P, Diaz-Cruz C et al (2018) Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis. Ann Clin Transl Neurol 5:1478–1491
Cohen JA, Arnold DL, Comi G, Bar-Or A, Gujrathi S, Hartung JP, Cravets M, Olson A, Frohna PA, Selmaj KW, Group RS (2016) Safety and efficacy of the selective sphingosine 1-phosphate receptor modulator ozanimod in relapsing multiple sclerosis (RADIANCE): a randomised, placebo-controlled, phase 2 trial. Lancet Neurol 15:373–381
Coisne C, Mao W, Engelhardt B (2009) Cutting edge: Natalizumab blocks adhesion but not initial contact of human T cells to the blood-brain barrier in vivo in an animal model of multiple sclerosis. J Immunol 182:5909–5913
Confavreux C, Vukusic S (2006) Age at disability milestones in multiple sclerosis. Brain 129:595–605
Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T (1998) Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. N Engl J Med 339:285–291
Confavreux C, Vukusic S, Moreau T, Adeleine P (2000) Relapses and progression of disability in multiple sclerosis. N Engl J Med 343:1430–1438
De Stefano N, Stromillo ML, Giorgio A, Bartolozzi ML, Battaglini M, Baldini M, Portaccio E, Amato MP, Sormani MP (2016) Establishing pathological cut-offs of brain atrophy rates in multiple sclerosis. J Neurol Neurosurg Psychiatry 87:93–99
Dean G, Elian M (1997) Age at immigration to England of Asian and Caribbean immigrants and the risk of developing multiple sclerosis. J Neurol Neurosurg Psychiatry 63:565–568

Detels R, Visscher BR, Haile RW, Malmgren RM, Dudley JP, Coulson AH (1978) Multiple sclerosis and age at migration. Am J Epidemiol 108:386–393

Disanto G, Barro C, Benkert P, Naegelin Y, Schadelin S, Giardiello A, Zecca C, Blennow K, Zetterberg H, Leppert D et al (2017) Serum Neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol 81:857–870

Dobson R, Giovannoni G, Ramagopalan S (2013) The month of birth effect in multiple sclerosis: systematic review, meta-analysis and effect of latitude. J Neurol Neurosurg Psychiatry 84:427–432

Durelli L, Verdun E, Barbero P, Bergui M, Versino E, Ghezzi A, Montanari E, Zaffaroni M, Independent Comparison of Interferon Trial Study, G (2002) Every-other-day interferon beta-1b versus once-weekly interferon beta-1a for multiple sclerosis: results of a 2-year prospective randomised multicentre study (INCOMIN). Lancet (London, England) 359:1453–1460

Durelli L, Conti L, Clerico M, Boselli D, Contessa G, Ripellino P, Ferrero B, Eid P, Novelli F (2009) T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. Ann Neurol 65:499–509

Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, Gudz T, Macklin WB, Lewis DA, Fox RJ et al (2006) Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann Neurol 59:478–489

Ebers GC (2008) Environmental factors and multiple sclerosis. Lancet Neurol 7:268–277

Ebers GC, Sadovnick AD, Dyment DA, Yee IM, Willer CJ, Risch N (2004) Parent-of-origin effect in multiple sclerosis: observations in half-siblings. Lancet 363:1773–1774

Fang L, Isobe N, Yoshimura S, Yonekawa T, Matsushita T, Masaki K, Doi H, Ochi K, Miyamoto K, Kawano Y et al (2011) Interleukin-7 receptor alpha gene polymorphism influences multiple sclerosis risk in Asians. Neurology 76:2125–2127

Fang M, Yamasaki R, Li G, Masaki K, Yamaguchi H, Fujita A, Isobe N, Kira J-I (2018) Connexin 30 deficiency attenuates chronic but not acute phases of experimental autoimmune encephalomyelitis through induction of neuroprotective microglia. Front Immunol 9:2588

Ferguson B, Matyszak MK, Esiri MM, Perry VH (1997) Axonal damage in acute multiple sclerosis lesions. Brain 120(Pt 3):393–399

Fox RJ, Miller DH, Phillips JT, Hutchinson M, Havrdova E, Kita M, Yang M, Raghupathi K, Novas M, Sweetser MT et al (2012) Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med 367:1087–1097

Fujisao S, Matsushita S, Nishi T, Nishimura Y (1996) Identification of HLA-DR9 (DRB1∗0901)-binding peptide motifs using a phage fUSE5 random peptide library. Hum Immunol 45:131–136

Gabibov AG, Belogurov AA Jr, Lomakin YA, Zakharova MY, Avakyan ME, Dubrovskaya VV, Smirnov IV, Ivanov AS, Molnar AA, Gurtsevitch VE et al (2011) Combinatorial antibody library from multiple sclerosis patients reveals antibodies that cross-react with myelin basic protein and EBV antigen. FASEB J 25:4211–4221

Giovannoni G, Comi G, Cook S, Rammohan K, Rieckmann P, Soelberg Sorensen P, Vermersch P, Chang P, Hamlett A, Musch B et al (2010) A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. N Engl J Med 362:416–426

Gold R, Linington C, Lassmann H (2006) Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain 129:1953–1971

Gold R, Kappos L, Arnold DL, Bar-Or A, Giovannoni G, Selmaj K, Tornatore C, Sweetser MT, Yang M, Sheikh SI et al (2012) Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med 367:1098–1107

Gregory SG, Schmidt S, Seth P, Oksenberg JR, Hart J, Prokop A, Caillier SJ, Ban M, Goris A, Barcellos LF et al (2007) Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. Nat Genet 39:1083–1091

Group., T.I.M.S.S (1993) Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. The IFNB multiple sclerosis study group. Neurology 43:655–661

Hammond SR, English DR, McLeod JG (2000) The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. Brain 123(Pt 5):968–974

Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, Bar-Or A, Panzara M, Sarkar N, Agarwal S et al (2008) B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 358:676–688

Howell OW, Reeves CA, Nicholas R, Carassiti D, Radotra B, Gentleman SM, Serafini B, Aloisi F, Roncaroli F, Magliozzi R, Reynolds R (2011) Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain 134:2755–2771

Investigators, C.T, Coles AJ, Compston DAS, Selmaj KW, Lake SL, Moran S, Margolin DH, Norris K, Tandon PK, Panitch H et al (2008) Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. N Engl J Med 359:1786–1801

Jacobs LD, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar AM, Fischer JS, Goodkin DE, Granger CV, Simon JH et al (1996) Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The multiple sclerosis collaborative research group (MSCRG). Ann Neurol 39:285–294

Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, Myers LW, Panitch HS, Rose JW, Schiffer RB (1995) Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. the copolymer 1 multiple sclerosis study group. Neurology 45:1268–1276

Kappos L, Radue E-W, O'Connor P, Polman C, Hohlfeld R, Calabresi P, Selmaj K, Agoropoulou C, Leyk M, Zhang-Auberson L et al (2010) A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. N Engl J Med 362:387–401

Kappos L, Bar-Or A, Cree BAC, Fox RJ, Giovannoni G, Gold R, Vermersch P, Arnold DL, Arnould S, Scherz T et al (2018) Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. Lancet (London, England) 391:1263–1273

Kennedy J, O'Connor P, Sadovnick AD, Perara M, Yee I, Banwell B (2006) Age at onset of multiple sclerosis may be influenced by place of residence during childhood rather than ancestry. Neuroepidemiology 26:162–167

Kira J-I (2012) Genetic and environmental backgrounds responsible for the changes in the phenotype of MS in Japanese subjects. Mult Scler Relat Disord 1:188–195

Kotter MR, Li W-W, Zhao C, Franklin RJM (2006) Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. J Neurosci 26:328–332

Kremenchutzky M, Rice GPA, Baskerville J, Wingerchuk DM, Ebers GC (2006) The natural history of multiple sclerosis: a geographically based study 9: Observations on the progressive phase of the disease. Brain 129:584–594

Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Bruck W (2002) Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain 125:2202–2212

Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33:1444–1452

Kurtzke JF, Kurland LT, Goldberg ID (1971) Mortality and migration in multiple sclerosis. Neurology 21:1186–1197

Lassmann H, Raine CS, Antel J, Prineas JW (1998) Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. J Neuroimmunol 86:213–217

Levin LI, Munger KL, O'Reilly EJ, Falk KI, Ascherio A (2010) Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. Ann Neurol 67:824–830

Li G, Yamasaki R, Fang M, Masaki K, Ochi H, Matsushita T, Kira J-I (2018) Novel disease-modifying anti-rheumatic drug iguratimod suppresses chronic experimental autoimmune

encephalomyelitis by down-regulating activation of macrophages/microglia through an NF-kappaB pathway. Sci Rep 8:1933
Lunde HMB, Assmus J, Myhr K-M, Bo L, Grytten N (2017) Survival and cause of death in multiple sclerosis: a 60-year longitudinal population study. J Neurol Neurosurg Psychiatry 88:621–625
Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallstrom E, Khademi M, Oturai A, Ryder LP, Saarela J, Harbo HF et al (2007) Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nat Genet 39:1108–1113
Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, Reynolds R, Aloisi F (2007) Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 130:1089–1104
Magliozzi R, Howell OW, Reeves C, Roncaroli F, Nicholas R, Serafini B, Aloisi F, Reynolds R (2010) A gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Ann Neurol 68:477–493
Magliozzi R, Howell OW, Nicholas R, Cruciani C, Castellaro M, Romualdi C, Rossi S, Pitteri M, Benedetti MD, Gajofatto A et al (2018) Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. Ann Neurol 83:739–755
Mahad DJ, Ransohoff RM (2003) The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). Semin Immunol 15:23–32
Markoullis K, Sargiannidou I, Schiza N, Hadjisavvas A, Roncaroli F, Reynolds R, Kleopa KA (2012) Gap junction pathology in multiple sclerosis lesions and normal-appearing white matter. Acta Neuropathol 123:873–886
Marrosu MG, Cocco E, Lai M, Spinicci G, Pischedda MP, Contu P (2002) Patients with multiple sclerosis and risk of type 1 diabetes mellitus in Sardinia, Italy: a cohort study. Lancet 359:1461–1465
Masaki K, Suzuki SO, Matsushita T, Matsuoka T, Imamura S, Yamasaki R, Suzuki M, Suenaga T, Iwaki T, Kira J-I (2013) Connexin 43 astrocytopathy linked to rapidly progressive multiple sclerosis and neuromyelitis optica. PLoS One 8:e72919
Meinl E, Krumbholz M, Hohlfeld R (2006) B lineage cells in the inflammatory central nervous system environment: migration, maintenance, local antibody production, and therapeutic modulation. Ann Neurol 59:880–892
Mistry N, Dixon J, Tallantyre E, Tench C, Abdel-Fahim R, Jaspan T, Morgan PS, Morris P, Evangelou N (2013) Central veins in brain lesions visualized with high-field magnetic resonance imaging: a pathologically specific diagnostic biomarker for inflammatory demyelination in the brain. JAMA Neurol 70:623–628
Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, de Seze J, Giovannoni G, Hartung H-P, Hemmer B et al (2017) Ocrelizumab versus placebo in primary progressive multiple sclerosis. N Engl J Med 376:209–220
Montalban X, Gold R, Thompson AJ, Otero-Romero S, Amato MP, Chandraratna D, Clanet M, Comi G, Derfuss T, Fazekas F et al (2018) ECTRIMS/EAN guideline on the pharmacological treatment of people with multiple sclerosis. Eur J Neurol 25:215–237
Moore CS, Abdullah SL, Brown A, Arulpragasam A, Crocker SJ (2011) How factors secreted from astrocytes impact myelin repair. J Neurosci Res 89:13–21
Moutsianas L, Jostins L, Beecham AH, Dilthey AT, Xifara DK, Ban M, Shah TS, Patsopoulos NA, Alfredsson L, Anderson CA et al (2015) Class II HLA interactions modulate genetic risk for multiple sclerosis. Nat Genet 47:1107–1113
Nakamura Y, Matsushita T, Sato S, Niino M, Fukazawa T, Yoshimura S, Hisahara S, Isobe N, Shimohama S, Watanabe M et al (2016) Latitude and HLA-DRB1∗04:05 independently influence disease severity in Japanese multiple sclerosis: a cross-sectional study. J Neuroinflammation 13:239
Niino M, Sato S, Fukazawa T, Masaki K, Miyazaki Y, Matsuse D, Yamasaki R, Takahashi E, Kikuchi S, Kira J (2015) Decreased serum vitamin D levels in Japanese patients with multiple sclerosis. J Neuroimmunol 279:40–45

Nischwitz S, Muller-Myhsok B, Weber F (2011) Risk conferring genes in multiple sclerosis. FEBS Lett 585:3789–3797

Noonan CW, Kathman SJ, White MC (2002) Prevalence estimates for MS in the United States and evidence of an increasing trend for women. Neurology 58:136–138

O'Connor P, Wolinsky JS, Confavreux C, Comi G, Kappos L, Olsson TP, Benzerdjeb H, Truffinet P, Wang L, Miller A et al (2011) Randomized trial of oral teriflunomide for relapsing multiple sclerosis. N Engl J Med 365:1293–1303

Odoardi F, Sie C, Streyl K, Ulaganathan VK, Schlager C, Lodygin D, Heckelsmiller K, Nietfeld W, Ellwart J, Klinkert WEF et al (2012) T cells become licensed in the lung to enter the central nervous system. Nature 488:675–679

O'Gorman C, Lucas R, Taylor B (2012) Environmental risk factors for multiple sclerosis: a review with a focus on molecular mechanisms. Int J Mol Sci 13:11718–11752

Okuda DT, Mowry EM, Beheshtian A, Waubant E, Baranzini SE, Goodin DS, Hauser SL, Pelletier D (2009a) Incidental MRI anomalies suggestive of multiple sclerosis: the radiologically isolated syndrome. Neurology 72:800–805

Okuda DT, Srinivasan R, Oksenberg JR, Goodin DS, Baranzini SE, Beheshtian A, Waubant E, Zamvil SS, Leppert D, Qualley P et al (2009b) Genotype-phenotype correlations in multiple sclerosis: HLA genes influence disease severity inferred by 1HMR spectroscopy and MRI measures. Brain 132:250–259

Ontaneda D, Fox RJ, Chataway J (2015) Clinical trials in progressive multiple sclerosis: lessons learned and future perspectives. Lancet Neurol 14:208–223

Osoegawa M, Kira J, Fukazawa T, Fujihara K, Kikuchi S, Matsui M, Kohriyama T, Sobue G, Yamamura T, Itoyama Y et al (2009) Temporal changes and geographical differences in multiple sclerosis phenotypes in Japanese: nationwide survey results over 30 years. Mult Scler 15:159–173

Pandit L, Ban M, Sawcer S, Singhal B, Nair S, Radhakrishnan K, Shetty R, Misri Z, Hegde S, Bhat IG (2011) Evaluation of the established non-MHC multiple sclerosis loci in an Indian population. Mult Scler 17:139–143

Paty DW, Li DK (1993) Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/MRI study group and the IFNB multiple sclerosis study group. Neurology 43:662–667

Peterson JW, Bo L, Mork S, Chang A, Trapp BD (2001) Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol 50:389–400

Plavina T, Subramanyam M, Bloomgren G, Richman S, Pace A, Lee S, Schlain B, Campagnolo D, Belachew S, Ticho B (2014) Anti-JC virus antibody levels in serum or plasma further define risk of natalizumab-associated progressive multifocal leukoencephalopathy. Ann Neurol 76:802–812

Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A et al (2006) A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 354:899–910

Poser S, Poser W (1983) Multiple sclerosis and gestation. Neurology 33:1422–1427

Qiu W, James I, Carroll WM, Mastaglia FL, Kermode AG (2011) HLA-DR allele polymorphism and multiple sclerosis in Chinese populations: a meta-analysis. Mult Scler 17:382–388

Rae-Grant A, Day GS, Marrie RA, Rabinstein A, Cree BAC, Gronseth GS, Haboubi M, Halper J, Hosey JP, Jones DE et al (2018) Practice guideline recommendations summary: disease-modifying therapies for adults with multiple sclerosis: report of the guideline development, dissemination, and implementation Subcommittee of the American Academy of Neurology. Neurology 90:777–788

Ramagopalan SV, Dobson R, Meier UC, Giovannoni G (2010) Multiple sclerosis: risk factors, prodromes, and potential causal pathways. Lancet Neurol 9:727–739

Ransohoff RM, Engelhardt B (2012) The anatomical and cellular basis of immune surveillance in the central nervous system. Nat Rev Immunol 12:623–635

Reynolds R, Roncaroli F, Nicholas R, Radotra B, Gveric D, Howell O (2011) The neuropathological basis of clinical progression in multiple sclerosis. Acta Neuropathol 122:155–170

Rouach N, Avignone E, Meme W, Koulakoff A, Venance L, Blomstrand F, Giaume C (2002) Gap junctions and connexin expression in the normal and pathological central nervous system. Biol Cell 94:457–475

Sadovnick AD, Armstrong H, Rice GP, Bulman D, Hashimoto L, Paty DW, Hashimoto SA, Warren S, Hader W, Murray TJ et al (1993) A population-based study of multiple sclerosis in twins: update. Ann Neurol 33:281–285

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

Scalfari A, Neuhaus A, Daumer M, Muraro PA, Ebers GC (2014) Onset of secondary progressive phase and long-term evolution of multiple sclerosis. J Neurol Neurosurg Psychiatry 85:67–75

Shinoda K, Matsushita T, Nakamura Y, Masaki K, Yamasaki R, Yamaguchi H, Togao O, Hiwatashi A, Kira J-I (2018) HLA-DRB1*04:05 allele is associated with intracortical lesions on three-dimensional double inversion recovery images in Japanese patients with multiple sclerosis. Mult Scler 24:710–720

Skoog B, Runmarker B, Winblad S, Ekholm S, Andersen O (2012) A representative cohort of patients with non-progressive multiple sclerosis at the age of normal life expectancy. Brain 135:900–911

Sorensen PS, Lisby S, Grove R, Derosier F, Shackelford S, Havrdova E, Drulovic J, Filippi M (2014) Safety and efficacy of ofatumumab in relapsing-remitting multiple sclerosis: a phase 2 study. Neurology 82:573–581

Srivastava R, Aslam M, Kalluri SR, Schirmer L, Buck D, Tackenberg B, Rothhammer V, Chan A, Gold R, Berthele A et al (2012) Potassium channel KIR4.1 as an immune target in multiple sclerosis. N Engl J Med 367:115–123

Stadelmann C, Wegner C, Bruck W (2011) Inflammation, demyelination, and degeneration - recent insights from MS pathology. Biochim Biophys Acta 1812:275–282

Stoffels JMJ, de Jonge JC, Stancic M, Nomden A, van Strien ME, Ma D, Siskova Z, Maier O, Ffrench-Constant C, Franklin RJM et al (2013) Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. Brain 136:116–131

Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, Correale J, Fazekas F, Filippi M, Freedman MS et al (2018a) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17:162–173

Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O (2018b) Multiple sclerosis. Lancet (London, England) 391:1622–1636

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338:278–285

Tremlett H, Yinshan Z, Devonshire V (2008) Natural history of secondary-progressive multiple sclerosis. Mult Scler (Houndmills, Basingstoke, England) 14:314–324

Voskuhl RR, Peterson RS, Song B, Ao Y, Morales LBJ, Tiwari-Woodruff S, Sofroniew MV (2009) Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. J Neurosci 29:11511–11522

Wallin MT, Page WF, Kurtzke JF (2004) Multiple sclerosis in US veterans of the Vietnam era and later military service: race, sex, and geography. Ann Neurol 55:65–71

Yoshimura S, Isobe N, Yonekawa T, Matsushita T, Masaki K, Sato S, Kawano Y, Yamamoto K, Kira J, South Japan Multiple Sclerosis Genetics, C (2012) Genetic and infectious profiles of Japanese multiple sclerosis patients. PLoS One 7:e48592

Visualization of Myelin for the Diagnosis and Treatment Monitoring of Multiple Sclerosis

15

Jin Nakahara

Abstract

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) affecting more than two million people worldwide. As the exact etiology of MS remains elusive, the diagnosis of MS is made by referring to the McDonald diagnostic criteria, which utilizes MRI as a tool to identify "demyelinated" MS lesions. In particular, hyperintense lesions on T2-weighted images (T2WI) or so-called "T2-lesions" are considered to represent demyelinated MS lesions. T2WI, however, lacks myelin specificity, and moreover, remyelination could not be depicted by the use of such modality. For the accurate diagnosis and treatment decision-making, or for the future development of remyelination therapeutics, imaging tools to visualize myelin-specific signals are mandatory. In this chapter, the current use and the limitation of imaging modalities in MS diagnosis and treatment will be reviewed, with the introduction of new imaging method, namely *q*-space Myelin Map (qMM), to be used for visualization of demyelination and remyelination in MS.

Keywords

Multiple sclerosis · Demyelination · Remyelination · MRI · *q*-space Myelin Map

15.1 Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) affecting more than two million people worldwide. Without adequate treatment, the vast majority of MS patients have poor prognosis in terms of long-term disability: On average, they become cane-dependent in their 50s,

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wheelchair-dependent in their 60s, and their life expectancy is nearly 10 years shorter than that of healthy populations (Confavreux and Vukusic 2006; Scalfari et al. 2013). While "sclerosis" originally refers to the hardening of MS lesion by astrogliosis, the clinical definition of MS was determined in 1965 (Schumacher et al. 1965) in order to standardize "MS" for the sake of clinical studies. Since then, MS is clinically defined as a demyelinating CNS disease that disseminates in time and space, but with unknown causes. This classical definition of MS has not been modified up to present, but given the great utilization of magnetic resonance imaging (MRI) in our clinical practice, the McDonald criteria was made, as an "expert consensus" to how we determine "demyelination" that "disseminates in time and space" by the use of non-invasive MRI (McDonald et al. 2001). Such a consensus has been modified several times up to date (Polman et al. 2005, 2011; Thompson et al. 2018), largely due to the growing need to diagnose and treat early with the concomitant development of disease-modifying drugs (DMDs). The modification was only made to how we determine "dissemination in time and space" with more sensitivity and specificity, but the determination of "demyelination" by MRI has not been modified so far: The hyperintense lesion on T2-weighted images (T2WI), or so-called "T2-lesions" are considered as the sign of CNS demyelination (Polman et al. 2005, 2011; Thompson et al. 2018).

As most trained neurologists are aware of, T2WI is not a specific modality to detect myelin-related signals. Rather, T2-lesions generally stand for increased proton fluidity in the region of interest, which may include but not necessarily limited to demyelination. For example, we know from our clinical experience that cerebral infarctions, CNS vasculitis, or brain abscess also appears as "T2-lesions" on their MRI. Utilizing the McDonald criteria, we still encounter misdiagnosing patients with small vessel diseases or non-specific white matter lesions (e.g., migraine) as MS (Solomon et al. 2016). These misdiagnoses may be greatly eliminated if we had a more specific measure to detect demyelination.

One important clinical characteristic of MS is the remission of its symptom. The vast majority of MS patients initially develop relapsing-remitting MS (RRMS), with most of them develop secondary progressive MS (SPMS) if not treated appropriately. In the course of RRMS, there occurs "relapse" and "remission," but the remission mostly disappears in the phase of SPMS with the gradual increase of disability without apparent relapses. "Relapse" is clinically considered when the patient suffered from a newly onset symptom that persists for more than 24 h, according to the McDonald criteria (McDonald et al. 2001). In the real-world practice, most relapses last for weeks to months, which usually subside within months either spontaneously or by the use of anti-inflammatory therapy such as intravenous methylprednisolone therapy (IVMP). When the patient exhibit significant clinical improvement in their disability soon after the IVMP, it may be considered as the direct IVMP effect on inflammation. The majority of MS patients, however, continue to gradually improve even for months after the IVMP, which is generally considered as a sign of remyelination.

Albeit clinicians are aware of the fact, that remyelination seems to have a great contribution in the remission of symptoms, objective confirmation of the notion was

somewhat difficult. While T2WI sensitively depict newly evolving demyelination, nearly 95% of those T2-lesions remain hyperintense on T2WI even after the spontaneous resolution of clinical symptoms (Zivadinov 2007). The discrepancy between clinical and radiological "remission" is due to the fact that T2WI is not capable of depicting remyelination. As has been previously confirmed by radio-pathological analysis, T2-lesions may include already remyelinated MS lesions (Barkhof et al. 2003).

15.2 "Biochemical" Myelin Imaging

In order to improve the accuracy of diagnosis and visualize remyelination in multiple sclerosis, attempts had been made to develop more myelin-specific imaging modalities. Considering the clinical utility, such a myelin imaging should have a good quality (resolution high enough to detect millimeter-sized lesions), minimally invasive (short acquisition time and preferably no exposure to radiation), and economical (implementable with scanners already available at the clinic).

Two "biochemical" myelin-imaging modalities have fulfilled these criteria so far (Mallik et al. 2014). One of such was the myelin water fraction (MWF) method (MacKay et al. 1994). In principle, T2 relaxation in white matters is multi-exponential and "myelin water" also contribute to this T2 decay curve. The heat map reflecting the "fraction" of "myelin water" or MWF appears to be more myelin-specific imaging modality compared to ordinal T2-weighted images. Albeit the fact that this MWF (using 3D-GRASE) can be performed within minutes, there is limited evidence available with respect to its clinical correlation. The specificity of MWF signals to myelin remains somewhat uncertain, as argued by the study which showed increased MWF signals with the aging process (Billiet et al. 2015), contradicting to the wide-held view that myelin decreases with aging. More importantly, MWF is based on "biochemical" property of hydrophobic myelin, and therefore it cannot be used to discern myelin debris from intact myelin sheaths (MacKay and Laule 2016), which would be the major limitation of this method to be used for inflammatory demyelinating conditions including MS.

The other method is magnetization transfer ratio (MTR). Magnetization transfer is the exchange of energy between "macromolecular protons (i.e., immobile protons bound to macromolecules)" and "free water protons" (Edzes and Samulski 1977); myelin could serve as a source of "macromolecular protons" in this case. "Macromolecular protons" are largely invisible in ordinal MR images due to significantly shorter T2. In other words, ordinal MR images are heavily focused on "free water protons." When so-called "off-resonance pulse," which saturate "macromolecular protons" but leaving "free water protons" unaffected (Wolff et al. 1991), due to the magnetic transfer between the two proton pools, "free water protons" will be partially saturated resulting in decreased signals in the ordinal MR images. The ratio of signals with and without "off-resonance pulse" relates to the proportion of "macromolecular protons" or myelin in this case, in the particular region of interest. Therefore, MTR is considered as a "biochemical" myelin imaging method. While

MTR is the most popular imaging method to detect remyelination at present, this method also fails to differentiate myelin debris from intact myelin sheaths, as shown in the discrepancy between clinical recovery and "remyelination" indicated by MTR in acute optic neuritis cases (Hickman et al. 2004).

15.3 "Morphological" Myelin Imaging

If we were to accurately image myelin in inflammatory conditions, then we should be able to segregate signals of myelin debris from intact myelin sheath; that is, we need to develop a method in a more morphological fashion. In a morphological point of view, intact myelin sheaths act as an insulator, which will entrap the axonal water molecules or protons within the axon. Myelin debris, of course, has no axons beneath, and unmyelinated axons have more incomplete entrapment of protons within axons due to the presence of certain channels on axonal surfaces. In principle, therefore, if we were able to monitor the movement of protons in a particular region of interest, with a resolution to depict the difference under the size of axonal diameter (around 10 μm), then we should be able to "predict" the chance of myelin presence in the area.

q-Space imaging (QSI) was developed as a method to calculate the probability-diffusion function (PDF) of protons in non-Gaussian fashion, enabling us to estimate such diffusion of protons in an order of μm (King et al. 1994). The accuracy of PDF depends on the quality and the quantity of diffusion-weighted images that were taken with various *b*-value range and steps, thus it generally requires several hours to acquire fully accurate PDF for the entire brain, making QSI unpractical in a clinical setting. In order to reduce the acquisition time, diffusion kurtosis imaging (DKI) was developed, in which the QSI sequence was optimized for the calculation of kurtosis values, but not the entire PDF itself, by which *b*-value was reduced to six steps in the range of 0–3500 s/mm^2 (Jensen et al. 2005). The acquisition time for brain DKI is generally 10–20 min, however, its specificity to myelin signals is compromised by the omission of high *b*-value data, which is essentially important for the visualization of myelin (Cohen and Assaf 2002).

To skim the cream off the top, we focused on the development of high *b*-value QSI optimized for kurtosis values essential for myelin signals. At last, we successfully developed *q*-space Myelin Map (qMM), which could be acquired within 10 min using an ordinal MR scanner already available in the clinical setting. qMM employs lesser *b*-value steps compared to full-scale QSI to reduce the acquisition time, but higher *b*-value range compared to DKI to increase the myelin specificity (Fujiyoshi et al. 2016).

The myelin specificity of qMM has been studied rigorously by using several myelin-deficient mice and chemically induced demyelination model in common marmosets (Fujiyoshi et al. 2016). Finally, the superiority of qMM to visualize myelin signals when compared with conventional modalities or diffusion-tensor imaging (DTI) was confirmed in healthy human volunteers.

15.4 *q*-Space Myelin Map in Multiple Sclerosis

Theoretically speaking, qMM could be commonly utilized to visualize myelin signals regardless of disorders, although its clinical utility has been so far testified in MS.

As previously described, the conventional T2WI cannot depict remyelinated MS lesions: MS lesions remain hyperintense on T2WI regardless of remyelination afterward. Utilizing qMM along with T2WI, presence of "myelin signals" on the corresponding qMM is suggestive of already remyelinated MS lesions (Fig. 15.1). Thus, the use of qMM may allow clinicians to detect one's remyelination capacity, which may affect his or her long-term prognosis (Fig. 15.2).

As such, remyelination often takes place in MS patients, especially during the early phase. Even when MS patients become essentially symptom-free by anti-inflammatory therapy (e.g., IVMP), the most T2 lesions, if not all, remain unchanged thus producing "clinico-radiological paradox." qMM could be used to detect remyelination in parallel with clinical recovery, thus to solve such paradox. Moreover, certain DMDs including fingolimod have been suggested to improve physical disability in young patients with MS (Fragoso et al. 2015), while the underlying mechanism remained uncertain. Application of qMM in MS patients under fingolimod who showed improvement in their physical disability revealed signs of remyelination to a varying degree, therefore, remyelination may in part be associated with such improvement (Tanikawa et al. 2017).

Furthermore, several candidate medicines for remyelination therapy have been developed so far (reviewed in Nakahara 2017), but the lack of appropriate methods to detect remyelination has been a serious obstacle for the further clinical develop-

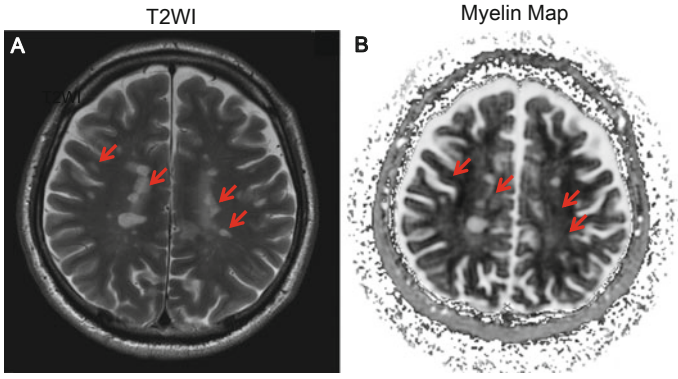

Fig. 15.1 q-space Myelin Map in an MS patient. Axial T2WI and qMM for the brain of RRMS patient (a 43-year-old male) are shown. Red arrows indicate MS lesions (i.e., "T2-lesions") with positive myelin signals in qMM, suggestive of remyelinated MS lesions. Figure reproduced from Fig. 8 in Fujiyoshi et al. (2016)

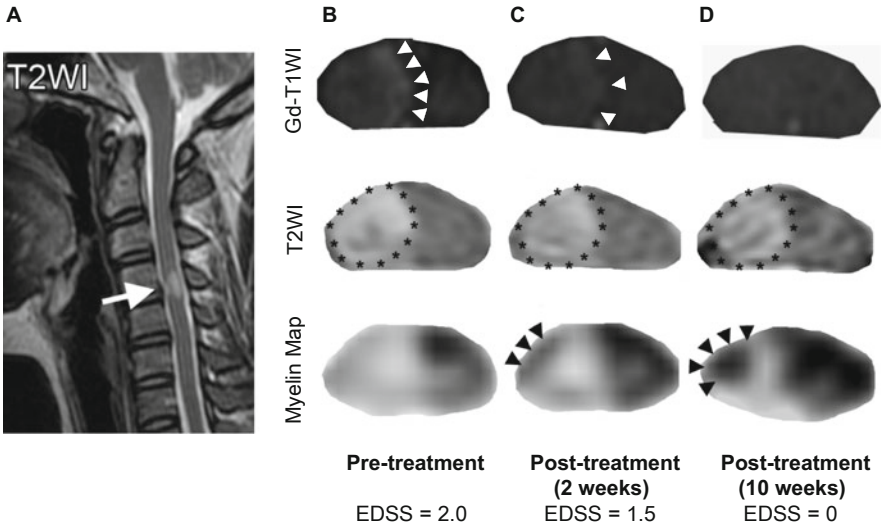

Fig. 15.2 q-space Myelin Map in an MS patient. Sequential qMM during recovery after relapse in an MS patient. Although T2WI shows essentially no differences throughout the course, the gadolinium (Gd)-enhancement disappears in T1-weighed images (T1WI) and qMM detects the reappearance of myelin signals suggestive of remyelination, which correlated with the clinical recovery. Figure reproduced from Fig. 10 in Fujiyoshi et al. (2016)

ment. Therefore, qMM may become a feasible and useful radiological marker for the future development of remyelination medicines.

15.5 Future Perspectives

So far, qMM is a promising novel myelin-specific imaging that can be utilized in clinical practice. qMM may allow more accurate and faster diagnosis of demyelinating diseases including MS. In MS, qMM may solve the "clinico-radiological paradox" and enable real-time monitoring of treatment response with anti-inflammatory therapy (e.g., IVMP) or DMDs. The clinical utility of qMM in other neurological diseases or comparison of qMM with "biochemical" myelin imaging (i.e., MTR and MWF) awaits further studies. Finally, qMM may serve as an important radiological marker in future clinical development of remyelination therapeutics.

References

Barkhof F, Bruck W, De Groot CJ, Bergers E, Hulshof S, Geurts J, Polman CH, van der Valk P (2003) Remyelinated lesions in multiple sclerosis: magnetic resonance image appearance. Arch Neurol 60(8):1073–1081

Billiet T, Vandenbulcke M, Madler B, Peeters R, Dhollander T, Zhang H, Deprez S, Van den Bergh BR, Sunaert S, Emsell L (2015) Age-related microstructural differences quantified using myelin water imaging and advanced diffusion MRI. Neurobiol Aging 36(6):2107–2121

Cohen Y, Assaf Y (2002) High *b*-value *q*-space analyzed diffusion-weighted MRS and MRI in neuronal tissues—a technical review. NMR Biomed 15(7–8):516–542

Confavreux C, Vukusic S (2006) Natural history of multiple sclerosis: a unifying concept. Brain 129(Pt 3):606–616

Edzes HT, Samulski RT (1977) Cross relaxation and spin diffusion in the proton NMR of dehydrated collagen. Nature 265:521–523

Fragoso YD, Alves-Leon SV, Barreira AA, Callegaro D, Ferreira BML, Finkelsztejn A, Gomes S, Goncalves MVM, Machado MIM, Marques VD, Matta APC, Papais-Alvarenga RM, Pereira SLA, Tauil CB (2015) Fingolimod prescribed for the treatment of multiple sclerosis in patients younger than age 18 years. Pediatr Neurol 53:166–168

Fujiyoshi K, Hikishima K, Nakahara J, Tsuji O, Hata J, Konomi T, Nagai T, Shibata S, Kaneko S, Iwanami A, Momoshima S, Takahashi S, Jinzaki M, Suzuki N, Toyama Y, Nakamura M, Okano H (2016) Application of *q*-space diffusion MRI for the visualization of white matter. J Neurosci 36(9):2796–2808

Hickman SJ, Toosy AT, Jones SJ, Altmann DR, Miszkeil KA, MacManus DG, barker GJ, Plant GT, Thompson AJ, Miller DH (2004) Serial magnetization transfer imaging in acute optic neuritis. Brain 127(3):692–700

Jensen JH, Helpern JA, Ramani A, Lu H, Kaczynski K (2005) Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging. Magn Reson Med 53(6):1432–1440

King MD, Houseman J, Roussel SA, van Bruggen N, Williams SR, Gadian DG (1994) *q*-space imaging of the brain. Magn Reson Med 32:707–713

MacKay AL, Laule C (2016) Magnetic resonance of myelin water: an in vivo marker for myelin. Brain Plasticity 2:71–91

MacKay A, Whittall K, Adler J, Li D, Paty D, Graeb D (1994) In vivo visualization of myelin water in brain by magnetic resonance. Magn Reson Med 31(6):673–677

Mallik S, Samson RS, Wheeler-kingshott CA, Miller DH (2014) Imaging outcomes for trials of remyelination in multiple sclerosis. J Neurol Neurosurg Psychiatry 85(12):1396–1404

McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van der Noort S, Weinshenker BY, Wolinsky JS (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann Neurol 50(1):121–127

Nakahara J (2017) Remyelination in multiple sclerosis: pathology and treatment strategies. Clinic Exp Neuroimmunol 8(Suppl 1):40–46

Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald criteria". Ann Neurol 58(6):840–846

Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havdova E, Huchinson M, Kappos L, Lublin FD, Montalban X, O'Connor P, Sandberg-Wollheim M, Thompson AJ, Waubant E, Weinshenker B, Wolinsky JS (2011) Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 69(2):292–302

Scalfari A, Knappertz V, Cutter G, Goodin DS, Ashton R, Ebers GC (2013) Mortality in patients with multiple sclerosis. Neurology 81(2):184–192

Schumacher GA, Beebe G, Kibler RF, Kurland LT, Kurtzke JF, McDowell F, Nagler B, Sibley WA, Tourtellotte WW, Willmon TL (1965) Problems of experimental trials of therapy in multiple sclerosis: report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. Ann N Y Acad Sci 122:552–568

Solomon AJ, Bourdette DN, Cross AH, Applebee A, Skidd PM, Howard DB, Spain RI, Cameron MH, Kim E, Mass MK, Yadav V, Whitham RH, Longbrake EE, Naismith RT, Wu GF, Parks BJ, Wingerchuk DM, Rabin BL, Toledano M, Tobin WO, Kantarci OH, Carter JL, Keegan BM, Weinshenker BG (2016) The contemporary spectrum of multiple sclerosis misdiagnosis: a multicenter study. Neurology 87(13):1393–1399

Tanikawa M, Nakahara J, Hata J, Suzuki S, Fujiyoshi K, Fujiwara H, Momoshima S, Jinzaki M, Nakamura M, Okano H, Takahashi S, Suzuki N (2017) *q*-space myelin map imaging for longitudinal analysis of demyelination and remyelination in multiple sclerosis patients treated with fingolimod: a preliminary study. J Neurol Sci 373:352–357

Thompson AJ, Banwell BL, Barkhof F, Carrol WM, Coetzee T, Comi G, Correale J, Fazekas F, Filippi M, Freedman MS, Fujihara K, Galetta SL, Hartung HP, Kappos L, Lublin FD, Marrie RA, Miller AE, Miller DH, Montalban X, Mowry EM, Sorensen PS, Tintore M, Traboulsee AL, Trojano M, Uitdehaag BMJ, Vukusic S, Waubant E, Weinshenker BG, Reingold SC, Cohen JA (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17:162–173

Wolff SD, Eng J, Balaban RS (1991) Magnetization transfer contrast: method for improving contrast in gradient-recalled-echo images. Radiology 179:133–137

Zivadinov R (2007) Can imaging techniques measure neuroprotection and remyelination in multiple sclerosis? Neurology 68(22 Suppl 3):S72–S82

Roads to Formation of Normal Myelin Structure and Pathological Myelin Structure

16

Yoshio Bando

Abstract

Demyelination and axonal damage are responsible for neurological deficits in demyelinating diseases including multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system. However, the pathology of demyelination and axonal damage in MS is not fully understood. While immunologists have accumulated evidence, which is involved in many immunological events in these diseases, neuroscientists and anatomists have also investigated morphological changes of myelin in these diseases. In this chapter, a new concept of demyelination will be described.

Keywords

MS · EAE · Demyelination · Oligodendrocyte · Myelin · Axonal degeneration

16.1 Introduction

In the central nervous system (CNS), oligodendrocytes are responsible for myelination by wrapping around the axon and maintaining saltatory conduction (Baumann and Pham-Dinh 2001). In addition, the myelin sheath plays an important role in protecting axons from various physical stresses, infections, and inflammation. The processes of oligodendroglial maturation and myelination are still in mystery. However, it is well known that dysregulation of these events is involved in the pathogenesis of many neurological/psychological diseases.

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Besides developmental dysregulation of myelination, damage to oligodendrocytes and the myelin sheath is termed demyelination. Once demyelination has occurred, various neurological symptoms with axonal degeneration will be induced. However, the mechanisms of demyelination and axonal degeneration have not been fully understood.

16.2 Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory demyelinating disease in the CNS characterized by immune-mediated disease with autoimmune responses against myelin antigens and inflammation contributing to the pathogenesis of demyelination in the CNS (Noseworthy et al. 2000; Hafler 2004). Although the major cause of the disease remains unknown, to date, it is widely accepted that immune cells attack myelinated axons in the CNS, followed by demyelination and axonal degeneration (Denic et al. 2011; Misko et al. 2010; Mayo et al. 2012; Dutta and Trapp 2011; Stadelmann et al. 2011; Schirmer et al. 2013). For instance, activated autoreactive T cells and myelin-specific T cells can facilitate the recruitment of macrophages by producing various cytokines and chemokines. Infiltrating inflammatory cells are activated within the CNS and interact with other immune cells and neuronal cells, resulting in oligodendroglial cell death-mediated demyelination, glial cell activation, and axonal degeneration (Noseworthy et al. 2000). Therefore, it has been suggested that demyelination and oligodendroglial cell death in MS is passively induced by infiltrating immune cells.

16.3 Animal Models for MS Research

To understand further the pathology of demyelination and axonal degeneration in MS, neurological and histological investigations in different animal models that mimic some aspects of MS are needed. There are several animal models for MS research including experimental autoimmune encephalomyelitis (EAE) and cuprizone-induced demyelination model (Bando et al. 2015, 2018). EAE is widely used as an animal model for studying aspects of human MS. According to these studies, therefore, the current concept of pathogenesis and progression of EAE has been based on immunological processes, which are associated with the infiltrating immune cells in the CNS. On the other hand, the cuprizone-induced mouse model has been used to induce demyelination and remyelination. Cuprizone is a copper chelator and it is known that the cuprizone feeding leads to oligodendroglial cell death and subsequent demyelination in the corpus callosum in mice. Endogenous and spontaneous remyelination can be observed after withdrawal of cuprizone (Blackmore 1972). This model is widely accepted in the field; however, it does not involve the breakdown of the blood–brain barrier or infiltration of peripheral immune cells into the CNS. It is totally different from the EAE model.

Although the classical scenario of MS and EAE pathology is based on T cell-mediated immune pathogenesis, it failed to explain fully some of the features of MS and EAE. For example, T cells alone do not appear to destroy myelin on a large scale, which requires additional help from B cells, humoral autoantibodies, and cytokines. Indeed, B lymphocytes and other factors were recently shown to be associated with demyelination in MS lesions (Krumbholz and Meinl 2014). A new concept of demyelination in MS and EAE was proposed suggesting factors other than immune cells might cause the disintegration and loss of periaxonal/myelin sheaths and large-scale demyelination.

16.4 Histological Analysis for Myelin Research

In the point of view from neuroscientists and anatomists, histological methods are also powerful tools for studying what is happened to myelin structures. Especially, transmission electron microscopy (TEM) is often used for the field of myelin research. TEM has been traditionally used to observe myelin morphology. For example, we have a demyelinating animal model such as EAE. If we cannot see any myelin around the axon in the EAE mice by TEM, we will think demyelination has occurred. This is a simple answer with no doubt for demyelination. However, it has been suggested that destruction of myelin structure owing to sample preparation for TEM study often occurs (Osawa et al. 2002). If we see this event, how can we think the principle of demyelination? Thus, it is difficult to judge whether the loose myelin appearance is a cause of demyelination. For this reason, the morphological changes to myelin during the process of demyelination have not been well studied. Recently, another morphological technique has been established (Bando et al. 2015). Scanning electron microscopy (SEM), combined with the osmium-maceration method, has been developed for the detection of ultrastructural abnormalities in myelin and axons at early stages of demyelination in experimental animal models (Tanaka and Mitsushima 1984; Koga and Ushiki 2006; Nomura et al. 2013). Although this technique strategically degrades protein components, membrane structures such as myelin are clearly visualized. This SEM technique enables morphological changes during demyelination to be observed more accurately. In fact, this technique demonstrated a variety of abnormal myelin structures with compact lamination of myelin surrounding axons, even in EAE mice.

16.5 Cuprizone-Induced Demyelination Model

In cuprizone-induced demyelination model, most myelin was gone from the axons in the corpus callosum because of demyelination caused by cuprizone-induced oligodendroglial cell death (Blackmore 1972). While few abnormal myelin structures were observed such as excess myelin folding, the frequency was low. Therefore, the histopathological changes in this model seem to be simple demyelination, namely loss of myelin.

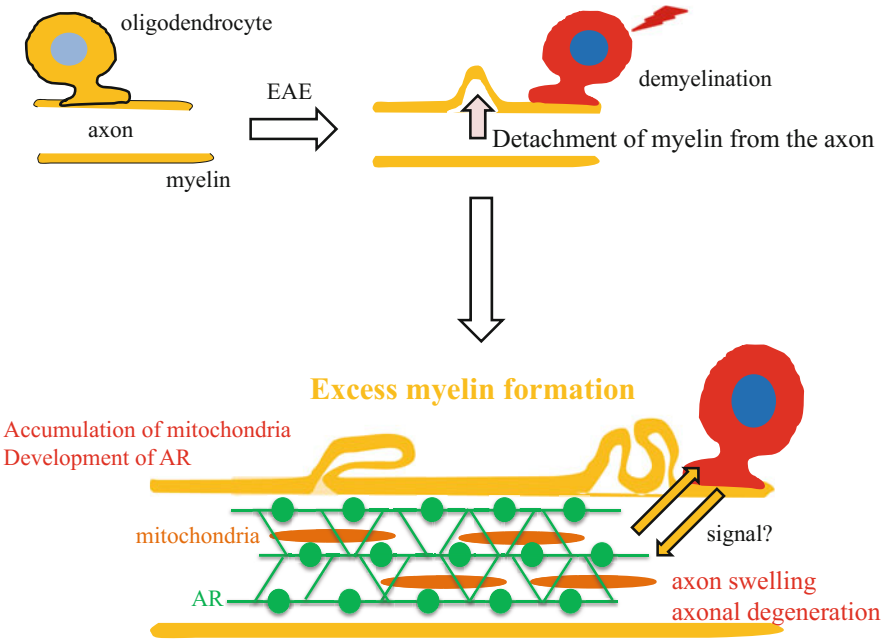

Fig. 16.1 Current hypothesis of demyelination and axonal injury in EAE. EAE induces myelin detachment from axons at the initial phase of demyelination. At this point, infiltration of immune cells is not necessary. At the peak of EAE disease, abnormal myelin structures including excess myelin formation have proceeded. Furthermore, complex myelin structures including multiple layered and obstructive myelin are formed at the chronic phase. Thus, myelin detachment from axons is the initial step of demyelination. In the axons of the EAE spinal cord, the spatial development of axoplasmic reticulum (AR) and accumulation of mitochondria can be observed. Thus, morphological changes of myelin trigger axonal degeneration in addition to morphological changes of axonal organelles. There might be some signaling between demyelination and axonal degeneration

16.6 Demyelination in EAE

EAE induces inflammatory demyelination in the CNS white matter (Martin et al. 1992; Noseworthy et al. 2000; Dhib-Jalbut 2007; Greenstein 2007). MOG-induced EAE mice developed demyelination, inflammation with infiltrating peripheral immune cells and glial cell activation in spinal cord white matter. Myelin detachment from the axon and excess myelin formation were typical myelin abnormalities in MOG-EAE mice (Fig. 16.1, Bando et al. 2015). Thus, it is hypothesized that excess myelin formation is induced by the dysfunction/dysregulation of oligodendrocytes in the EAE spinal cord. Myelin abnormalities in MOG-induced EAE were complicated and different from those in cuprizone-induced demyelination.

In the EAE mice, myelin detachment from axons was already present in the lumbar region of the spinal cord at a preclinical stage of EAE (Bando et al. 2015). At this point, CNS infiltrating immune cells were not observed. Besides this morphological change, abnormal myelin structures such as double myelin were also observed at the peak of EAE disease. At the chronic phase, complex myelin structures including multiple layered and obstructive myelin were formed. Thus, myelin detachment from axons is the initial step of demyelination (Fig. 16.1).

Involvement of gray matter and axonal damage in normal-appearing white matter (NAWA) has recently been reported in MS patients, indicating that axonal and neuronal damage occurs in NAWA. Non-characteristic morphological changes to myelin can partly explain the pathogenesis in the NAWA. These pathological abnormalities in NAWA may contribute to clinical disability in MS patients. Therefore, it is important to understand the pathological contribution of NAWA in MS. In the EAE model, myelin abnormalities were also observed in NAWA (Bando et al. 2015). These abnormalities may be associated with the pathogenesis of axonal damage in NAWA.

16.7 Axonal Degeneration in EAE

What happened to the axon after demyelination? Bando et al. have reported that morphological abnormalities of myelin preceded axonal degeneration and morphological changes of axonal organelles such as axoplasmic reticulum-like structures (ARLS) (Fig. 16.1, Bando et al. 2015). These structures are considered as axoplasmic reticulum (AR), which plays important roles in intra-calcium stores in secondary axonal degeneration via ryanodine and inositol triphosphate receptors (IP3R, Stirling et al. 2014). In the axons of the EAE spinal cord, the spatial development of ARLS and accumulation of mitochondria can be observed (Bando et al. 2015). This study indicates that morphological changes of myelin trigger axonal degeneration in addition to morphological changes of axonal organelles. Furthermore, axons in the white matter of the EAE spinal cord are tortuous. Although further analysis will be required, it is considerable relationship between tortuous axons and axonal degeneration. By contrast, abnormalities of axonal organelles were not observed in cuprizone-induced demyelination (Bando et al. 2015). Therefore, there are different mechanisms underlying these two murine models. Nikic et al. observed focal axonal degeneration (FAD) with focal axonal swellings and mitochondrial pathology in EAE and acute human MS lesions. Interestingly, they also proposed that intra-axonal mitochondrial pathology in FAD might be the earliest ultrastructural sign of damage, which also precedes changes in axon morphology (Nikić et al. 2011). Several studies of autopsied human MS brains and in vitro models also reported mitochondrial dysfunction might be a cause of axonal degeneration (Dutta et al. 2006; Kiryu-Seo et al. 2010; Ohno et al. 2011). Furthermore, myelin-associated glycoprotein-, 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP)-, and proteolipid protein (PLP)-null mice also showed axonal pathologies (Yin et al. 1998; Lappe-Siefke et al. 2003; Edgar et al. 2004), indicating that oligodendrocyte–axon

interactions are important for structural and functional modulation between myelin and axons.

Accumulating evidence suggests that axonal loss in MS and EAE is caused by impairment of axonal AR and mitochondrial function followed by increasing axonal Ca^{2+} levels released from AR and mitochondria. Stirling et al. reported that axoplasmic reticulum Ca^{2+} release caused secondary degeneration of spinal axons (Stirling and Stys 2010; Stirling et al. 2014). In fact, the EAE mice shows an increase of mitochondria with abnormal morphology in the axons, indicating dysfunction of these mitochondria with abnormal morphology. In addition, it has been shown an increased intensity of a mitochondrial fission-related protein Drp1/Dlp1 and decreased intensity of a mitochondrial fusion-related protein MFN1 in EAE spinal cords (Bando et al. 2015). Ohno et al. previously reported that larger axonal mitochondria were not mobile and more than 90% of axonal mitochondria were observed in intermodal stationary sites (Ohno et al. 2011; Campbell et al. 2012). Long mitochondrial stationary sites in internodes are the major source of axonal ATP, and the intermodal enrichment of stationary mitochondria helps facilitate axonal transport (Trapp and Stys 2009; Zhang et al. 2010; Kiryu-Seo et al. 2010; Ohno et al. 2011). Dutta et al. proposed a mechanism whereby reduced ATP production in demyelinated segments of upper motor neuron axons impacts ion homeostasis, induces Ca^{2+}-mediated axonal degeneration and contributes to progressive neurological disability in MS patients (Dutta et al. 2006; Ohno et al. 2011). Interestingly, in *shiverer* mice, the length of mitochondria in the spinal cord mice is longest among the three animal models such as EAE and cuprizone-induced demyelination (Bando et al. 2015). These results indicate that mitochondria play a crucial role in axonal degeneration during demyelination.

16.8 Summary

Proof of principle of demyelination in EAE has emerged from recent studies, indicating that morphological features of EAE-induced demyelination are more complex. Myelin detachment and excess myelin formation, but not loose myelin, are typical myelin abnormalities at inflammatory sites in MOG-induced EAE mice. In addition, detachment of myelin from axons and excess myelin formation are essential for the initial demyelination process (Fig. 16.1). Moreover, excess myelin formation is induced by oligodendrocyte dysfunction/dysregulation in the EAE spinal cord. Furthermore, abnormalities to myelin structures observed in EAE mice are similar to those observed in human MS brains. It seemed to be oligodendrocytopathy. These observations indicate the central role of mitochondria and ARLS in axonal degeneration to the pathogenesis of MS. Although dysregulation of the immune system has been the main focus of MS pathology, oligodendrocytes are also a considerable new target for MS therapy.

In conclusion, a new concept of myelin abnormalities followed by axonal degeneration in EAE mice and MS patients has been established. In EAE and MS, myelin abnormalities and morphological changes in axonal ARLS and mitochondria may be

a critical step in axonal degeneration. Therefore, understanding oligodendroglial behavior in demyelination and remyelination may open new avenues for the treatment of MS.

References

Bando Y, Nomura T, Bochimoto H, Murakami K, Tanaka T, Watanabe T, Yoshida S (2015) Abnormal morphology of myelin and axonal pathology in murine models of multiple sclerosis. Neurochem Int 81:16–27

Bando Y, Hagiwara Y, Suzuki Y, Yoshida K, Aburakawa Y, Kimura T, Murakami C, Ono M, Tanaka T, Jiang YP, Mitrovi B, Bochimoto H, Yahara O, Yoshida S (2018) Kallikrein 6 secreted by oligodendrocytes regulates the progression of experimental autoimmune encephalomyelitis. Glia 66(2):359–378

Baumann N, Pham-Dinh D (2001) Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev 81:871–927

Blackmore WF (1972) Observations on oligodendrocyte degeneration, the resolution of status spongiosus and remyelination in cuprizone intoxication in mice. J Neuro-Oncol 1:413–426

Campbell GR, Ohno N, Turnbull DM, Mahad DJ (2012) Mitochondrial changes within axons in multiple sclerosis: an update. Curr Opin Neurol 25:221–230

Denic A, Johnson AJ, Bieber AJ, Warrington AE, Rodriguez M, Pirko I (2011) The relevance of animal models in multiple sclerosis research. Pathophysiology 18:21–29

Dhib-Jalbut S (2007) Pathogenesis of myelin/oligodendrocyte damage in multiple sclerosis. Neurology 68:S13–S21

Dutta R, Trapp BD (2011) Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. Prog Neurobiol 93:1–12

Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, Gudz T, Macklin WB, Lewis DA, Fox RJ, Rudick R, Mirnics K, Trapp BD (2006) Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann Neurol 59:478–489

Edgar JM, McLaughlin M, Yool D, Zhang SC, Fowler JH, Montague P, Barrie JA, McCulloch MC, Duncan ID, Garbern J, Nave KA, Griffiths IR (2004) Oligodendroglial modulation of fast axonal transport in a mouse model of hereditary spastic paraplegia. J Cell Biol 166:121–131

Greenstein JI (2007) Current concepts of the cellular and molecular pathophysiology of multiple sclerosis. Dev Neurobiol 67:1248–1265

Hafler DA (2004) Multiple sclerosis. J Clin Invest 113:788–794

Kiryu-Seo S, Ohno N, Kidd GJ, Komuro H, Trapp BD (2010) Demyelination increases axonal stationary mitochondrial size and the speed of axonal mitochondrial transport. J Neurosci 30:6658–6666

Koga D, Ushiki T (2006) Three-dimensional ultra-structure of the Golgi apparatus in different cells: high-resolution scanning electron microscopy of osmium-macerated tissues. Arch Histol Cytol 69(5):357–374

Krumbholz M, Meinl E (2014) B cells in MS and NMO: pathogenesis and therapy. Semin Immunopathol 36:339–350

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA (2003) Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet 33:366–374

Martin R, McFarland HF, McFarlin DE (1992) Immunological aspects of demyelinating diseases. Annu Rev Immunol 10:153–187

Mayo L, Quintana FJ, Weiner HL (2012) The innate immune system in demyelinating disease. Immunol Rev 248:170–187

Misko A, Jiang S, Wegorzewska I, Milbrandt J, Baloh RH (2010) Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex. J Neurosci 30:4232–4240

Nikić I, Merkler D, Sorbara C, Brinkoetter M, Kreutzfeldt M, Bareyre FM, Brück W, Bishop D, Misgeld T, Kerschensteiner M (2011) A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. Nat Med 17:495–499

Nomura T, Bando Y, Bochimoto H, Koga D, Watanabe T, Yoshida S (2013) Three-dimensional ultra-structures of myelin and the axons in the spinal cord: application of SEM with the osmium maceration method to the central nervous system in two mouse models. Neurosci Res 75:190–197

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG (2000) Multiple sclerosis. N Engl J Med 343:938–952

Ohno N, Kidd GJ, Mahad D, Kiryu-Seo S, Avishai A, Komuro H, Trapp BD (2011) Myelination and axonal electrical activity modulate the distribution and motility of mitochondria at CNS nodes of Ranvier. J Neurosci 31:7249–7258

Osawa T, Ishida K, Obodera M, Feng XY, Hayashi S, Nozaka Y (2002) Measurement of the repeat period of myelin sheath using ultrathin frozen sections. J Electron Microscopy 51(3):195–197

Schirmer L, Merkler D, König FB, Brück W, Stadelmann C (2013) Neuroaxonal regeneration is more pronounced in early multiple sclerosis than in traumatic brain injury lesions. Brain Pathol 23:2–12

Stadelmann C, Wegner C, Brück W (2011) Inflammation, demyelination, and degeneration - recent insights from MS pathology. Biochim Biophys Acta 1812:275–282

Stirling DP, Stys PK (2010) Mechanisms of axonal injury: internodal nanocomplexes and calcium deregulation. Trends Mol Med 16:160–170

Stirling DP, Cummins K, Wayne Chen SR, Stys P (2014) Axoplasmic reticulum ca(2+) release causes secondary degeneration of spinal axons. Ann Neurol 75:220–229

Tanaka K, Mitsushima A (1984) A preparation method for observing intracellular structures by scanning electron microscopy. J Microsc 133:213–222

Trapp BD, Stys PK (2009) Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol 8:280–291

Yin X, Crawford TO, Griffin JW, Tu P-H, Lee VMY, Li C, Roder J, Trapp BD (1998) Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. J Neurosci 18:1953–1962

Zhang CL, Ho PL, Kintner DB, Sun D, Chiu SY (2010) Activity-dependent regulation of mitochondrial motility by calcium and Na/K-ATPase at nodes of Ranvier of myelinated nerves. J Neurosci 30:3555–3566

Therapeutic Strategies for Oligodendrocyte-Mediated Remyelination

17

Toru Ogata

Abstract

Given recent progress in our understanding of oligodendrocyte biology, significant attention has been directed toward cell therapy for myelin repair and remyelination. This trend has been reinforced by findings about the importance of white matter lesions in a variety of central nervous system (CNS) diseases, including demyelinating diseases as well as brain or spinal cord trauma and degenerative disorders such as Alzheimer's disease. Remyelination strategies include the implementation of myelin forming cells and the surrounding conditions and pathological disease context. Successful remyelination requires proper number of cells at the required location and subsequent maturation. Those processes involve variety of molecules, related to oligodendrocyte development or inflammation in the lesion. Understanding and manipulation of the functions of those molecules may improve the outcome of the cell therapies toward remyelination. Furthermore, the development of monitoring method for myelination is also anticipated to evaluate the effects of therapeutic interventions.

Keywords

Transplantation · Endogenous progenitors · Naked axon · Inflammation · Pharmacological intervention · Biomarkers · Diffusion tensor imaging

17.1 Remyelination Therapy for White Matter Lesions

Unlike gray matter that includes neuronal cell bodies and sites of synaptic activity, white matter mainly consists of axons and myelin-forming cells and functions to convey action potentials from neuron to neuron. Demyelination is the degradation of

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myelin structure with or without the death of myelin-forming cells (Barnett and Prineas 2004; Beattie et al. 2000). Myelin-forming cells also provide support to axons by supplying humoral factors or metabolites such as lactate (Lee et al. 2012). Therefore, the rationale of myelin conservation or repair has two aspects (1) facilitating the conduction of neuronal signals and (2) maintaining axons. Although recent findings underscore the importance of the latter function, it is not yet clear how long naked axons can survive in the human CNS before they eventually degenerate. The presence of viable naked axons is important for remyelination therapy as a prerequisite. The presence and viability of naked axons may vary by disease state; postmortem analyses of multiple sclerosis (MS) have revealed the presence of naked axons in plaques, while naked axons are not well documented in traumatic disorders. Additionally, timing of the intervention is another important consideration for remyelination therapy, including when the intervention is administered (in the early or late phases of injury) and how long treatments are required. These questions should be considered in the unique pathological context of each disease to achieve intervention success.

17.1.1 Myelin Protection and Remyelination

Many clinical trials for demyelinating diseases have investigated strategies to prevent demyelination, especially in MS. Because myelin-forming cells are vulnerable to extracellular stresses, these cells are commonly damaged by inflammatory cytokines, excitotoxicity, and oxidative stresses in immune-mediated and traumatic demyelination. Interleukin (IL)-1β, IL-2, interferon-gamma (IFNγ), and tumor necrosis factor (TNF)-α promote oligodendrocyte cell death in vitro. Some cytokines directly activate apoptotic signals within oligodendrocytes, while they induce inflammation in vivo, which leads to the production of free radicals. The free radicals, in turn, damages oligodendrocytes through oxidative stress. Alternatively, glutamate is an excitatory neurotransmitter that is released from axons, microglia, and macrophages after CNS insult. Elevations in extracellular glutamate lead to the activation of *α*-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptors, which subsequently produce increases in intracellular calcium and associated cellular damage (McTigue and Tripathi 2008). Accordingly, several myelin-protective strategies are designed to attenuate the inflammatory response. Methylprednisolone is a common steroidal anti-inflammatory drug that is used to treat acute episodes of MS. Given the side effects associated with steroid use, minocycline has also gained recent attention for its ability to attenuate microglial activation, although this mechanism requires further study. Several other molecules such as rolipram and atorvastatin are hypothesized to protect oligodendrocytes by interfering with the apoptosis signaling cascade (Mekhail et al. 2012).

While myelin protection is distinct from remyelination, it is sometimes difficult to separate these phenomena. In several cases, agents for myelin protection also promote remyelination. The demyelination and remyelination processes are linked

to some extent and can therefore occur simultaneously. Furthermore, several cytokines are relevant for both the survival and maturation of oligodendrocytes. For example, leukemia inhibitory factor (LIF) binds to the LIFR-beta receptor on oligodendrocytes and upregulates the anti-apoptotic molecule 14-3-3 or the cellular inhibitor of apoptosis proteins 2 (cIAP2), a caspase antagonist, to promote cell survival, while it also promotes cell differentiation through different downstream pathways (Mekhail et al. 2012). In the same manner, it is possible that remyelination interventions can simultaneously affect myelin protection. This is an important issue in evaluating drug interventions for white matter disorders. Observed increases in myelin structures at the end point can represent better oligodendrocyte survival, better remyelination, or both, unless white matter structures are evaluated repeatedly during the treatment course.

17.1.2 Effects of Remyelination on Functional Recovery

In contrast to the established rationale for myelin protection as a therapeutic strategy, strategies for remyelination are controversial. An important issue is whether there are enough viable naked axons in damaged white matter to yield an improvement in clinical symptoms after remyelination. In both the rodent and the human CNS, some populations of oligodendrocytes remain in the progenitor state after development and even in adulthood. These progenitors (oligodendrocyte progenitor cells, OPCs) are activated after white matter insult and contribute to spontaneous remyelination (Lopez Juarez et al. 2016). Spontaneous remyelination is now recognized as a main factor for spontaneous recovery in white matter diseases including traumatic injury and MS. In a murine model of spinal cord injury, preventing OPC remyelination with time-specific gene modulation retards motor recovery from 2 weeks after injury (Doi et al. 2017). Considering other reports showing that OPC-mediated spontaneous remyelination begins at 2 weeks after injury (McTigue et al. 2001), the current understanding is that OPCs have the potential to provide remyelination sufficient for functional recovery at several weeks after injury, at least in rodents.

Despite the beneficial effects of spontaneous remyelination early after a demyelinating insult, the utility of therapeutic interventions to promote remyelination of naked axons at later time points is unclear. As aforementioned, it is unclear whether axons that are not spontaneously remyelinated are viable or sufficient in number to produce clinical effects. Some reports suggest that there are very few naked axons late after chronic spinal cord injury in mice, either due to efficient spontaneous remyelination or poor survival of naked axons (Powers et al. 2012). In human, naked axons are observed in brain tissue from patients with MS, but little is known in the context of spinal cord injury. Later in this review, we will discuss a novel imaging technique that might provide information about the condition of naked axons in clinical patients.

17.1.3 Timing of Remyelination

The timing of remyelination therapy is important given the sensitivity of myelination processes to the local microenvironment and the need for completion within a certain timeframe to ensure success. Damaged tissue is exposed to various inflammatory cytokines in the acute phases of many diseases. Several cytokines such as TNF-α and IFNγ have been reported to interfere myelination or induce oligodendrocyte apoptosis (McTigue and Tripathi 2008). Therefore, remyelination strategies are not ideally feasible during the acute phase in the presence of uncontrolled inflammation. In the later stage, white matter is reconstructed by gliosis or glial scarring involving astrocytes, microglia, macrophages, and fibroblasts. The process of glial scar formation involves cytokines such as bone morphogenetic proteins and the induction of extracellular matrix molecules like chondroitin sulfate proteoglycan (CSPG). In both spinal cord injury and MS, CSPG prevents axonal regeneration and OPC migration and differentiation. Astrocytes are a main source of CSPG and, in the case of MS plaques, also express the Jagged1 ligand that activates canonical Notch signaling to inhibit oligodendrocyte differentiation (Dulamea 2017). The abundance of anti-myelination signals surrounding axons in the chronic stage of injury is thought to be main obstacle for recovery. Therefore, the ideal time window for remyelination should be the subacute phase of disease or injury when inflammation is resolving and gliosis is not yet prominent. Alternatively, remyelination therapy should be accompanied by co-therapies designed to suppress anti-myelination signals in the environment.

17.2 Cell Sources for Remyelination

The presence of an adequate number of myelinating cells is important for achieving remyelination sufficient to produce functional improvement. Possible candidates for CNS remyelination include oligodendrocytes and OPCs, Schwann cells, and olfactory ensheathing glia. While Schwann cells originate outside of the CNS, they migrate from the dorsal root entry zone into the spinal cord after injury. The utilization of these cells for therapeutic myelination requires an understanding of the characteristics and limitations of each cell type.

17.2.1 Endogenous OPCs

As described above, subpopulations of quiescent OPCs are responsible for spontaneous remyelination in the CNS, especially in the spinal cord. Stem cells in the subventricular zone of the brain also have the potential to differentiate into oligodendrocytes. In general, adult OPCs are diffusely located in white and gray matter and proliferate slowly. The rate of the OPC cell cycle decreases with advancing age. For example, in rodents, the cell cycle of OPCs in the corpus callosum is as short as 3 days during first several weeks and is extended to more

than 100 days after 1 year of age. Age-related cell cycle delay is attributed to a prolonged G1 period and the gradual accumulation of cell cycle inhibitory molecules such as p27 and p57 (Bergles and Richardson 2015). The initial trigger converting quiescent cells to proliferative and migratory progenitors is still unknown. It is likely that growth factors, such as platelet-derived growth factor (PDGF) α, are secreted from inflammatory cells to reactivate quiescent cells.

While remyelination by endogenous progenitors is an important mechanism for symptomatic recovery after MS, this myelination is incomplete and provides a rationale for strategies to support or enhance endogenous remyelination as a therapeutic intervention. Pharmacological agents can target both cell surface receptors and cytoplasmic signaling in OPCs as well as modulate the microenvironment surrounding axons to permit myelination.

17.2.2 Transplantation

Recent improvements in our understanding of neural stem cell biology together with the advancement of cell culture techniques have yielded cultured cell transplantation as a feasible therapeutic intervention. Various cell types have been proposed as candidates for cell therapy in CNS diseases. Regarding remyelination, transplantation therapies can be categorized in accordance with the type of cell to be transplanted and how the cells are transplanted (Table 17.1). The most direct approach uses OPCs as a cell source. So far, several protocols have been established to obtain high-purity OPCs. Among them, embryonic stem (ES) cell-derived OPCs are an attractive option for therapeutic purposes. Human ES cell-derived OPC transplantation has been reported in a rodent model of spinal cord injury with successful recovery when transplanted in 7 days after injury (Keirstead et al. 2005). Such studies demonstrate the ability of transplanted OPCs to remyelinate naked axons, as well as the presence of remaining axons to be remyelinated in the subacute phase of injury. A company reported clinical application of this approach in 2010 using a human ES cell known as GRNOPC1. The transplantation procedure was performed in several patients, but the company did not complete the clinical trial for financial reasons. More recently, another company developed the human ES cell-derived OPC line AST-OPC1, which is now being examined in a clinical trial for subacute spinal cord injury.

Undifferentiated stem cells represent another important option for CNS transplant and differentiation into oligodendrocyte lineage cells after transplantation. Neural stem cells and bone marrow-derived stem cells are two major cell sources for transplantation. Transplanted neural stem cells can differentiate into neurons, astrocytes, and oligodendrocytes. Although the key determinant for lineage selection is still unclear, signals from naked axons such as ATP and growth factors may promote oligodendrocyte differentiation and subsequent remyelination. The importance of oligodendrocyte lineage in neural stem cell transplantation was clearly shown by the study of spinal cord injury in which neural stem cells from wild-type mice produced better motor recovery than those from shiverer mutant mice

Table 17.1 Overview of cell transplantation therapy for myelination in the central nervous system

Cell source	Auto- or allo-graft	Method for transplantation	How to contribute to myelination?[a]
Bone marrow stem cell	Autograft	Local injection	Possible differentiation into oligodendrocytes after transplantation
		Intrathecal delivery	
		Intravenous delivery	
Human ES cell (Keirstead et al. 2005)	Allograft	Local injection	Possible differentiation into oligodendrocytes after transplantation
			Pre-treated to become oligodendrocyte lineage cells
Human iPS cell (Kawabata et al. 2016)	Allograft	Local injection	Possible differentiation into oligodendrocytes after transplantation
			Pre-treated to become oligodendrocyte lineage cells
Olfactory mucosa tissue (Lima et al. 2010)	Autograft	Local transplantation	Olfactory ensheathing cells within the olfactory mucosa
Peripheral nerve (Bunge et al. 2017)	Autograft	Local injection	Schwann cell cultured from the peripheral nerves

[a]All kind of cell therapy may contribute to myelination through the humoral factors secreted from the transplanted cells

lacking a gene for myelination (Yasuda et al. 2011). Alternatively, it is thought that OPCs can be differentiated from neural stem cells derived from inducible pluripotent stem cells (iPS cells). Because iPS cell transplantation circumvents several ethical issues associated with the use of ES cells or embryo-derived neural stem cells, iPS cell therapy is a promising approach for white matter lesions. On the other hand, one of the obstacles to be overcome to use iPS cell in clinical conditions is tumor genesis from the transplanted cells. The genomic and epigenetic characterization of each iPS cell line is needed to choose "safe" iPS cell line, which will be stored in iPS cell bank for clinical application.

The fate of transplanted cells is mostly governed by environmental cues unless the cells are genetically modified. Further interventions to promote OPC differentiation and/or myelin formation can enhance the success of transplantation strategies. Parts of these approaches may be similar to those for endogenous progenitor strategies because the environment of transplanted cells is similar to that of endogenous progenitors. Therefore, one might expect a combination transplantation and pharmacological therapy to produce greater success than either strategy alone in CNS diseases.

17.3 Stratified Steps for Remyelination

Regardless of the cellular source of myelination, progenitors located in damaged CNS tissue are subject to several steps before the completion of myelination. First, progenitors must proliferate to yield a quantity of cells sufficient to remyelinate a given lesion size and migrate to the lesion site. Then, progenitors undergo the process of axon myelination (Fig. 17.1). It should be emphasized that progenitors must remain undifferentiated and avoid apoptosis during all steps by resisting cellular stressors from the lesion environment.

17.3.1 Cell Number

OPC proliferation is mainly governed by growth factors such as PDGFα and basic fibroblast growth factor (bFGF). Under culture conditions, both PDGFα and bFGF are required for cell expansion and the suppression of maturation. Inflammation and the release of growth factors are characteristic features in the acute phase of CNS disorders. Endogenous OPCs proliferate from 2 to 4 weeks after injury in a rodent model of spinal cord injury (McTigue et al. 2001). Total cell number of OPCs is determined by the sum of proliferated cells and dead cells. Yet, it is unclear whether increased OPC proliferation after injury is sufficient to compensate for injury-associated demyelination. In the chronic phase of CNS disorders, OPC proliferation is limited by the absence of growth cues. In such cases, transplantation with a sufficient number of cells is a logical approach.

17.3.2 Migration

Transplant location is as important as the total cell number. Yet, little is known about the migration cues that are crucial for guiding OPCs toward naked axons. In the case of transplantation, local delivery adjacent to demyelinated axon seems to be a

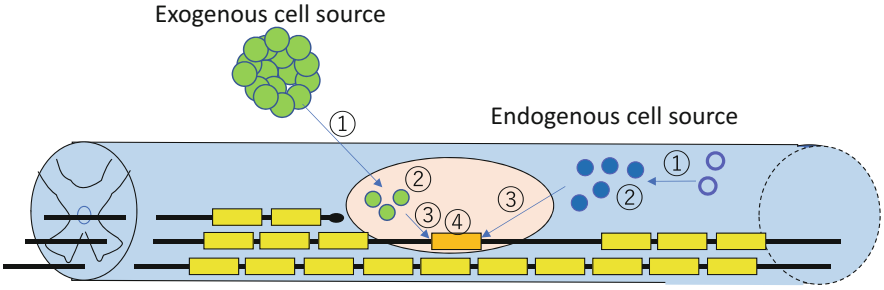

Fig. 17.1 Sequential steps for remyelination. ① Transplantation of the cell or activation of endogenous progenitors. ② Proliferation and/or survival of the cell. ③ Migration toward the demyelinated lesions. ④ Remyelination

reasonable strategy. Yet, this delivery method is not feasible from a practical viewpoint because (1) it is difficult to determine the precise locations of demyelinating lesions with currently available imaging and/or surgical techniques and (2) some demyelinating lesions are located deep or dispersed in CNS tissue. Therefore, it is necessary for cells to migrate some distance to naked axons.

So far, the most reliable guidance cues (e.g., ATP) are released from naked axons. Active axons are more likely to be myelinated by oligodendrocytes than quiescent axons (Fields 2015). Several other humoral factors such as neuregulin are responsible for axon–OPC cross talk. In addition to axon-specific guidance molecules, other extrinsic cellular factors can also affect progenitor migration. The extracellular matrix is one such factor that can prevent migration as a component of glial scarring around demyelinating lesions. Similar to the issue of axon regeneration through scar tissue, additional strategies are necessary to create a permissive environment for progenitors in this context.

17.4 Induction of Remyelination

After meeting the prerequisites of cell number and proper progenitor allocation, the last but most important step is myelination itself. Although the process of myelination is mainly governed by interactions between axons and OPCs, OPC maturation is affected by various extracellular and intracellular molecules. The initiation of myelin sheath formation around the axon is not always optimal, especially in pathological contexts. To obtain successful myelination, the conditions surrounding progenitors (whether transplanted or endogenous) must be suitable for maturation. That is, progenitors will remain undifferentiated until environmental conditions are conducive to differentiation. Otherwise, precocious maturation can lead to cells that are vulnerable to stressors and cell death. Therefore, the induction of myelination with proper timing is an important issue for consideration. Importantly, many molecules related to maturation are candidates as pharmacological agents in demyelinating disease.

17.4.1 Extracellular Targets for Promoting Oligodendrocyte Maturation

Myelination is affected by numerous extrinsic stimuli including humoral factors and cell adhesion molecules. Relevant humor factors include various types of receptor tyrosine kinases such as PDGF, hepatocyte growth factor, epidermal growth factor, neurotrophins, FGF, and neuregulin. Some of these molecules play roles in proliferation and maturation by activating intracellular ERK1/2 and AKT signaling. Extracellular signals can also come from G-protein-coupled receptors and their ligands; both GPR17 and GPR56 inhibit oligodendrocyte maturation via undetermined mechanisms (Bothwell 2017). In the developmental period, these molecules orchestrate the myelinating process, although the role of each molecule in pathological

contexts is poorly understood. It is possible that a lack of pro-myelinating factors and the presence of anti-myelinating factors inhibit remyelination in lesions. Consistent with this hypothesis, undifferentiated OPCs are often seen in demyelinating MS lesions. Leucine-rich repeat and immunoglobin-like domain-containing protein 1 (LINGO1) is a component of a receptor complex containing LINGO1, Nogo-66, and p75 neurotrophin receptor (or Tryo/Taj) that mediates the inhibitory effects of myelin protein on axonal growth. LINGO1 also inhibits myelin formation presumably via the LNGOI1/Nogo-66/p75NTR complex (Bothwell 2017). These findings provide a rationale for the development of a LINGO1 antibody to inhibit LINGO1 function and promote remyelination, especially in MS. Strategies applying pro-myelinating factors to demyelinating lesions are also under development. In this scenario, ligands for receptor tyrosine kinases are unlikely candidates because of non-selectivity, potentially promoting the proliferation of other cells or failing to promote the maturation of OPCs. Alternatively, quetiapine is an atypical antipsychotic drug and non-selective agonist for several GPCRs including D2 dopamine receptors, 5-HT2A and 5-HT 1A serotonin receptors, and adrenergic receptors that promotes oligodendrocyte maturation via the activation of ERK1/2 (Bothwell 2017). Given a multitude of potential targets and agents, several phase I/II clinical trials are underway in the context of MS.

17.4.2 Intracellular Targets

Multiple studies have demonstrated that the timing of OPC maturation is regulated by intrinsic cellular mechanisms such as the cell cycle inhibitor p57. The accumulation of p57 is a trigger for the genetic orchestration of myelination. Recent studies have also revealed the importance of epigenetic molecules that open or close the transcription regulatory site at appropriate times. Histone deacetylases (HDACs) are a class of epigenetic enzymes that modify histones to bind DNA more tightly, leading to a decrease in gene expression from the related locus. Administration of valpronic acid, a HDAC inhibitor, induces hypomyelination in the developing rat brain, indicating that HDACs are necessary for myelination (Shen et al. 2005). The definite targets of HDACs for myelination are still elusive, but several suppressor genes for maturation have been proposed including Id2, Egr1, and Sox11 (Swiss et al. 2011).

Alternatively, the chromodomain helicase DNA binding protein (Chd) family of molecules is a class of ATP-dependent chromatin-remodeling enzymes that regulates gene expression in neural cells. Among them, Chd7 regulates both proliferation and differentiation by forming complexes with transcription molecules such as those from the Sox family. During progenitor proliferation, Chd7 complexes with Sox2 to induce gene expression for oligodendrocyte lineage maintenance and proliferation. In a later phase, Chd7 induces myelinating genes presumably by forming a complex with Sox10 (Doi et al. 2017). Epigenetic molecules are now important targets of drug development, especially in cancer research. Therefore,

understanding the function of each epigenetic molecule is important for determining its exact utility for remyelination.

17.4.3 Role of Astrocytes in Remyelination

Astrocytes are the most abundant cell type in remyelinating lesions. Under physiological conditions, astrocytes maintain the local microenvironment through functions such as neurotransmitter reuptake and supporting the blood–brain barrier. Upon insult, astrocytes become activated and secrete various factors including growth factors. Activated astrocytes can become hypertrophic and express a scar-forming phenotype. Astrocytes also release several cytokines such as LIF, ciliary neurotrophic factor, neurotrophin-3, and insulin-like growth factor 1 (Domingues et al. 2016). Additionally, cell contact between astrocytes and oligodendrocytes facilitates myelination. This interaction is in part mediated by the binding of alpha6-beta1 integrin on oligodendrocytes and laminin on astrocytes. Accordingly, astrocytes are indispensable for remyelination, and irradiation of astrocytes in a given lesion prohibits successful remyelination in vivo (Harrison 1985). Alternatively, astrocytes also regulate inflammation. Recent findings show that paracrine interactions between astrocytes and microglia through chemokine messengers such as CCL-2 exacerbate inflammation and worsen demyelination (Okazaki et al. 2016). The supportive versus inhibitory functions of astrocytes for remyelination may depend on the tissue context. Considering the potential for astrocytes to support myelination, these cells represent a feasible therapeutic target for future investigation.

17.4.4 Role of Axons in Remyelination

Axon characteristics are also important for myelination. In addition to the role of axons as a structural target for wrapping of the myelin sheath, recent reports indicate that neural activation promotes myelination. In rodents, learning induces structural changes in white matter areas relevant to memory function (Sampaio-Baptista et al. 2013). This phenomenon is consistent with the idea that repeated activation optimizes connectivity between neurons; axons connecting active neurons gain a better transduction efficacy over time, which subsequently increases the probability that related synapses will be maintained. Several factors are proposed to mediate signals between axons and oligodendrocytes. For example, vesicular neurotransmitter release occurs not only at synapses but also at the axonal membrane. Electrical stimulation of axons causes the release of vesicular glutamate from axons and the activation of NMDA receptors and metabotropic glutamate receptors on oligodendrocytes. This axonal input leads to the activation of FYN and local translation of myelin molecules in oligodendrocytes. Axons also release non-vesicular messengers; for example, volume-regulated anion channels on axons facilitate adenosine or ATP release following axon activation. These neurotransmitters may promote oligodendrocyte maturation directly or indirectly by signaling astrocytes to secrete growth factors (Fields 2015).

Thus far, activation-dependent axon–oligodendrocyte interactions have primarily been investigated under physiological (healthy) conditions, such that the relevance of these pathways in pathological contexts remains unclear. From a rehabilitation perspective, temporal-dependent plasticity is critical for stabilizing synapses for motor learning. If neuronal activity is also important for remyelination, approaches to enhance plasticity can be also introduced for rehabilitation. Potential strategies include not only physical rehabilitation with a therapist but also physical stimuli such as electrical or magnetic stimulation to activate neurons and promote myelination.

17.5 Evaluation of Myelin Repair

Methods for the accurate determination of myelin integrity and repair are critical for identifying effective therapeutic interventions. In neurological disorders, clinical symptoms such as motor dysfunction and pain are frequently used as primary endpoints to measure drug efficacy. Yet, secondary endpoints that explain the reason for clinical improvements are also necessary for the drug development process. While remyelination evaluation in an animal model has been established by histological analysis, such evaluations are difficult or not feasible in human studies. Therefore, the need for better techniques to assess myelination is a critical issue in this research field.

17.5.1 Imaging

Magnetic resonance imaging (MRI) is one of the most powerful tools for detecting changes in CNS. Recent advancements in MRI and especially in diffusion imaging have enabled the observation of myelin structure in clinical cases. Fujiyoshi et al. (2016) reported a novel method known as myelin mapping using diffusion tensor imaging to detect white matter volume on MRI. The movement of water is tightly constrained with specific alignment in the myelin sheath and can be evaluated with a specific water diffusion protocol. In a primate model of spinal cord injury, myelin map images were virtually identical to corresponding histological samples. While images do not distinguish between newly formed myelin (remyelination) and surviving myelin after an insult, repetitive imaging has the potential for assessing post-injury remyelination. Moreover, combined assessments of axons by tractography, another subtype of diffusion imaging, can provide additional information about the therapeutic effects of remyelination interventions.

Other imaging techniques relevant to remyelination assessment include positron emission tomography (PET). In PET imaging, the target tissue is labeled with a specific radiolabeled compound with high affinity and specificity. Several myelin-specific tracers such as thioflavin-based compounds are currently under investigation for clinical. One longitudinal study has already reported that the accumulation of the tracer shows a good correlation with patient symptoms (Bodini et al. 2016).

17.5.2 Cerebrospinal Fluid (CSF) Biomarkers

Blood and CSF biomarkers are widely used for the diagnosis and evaluation of many diseases. For example, the presence of oligoclonal bands of IgG in CSF is a classic and important diagnostic factor for MS. Recently, several promising biomarkers have been proposed in myelin-related disorders. Chitinase 3 and the light subunit of neurofilaments have been proposed as prognostic indicators in demyelinating disease (Comabella et al. 2016). Several other inflammatory biomarkers have been posited detect demyelinating disease progression. Yet, little is known about remyelination markers. Recent advancements suggest that microRNAs are useful indicators of various biological processes. Among them, miR-219 is required for oligodendrocyte maturation and can be detected in CSF. One study demonstrated that the level of miR-219 in CSF was lower in patients with MS than in healthy control subjects, indicating the consumption of cell-free miR-219 in the site of remyelination (Bruinsma et al. 2017). Yet, there are no established biochemical markers for evaluating the remyelination process directly. Additional knowledge about the myelin formation processes may yield novel candidates in the future.

17.5.3 Electrophysiology

Given the role of myelin as an insulator for the propagation of axon actions potentials, changes in myelin are reflected in the pulse waves conducted along myelinated axons. Demyelination results in decreased amplitudes and dispersed waveforms beyond the lesioned areas. Therefore, improvements in myelin structure are reflected by electrophysiological parameters. In CNS disorders, transcranial magnetic stimulation can be used to activate descending tracts for peripheral nerve conduction studies that then reflect the integrity of upstream tracts. Activated descending tracts subsequently activate motor neurons at the spinal segment, leading to motor evoked potentials at the peripheral target. Importantly, this type of study reflects the overall activity of the corticospinal tract. Therefore, this technique does not indicate whether lesions are at the level of the brain or spinal cord. More invasive measurements, such as spinal stimulation, are necessary for detail examinations, but not impractical in a clinical setting. As an alternative, new methods such as magnetospinography have been proposed for the non-invasive analysis of conduction within the spinal cord. Like magnetoencephalography, magnetospinography permits the detection of weak magnetic fields associated with spinal cord neuronal activity with low-temperature superconducting quantum interference device (SQUID)-based magnetic flux sensors (Adachi et al. 2013). So far, it is not clear whether this technique provides adequate resolution for detecting therapeutic changes in white matter lesions.

17.5.4 Symptoms

Perhaps most important is the relevance of white matter recovery to disease symptoms and patient quality of life, especially compared to currently available treatment options. Motor functional scores from manual muscle testing and spinal cord independence measures evaluating functional independence are common endpoints in spinal cord injury. Neurological function is commonly assessed based on a variety of neural functions and action potential characteristics. Although expected changes to white matter lesions after therapy can be clearly defined, it is difficult to predict the expected degree of symptomatic recovery. Taken together, the clinical evaluation of therapeutic interventions targeting white matter lesions should pay careful attention to the selection of outcomes, including structural and physiological changes in white matter as well as adequate assessment of functions in daily life.

17.6 Other Issues for Clinical Application of Remyelination

While numerous approaches to the treatment of white matter disease have been proposed from basic research, it is important to be aware of the differences between rodents and humans in terms of glial and specifically oligodendrocyte function, especially in the adult life stage. Although evidence has identified dormant OPCs in the adult human brain and spinal cord that are capable of reentering the cell cycle and contributing to remyelination following a pathological insult, the capacity of these cells for proliferation may be limited compared to that of rodent cells. One study reported that remyelination in humans is achieved with little contribution from OPCs and that preexisting oligodendrocytes are predominantly responsible for remyelination after injury (Bothwell 2017). Furthermore, the distribution of OPCs in humans differs from that in rodents. These considerations are important for therapeutic attempts to enhance the activity of endogenous progenitors. As for cell transplantation strategies, there are important inter-species differences in the efficacy of myelin wrapping. In general, human OPCs take longer than rodent OPCs to complete the myelination process. A longer duration of remyelination may be associated with a higher risk of interference by processes derived from pathological contexts. Future studies are required to investigate the characteristics of human OPCs in therapeutic contexts and specifically their capacities for expansion and maturation.

17.7 Summary

This chapter provides an overview of therapeutic approaches to promote oligodendrocyte remyelination in the CNS. Recent drug development for remyelination and emerging cell engineering techniques will hopefully lead to clinical trials in which remyelination is assessed both directly through techniques such as MRI and

indirectly through patient symptoms. Regardless of the approach, using endogenous cells or transplanting cells, an important issue is how to understand the conditions of OPCs within the body, and what co-strategies are necessary to support endogenous or transplanted OPCs. There is a need for additional information about the extent to which OPCs are able to remyelinate lesions on their own and obstacles impeding these processes. There is also a need for improvement in the available methodology for monitoring OPCs and myelination in vivo. This information will ultimately contribute to the development of oligodendrocyte-based strategies for demyelinating neurological disorders.

References

Adachi Y, Oyama D, Kawai J, Kawabata S, Uehara G (2013) Spinal cord evoked magnetic field measurement using a magnetospinography system equipped with a cryocooler. Conf Proc IEEE Eng Med Biol Soc 2013:4426–4429. https://doi.org/10.1109/EMBC.2013.6610528

Barnett MH, Prineas JW (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 55:458–468. https://doi.org/10.1002/ana.20016

Beattie MS, Farooqui AA, Bresnahan JC (2000) Review of current evidence for apoptosis after spinal cord injury. J Neurotrauma 17:915–925. https://doi.org/10.1089/neu.2000.17.915

Bergles DE, Richardson WD (2015) Oligodendrocyte development and plasticity. Cold Spring Harb Perspect Biol 8:a020453. https://doi.org/10.1101/cshperspect.a020453

Bodini B et al (2016) Dynamic imaging of individual remyelination profiles in multiple sclerosis. Ann Neurol 79(5):726–738. https://doi.org/10.1002/ana.24620

Bothwell M (2017) Mechanisms and medicines for remyelination. Annu Rev Med 68:431–443. https://doi.org/10.1146/annurev-med-050715-104400

Bruinsma IB et al (2017) Regulator of oligodendrocyte maturation, miR-219, a potential biomarker for MS. J Neuroinflammation 14:235. https://doi.org/10.1186/s12974-017-1006-3

Bunge MB, Monje PV, Khan A, Wood PM (2017) From transplanting Schwann cells in experimental rat spinal cord injury to their transplantation into human injured spinal cord in clinical trials. Prog Brain Res 231:107–133. https://doi.org/10.1016/bs.pbr.2016.12.012

Comabella M, Sastre-Garriga J, Montalban X (2016) Precision medicine in multiple sclerosis: biomarkers for diagnosis, prognosis, and treatment response. Curr Opin Neurol 29:254–262. https://doi.org/10.1097/WCO.0000000000000336

Doi T, Ogata T, Yamauchi J, Sawada Y, Tanaka S, Nagao M (2017) Chd7 collaborates with Sox2 to regulate activation of oligodendrocyte precursor cells after spinal cord injury. J Neurosci 37:10290–10309. https://doi.org/10.1523/JNEUROSCI.1109-17.2017

Domingues HS, Portugal CC, Socodato R, Relvas JB (2016) Oligodendrocyte, astrocyte, and microglia crosstalk in myelin development, damage, and repair. Front Cell Dev Biol 4:71. https://doi.org/10.3389/fcell.2016.00071

Dulamea AO (2017) The contribution of oligodendrocytes and oligodendrocyte progenitor cells to central nervous system repair in multiple sclerosis: perspectives for remyelination therapeutic strategies. Neural Regen Res 12:1939–1944. https://doi.org/10.4103/1673-5374.221146

Fields RD (2015) A new mechanism of nervous system plasticity: activity-dependent myelination. Nat Rev Neurosci 16:756–767. https://doi.org/10.1038/nrn4023

Fujiyoshi K et al (2016) Application of q-space diffusion MRI for the visualization of white matter. J Neurosci 36:2796–2808. https://doi.org/10.1523/JNEUROSCI.1770-15.2016

Harrison B (1985) Schwann cell and oligodendrocyte remyelination in lysolecithin-induced lesions in irradiated rat spinal cord. J Neurol Sci 67:143–159

Kawabata S et al (2016) Grafted human iPS cell-derived oligodendrocyte precursor cells contribute to robust remyelination of demyelinated axons after spinal cord injury. Stem Cell Rep 6:1–8. https://doi.org/10.1016/j.stemcr.2015.11.013

Keirstead HS, Nistor G, Bernal G, Totoiu M, Cloutier F, Sharp K, Steward O (2005) Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 25:4694–4705. https://doi.org/10.1523/JNEUROSCI.0311-05.2005

Lee Y et al (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature 487:443–448. https://doi.org/10.1038/nature11314

Lima C et al (2010) Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. Neurorehabil Neural Repair 24:10–22. https://doi.org/10.1177/1545968309347685

Lopez Juarez A, He D, Richard Lu Q (2016) Oligodendrocyte progenitor programming and reprogramming: toward myelin regeneration. Brain Res 1638:209–220. https://doi.org/10.1016/j.brainres.2015.10.051

McTigue DM, Tripathi RB (2008) The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107:1–19. https://doi.org/10.1111/j.1471-4159.2008.05570.x

McTigue DM, Wei P, Stokes BT (2001) Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord. J Neurosci 21:3392–3400

Mekhail M, Almazan G, Tabrizian M (2012) Oligodendrocyte-protection and remyelination post-spinal cord injuries: a review. Prog Neurobiol 96:322–339. https://doi.org/10.1016/j.pneurobio.2012.01.008

Okazaki R et al (2016) The crucial role of Erk2 in demyelinating inflammation in the central nervous system. J Neuroinflammation 13:235. https://doi.org/10.1186/s12974-016-0690-8

Powers BE, Lasiene J, Plemel JR, Shupe L, Perlmutter SI, Tetzlaff W, Horner PJ (2012) Axonal thinning and extensive remyelination without chronic demyelination in spinal injured rats. J Neurosci 32:5120–5125. https://doi.org/10.1523/JNEUROSCI.0002-12.2012

Sampaio-Baptista C et al (2013) Motor skill learning induces changes in white matter microstructure and myelination. J Neurosci 33:19499–19503. https://doi.org/10.1523/JNEUROSCI.3048-13.2013

Shen S, Li J, Casaccia-Bonnefil P (2005) Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain. J Cell Biol 169:577–589. https://doi.org/10.1083/jcb.200412101

Swiss VA, Nguyen T, Dugas J, Ibrahim A, Barres B, Androulakis IP, Casaccia P (2011) Identification of a gene regulatory network necessary for the initiation of oligodendrocyte differentiation. PLoS One 6:e18088. https://doi.org/10.1371/journal.pone.0018088

Yasuda A et al (2011) Significance of remyelination by neural stem/progenitor cells transplanted into the injured spinal cord. Stem Cells 29:1983–1994. https://doi.org/10.1002/stem.767

Brain Tumors of Glial Origin

18

Christopher A. Waker and Robert M. Lober

Abstract

Gliomas are a heterogeneous group of tumors with evolving classification based on genotype. Isocitrate dehydrogenase (IDH) mutation is an early event in the formation of some diffuse gliomas, and is the best understood mechanism of their epigenetic dysregulation. Glioblastoma may evolve from lower-grade lesions with IDH mutations, or arise independently from copy number changes in platelet-derived growth factor receptor alpha (*PDGFRA*) and phosphatase and tensin homolog (*PTEN*). Several molecular subtypes of glioblastoma arise from a common proneural precursor with a tendency toward transition to a mesenchymal subtype. Following oncogenic transformation, gliomas escape growth arrest through a distinct step of aberrant telomere reverse transcriptase (TERT) expression, or mutations in either alpha thalassemia/mental retardation syndrome (*ATRX*) or death-domain associated protein (*DAXX*) genes. Metabolic reprogramming allows gliomas to thrive in harsh microenvironments such as hypoxia, acidity, and nutrient depletion, which contribute to tumor initiation, maintenance, and treatment resistance.

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Glioma · Metabolism · Cancer · Stem cell · Glycolysis · Hypoxia · Pediatric · Tumor

18.1 Introduction

Gliomas represent a diverse, heterogeneous group of tumors with evolving nosological classification based on recent understanding of genotypic features. Several putative origins are primitive neural stem cells, glial progenitor cells, or dedifferentiated mature astrocytes, oligodendrocytes, or ependymal cells (Ferris et al. 2017). Children and adolescents are more likely to have well-circumscribed low-grade types, whereas adults are more likely to have diffuse, infiltrating tumors without a clear margin (Ferris et al. 2017) (Fig. 18.1).

18.1.1 Classification

In 2016, the World Health Organization provided a landmark update to glioma classification, incorporating molecular parameters and emphasizing genotype for diagnosis (Louis et al. 2016). Prior to this, clinical grading was performed solely on histology (Table 18.1), identifying glioblastoma solely by angiogenesis and necrosis (Ferris et al. 2017). Diagnosis has now been refined with several recognized

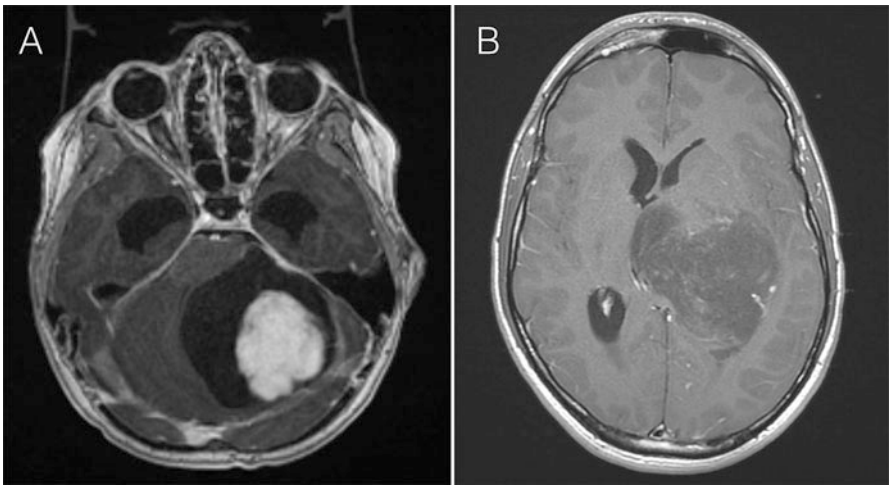

Fig. 18.1 Magnetic resonance images of gliomas. (**a**) Well-circumscribed cerebellar pilocytic astrocytoma with cystic and solid enhancing components, resulting in obstructive hydrocephalus in an 11-year-old boy. (**b**) Diffuse, infiltrative glioblastoma with heterogeneous enhancement involving the thalamus of a 16-year-old boy

Table 18.1 Conventional morphologic classification of diffuse gliomas[a]

Grade	Classification	Features
I	Not applicable for diffuse glioma	This grade is reserved for generally well-circumscribed low-grade tumors such as pilocytic astrocytoma, ganglioglioma, dysembryoplastic neuroepithelial tumor, and pleomorphic xanthoastrocytoma
II	Diffuse astrocytoma	Mild hypercellularity and nuclear atypia (e.g., enlarged, irregular, elongated, or hyperchromatic)
	Oligodendroglioma	Uniform nuclei with coarse chromatin and cytoplasm. Artifactual perinuclear clearing (i.e., "fried egg" appearance on formalin fixation. Prominent branching capillary network (i.e., "chicken wire" appearance), with calcifications and minigemistocytes
III	Anaplastic astrocytoma or oligodendroglioma	Increased cellularity and increased mitoses
IV	Glioblastoma	Microvascular proliferation and necrosis

[a]Adapted from Ferris et al. (2017)

Table 18.2 Glioblastoma subtypes based on transcriptome and epigenomic profiling[a]

Phenotype		Characteristic aberration
Proneural	Glioma CpG island methylator phenotype (G-CIMP)	IDH1 and IDH2 mutation
		TP53 mutation
		ATRX mutation
	Non-G-CIMP proneural	IDH1 wild type
		PDGFRA amplification
		CDK4 amplification
		DLL3 and OLIG2
Classical (proliferative)		EGFR amplification, EGFRvIII
Mesenchymal		NF1 mutation
		CD40
		CHI3LI/YKL-40
Neural		Variable

[a]Bhat et al. (2013) and Patel et al. (2014)

subtypes from global transcript profiling and DNA methylation analysis (Table 18.2) (Verhaak et al. 2010).

The more prognostically favorable glioma CpG island methylator phenotype (G-CIMP), which is tightly associated with IDH mutation and functional loss of TP53 and ATRX, is characteristic of "secondary" glioblastoma evolving from lower-grade gliomas (Ohgaki and Kleihues 2013; Noushmehr et al. 2010). Most G-CIMP tumors have a proneural transcription profile, defined by high expression of oligodendrocyte development genes such as *PDGFRA*, *neurokinin receptor homeobox 2* (*NKX2–2*), and *oligodendrocyte transcription factor 2* (*OLIG2*) (Verhaak et al. 2010).

In contrast, non-G-CIMP tumors arise de novo as "primary" glioblastoma and have worse clinical outcomes. These are subdivided into proneural, neural, classical, and mesenchymal subtypes (Verhaak et al. 2010) of variable prognostic significance (Nakano 2014). Proneural and mesenchymal transcriptional profiles are the most distinct, while the others are poorly demarcated (Nakano 2015). All likely evolve from a common proneural-like precursor with copy number changes in *PDGFRA* and *PTEN* (Ozawa et al. 2014).

There is plasticity between subtypes, with a tendency toward proneural-to-mesenchymal transition under selection pressure from cytotoxic therapy, or at the time of tumor recurrence (Halliday et al. 2014; Bhat et al. 2013; Piao et al. 2013; Phillips et al. 2006). Intratumoral mosaicism occurs, with individual gene signatures found within different histologic patterns (Jin et al. 2017). For example, vascular areas may have a proneural profile, whereas necrotic or hypoxic areas may have a mesenchymal pattern (Jin et al. 2017).

18.1.2 Epigenetic Dysregulation in Gliomas

Epigenetic reprogramming of DNA methylation and histone modifications is characteristic of all gliomas. The most commonly studied mechanisms are DNA methylation at long repeats of cytosine-guanine dinucleotide pairs, so-called "CpG islands" within gene promoters (Gusyatiner and Hegi 2017). Hypermethylation of CpG islands attracts methyl-CpG-binding protein 2 (MECP2) and additional machinery to silence the expression of numerous tumor suppressors in glioblastoma, such as WNT inhibitory factor 1 and negative regulators of the Ras pathway (Lambiv et al. 2011; Gotze et al. 2010; Horiguchi et al. 2003).

Histone proteins, the core components of nucleosomes and heterochromatin, are subject to highly specific and dynamically reversible posttranslational modifications, termed "histone marks," which modulate gene expression. Histone 3 proteins at active transcription sites are trimethylated at lysine 4 (H3K4me3) by trithorax protein complexes (Nishioka et al. 2002), whereas those at inactive sites are trimethylated at lysine 27 (H3K27me3) by polycomb repressive complex 2 (PRC2) (Gusyatiner and Hegi 2017). The chromobox homolog 7 (CBX7) protein, which recognizes H3K27me3 marks and facilitates transcriptional repression for cell cycle control, is a tumor suppressor that has reduced expression in high-grade gliomas (Yu et al. 2017).

Embryonic stem cells and glioma stem-like cells (GSCs) display simultaneous enhancing and repressive marks at certain gene promoters in a so-called "bivalent state," allowing for paused transcription rather than full repression (Lin et al. 2015). This state can be induced by hypoxia through inactivation of oxygen-dependent JumonjiC (JmjC) domain-containing histone demethylases (Prickaerts et al. 2016). JmjC domain-containing protein 3 (JMJD3) is an H3K27me3-specific demethylase overexpressed in gliomas, and its inhibition has antitumor activity in preclinical studies (Sui et al. 2017).

18.1.3 Isocitrate Dehydrogenase Mutants and the Glioma CpG Island Methylator Phenotype

Although most cases are IDH wild type (Yan et al. 2009; Noushmehr et al. 2010), the IDH mutation is the best understood modifier of the glioblastoma epigenome (Turcan et al. 2017). Two IDH isoforms (cytosolic IDH1 and mitochondrial IDH2) catalyze $NADP^+$-dependent enzymatic reduction of isocitrate to α-ketoglutarate (α-KG), producing NADPH necessary for glutathione to defend against reactive oxygen species and DNA damage (Miller et al. 2017; Parsons et al. 2008; Watanabe et al. 2009; Yan et al. 2009; Jo et al. 2002; Lee et al. 2002). Mutation of IDH is sufficient to establish the G-CIMP (Turcan et al. 2012), as neo-enzymatic conversion of α-KG to the oncometabolite D-2-hydroxyglutarate (2HG) inhibits α-KG-dependent enzymes, including the ten-eleven translocation (TET) family of 5′-methylcytosine (5mC) hydroxylases and JmJC domain-containing histone demethylases (Adam et al. 2014).

The *MGMT* gene encodes O^6-methylguanine-DNA methyltransferase, a protein that repairs the damage of alkylating agents (Tano et al. 1990; Cabrini et al. 2015; Esteller et al. 2000; Belanich et al. 1996). Methylation of the *MGMT* promoter is closely associated with IDH mutation and the G-CIMP phenotype, and has been one of the most clinically relevant predictors of therapeutic response (Houdova Megova et al. 2017; Mur et al. 2015; Hegi et al. 2005). However, IDH1 mutation has now surpassed *MGMT* promoter methylation as a prognostic factor for survival and *MGMT* promoter mutation is now thought to be independently predictive of treatment response only in the classical subtype of glioblastoma (Houdova Megova et al. 2017; Brennan et al. 2013).

IDH mutations are early events in the majority of low-grade gliomas in adult patients, affecting 80–90% of grade II and III tumors and only 5% of grade IV tumors, defining a subtype with a favorable prognosis (Hartmann et al. 2009; Johnson et al. 2014; Yan et al. 2009; Miller et al. 2017; Parsons et al. 2008; Watanabe et al. 2009). They are not typically seen in pediatric gliomas, which instead are often driven by aberrant mitogen-activated protein kinase (MAPK) pathways (Venneti and Huse 2015).

18.1.4 Histone 3 Lysine 27 Mutants

Point mutations in the histone genes *H3F3A* or *HIST1H3B*, substituting lysine 27 for methionine (H3 K27 M), likely occur in actively dividing neural precursors during central nervous system development (Wright et al. 2015; Pathania et al. 2017). They are mutually exclusive of IDH mutations, and characteristic for gliomas of the brain midline, frequently diffuse intrinsic pontine gliomas (DIPG) that primarily occur in children (Yan et al. 2009). The effect is inhibition of the polycomb repressive complex 2 (PRC2), leading to a global reduction of the H3K27me3 repressive mark (Lewis et al. 2013; Bender et al. 2013). Despite their exclusivity, H3 K27 M tumors and IDH mutant gliomas share common partner mutations, like *TP53* and

ATRX (Nikbakht et al. 2016). They commonly occur with *PDGFRA* amplification and mutations in *PIK3R1*, *PTEN*, and *ACVR1* (Nikbakht et al. 2016).

18.1.5 Maintenance of Telomere Length

Replicative immortality is a tumor property separate from proliferative capacity, requiring avoidance of natural telomere shortening. As with other tumor types, glioma cells achieve this through several mechanisms outside of the initial events of oncogenic transformation (Bell et al. 2015; Hahn et al. 1999; Ohba et al. 2016).

Maintenance of telomere length may occur through mutations in the telomere reverse transcriptase (*TERT*) promoter, which is characteristic of IDH wild type, that is, non-G-CIMP glioblastoma, as well as IDH mutant and 1p/19q-co-deleted oligodendrogliomas (Killela et al. 2013). The *TERT* gene is normally silenced in adult somatic cells but is aberrantly expressed when mutations in its promoter unmask novel binding sites for E-twenty-six/ternary complex factors (Ets/TCF) (Huang et al. 2013; Ahmad et al. 2017). This mechanism is rare in pediatric brain tumors, which are more likely to have developmentally appropriate telomerase activity, harbor *TERT* promoter hypermethylation, or have rearrangements placing the *TERT* gene next to strong enhancer elements (Peifer et al. 2015; Castelo-Branco et al. 2013; Koelsche et al. 2013).

In IDH-mutated astrocytomas, for example, G-CIMP glioblastoma in adults and H3 histone-mutated gliomas in children, telomerase-independent alternate lengthening of telomeres (ALT) may occur through mutation of alpha thalassemia/mental retardation syndrome (*ATRX*) or death domain-associated protein (*DAXX*) genes (Koschmann et al. 2016; Leung et al. 2013; Killela et al. 2013). In G-CIMP and H3 K27 M tumors, loss of ATRX is frequently found in association with G1/S checkpoint disruption by *TP53* mutation (Nikbakht et al. 2016).

18.2 Glioma Stem Cells

A fraction of glioma cells possesses self-renewal and multi-lineage differentiation properties with a gene expression pattern similar to that of non-neoplastic stem cells (Reya et al. 2001). While these glioma stem-like cells (GSCs) have important roles in tumor initiation, maintenance, and treatment resistance, they might be a tumor epiphenomenon rather than its source (Bakhshinyan et al. 2018). As examples, proneural subtype gliomas probably arise from neural stem cells, while mesenchymal subtype gliomas and oligodendrogliomas probably arise from dedifferentiated mature non-stem cells (Nakano 2015).

Confirmation of GSC isolation from tumors requires demonstrated capacity for tumor initiation, clonal expansion, and production of more differentiated progeny (Meacham and Morrison 2013). The cell surface antigen Cluster of Differentiation 133 (CD133) was originally proposed as a definitive marker for GSCs, followed by several others, for example, CD15, L1 Cell Adhesion Molecule (L1CAM), and

integrin α6, but none of these have proven to be universal or exclusive markers (Nakano 2015). Proneural subtype GSCs express CD133 and CD15, a marker of embryonic and adult neural stem cells, whereas mesenchymal subtype GSCs lack these markers and express CD44, a marker of astrocyte-restricted precursor cells (Nakano 2015).

Single-cell RNA sequencing of gliomas demonstrates intratumoral variation in transcription profiles with a fraction of proliferating GSCs found in various histologic niches (Jin et al. 2017). GSCs are not uniformly distributed, but preferentially localize to perivascular and hypoxic areas and are maintained by microenvironmental stressors such as hypoxia, low pH, and nutrient depletion (Li et al. 2009; Flavahan et al. 2013; Calabrese et al. 2007; Bao et al. 2006; Hjelmeland et al. 2011). It is unknown whether GSCs have an irreversible state whose population varies or a dynamic state with switching between cell types in response to environmental cues (Nakano 2014). As harsh microenvironmental conditions such as glucose restriction lead to preferential GSC survival and acquisition of stem-like features in non-GSCs, it is plausible that progeny cells that have lost stem cell properties might be able to reacquire the GSC phenotype, similar to what has been seen in breast cancer cells subjected to radiation therapy (Flavahan et al. 2013; Lagadec et al. 2012). This type of switching significantly complicates the study of glioma genetics, as mutations found in non-proliferative end-stage progeny, for example, non-GSCs, might be considered "passenger mutations" that decrease the signal-to-noise ratio in detecting mutations that drive gliomagenesis (Baysan et al. 2017).

The subventricular zone (SVZ) is one of several anatomic niches supporting stem cell maintenance in the adult brain (Sanai et al. 2011; Paredes et al. 2016). It is located adjacent to the lateral ventricles within the cerebrum, and normally produces interneurons destined for either the olfactory bulb or the ventromedial prefrontal cortex in the early postnatal human (Sanai et al. 2011; Paredes et al. 2016). Its ependymal cells support both neural stem cells (NSCs) and GSCs by producing pigment epithelium-derived factor (PEDF), which promotes proliferation, suppression of differentiation genes, and expression of sex-determining region Y-box 2 (SOX2) transcription factor (Yin et al. 2015). Other SVZ ependymal signals that create a favorable environment for GSCs include noggin and stromal-derived factor 1 (SDF-1 or CXCL12) (Lim et al. 2000; Piccirillo et al. 2006; Kokovay et al. 2010; Goffart et al. 2015).

Gliomas may arise within the SVZ, or migrate toward SDF-1/CXCL12 to "co-opt" this niche that is favorable for GSC proliferation and cell migration (Sinnaeve et al. 2017; Shiozawa et al. 2011; Goffart et al. 2017). The SVZ has a rich vasculature around which NSCs and GSCs tend to cluster (Calabrese et al. 2007). Endothelial cells within the SVZ are closely associated with NSCs, also secreting PEDF, as well as brain-derived neurotrophic factor (BDNF), placental growth factor 2 (PIGF-2), and vascular endothelial growth factor (VEGF) (Sinnaeve et al. 2017). Direct contact with endothelial cells supports NSC self-renewal through ephrinB2 and Jagged1 (Ottone et al. 2014).

18.3 Metabolism Within Normal and Neoplastic Glial Cells

Within the brain, lactate and glucose have similar concentrations (Schurr et al. 1999; Gallagher et al. 2009; Smith et al. 2003; Boumezbeur et al. 2010b). Astrocytes are likely the main source of high extracellular lactate due to a higher glycolytic rate and lower oxidative metabolism than neurons (Boumezbeur et al. 2010b; Belanger et al. 2011; Schurr et al. 1999). Neurons preferentially utilize astrocytic lactate for the pentose phosphate pathway (PPP), producing NADPH to maintain their antioxidant capacity (Lebon et al. 2002; Itoh et al. 2003; Bouzier-Sore et al. 2006; Boumezbeur et al. 2010a; Belanger et al. 2011).

Astrocytes and neurons also cooperate via the glutamate-glutamine cycle (Bak et al. 2006; Mckenna 2007; Belanger et al. 2011). Neurons release glutamate into the extracellular space, which is removed by astrocytes and converted into glutamine for restoration of neuronal neurotransmitter pools (Mckenna 2007; Bak et al. 2006; Belanger et al. 2011). Astrocytes also convert glutamate to glutathione precursors that are supplied to neurons to support resistance to oxidative damage (Belanger et al. 2011; Wilson 1997; Dringen 2000; Belanger and Magistretti 2009).

Astrocyte metabolism is accelerated in gliomas, which have increased glycolysis compared to normal tissue, even in the presence of normal oxygen levels (Agnihotri and Zadeh 2016; Warburg 1956a, b) in order to supply adenosine triphosphate (ATP) and biosynthetic precursors for increased cellular proliferation (Lunt and Vander Heiden 2011; Vander Heiden et al. 2011). The increased production of lactate also lowers extracellular pH (Martin and Jain 1994; Kallinowski and Vaupel 1986; Helmlinger et al. 1997; Kato et al. 2013) and induces expression of VEGF (Kato et al. 2013; Xu et al. 2002; Fukumura et al. 2001), stimulating neovascularization for continued tumor growth (Matsumoto and Ema 2014). Gliomas also increase glutamine uptake and utilization (Deberardinis et al. 2007; Wise and Thompson 2010; Venneti and Thompson 2017) to support production of ATP and biosynthetic precursors for growth and proliferation (Tardito et al. 2015; Deberardinis et al. 2007; Venneti and Thompson 2017; Wise et al. 2008; Wise and Thompson 2010).

18.3.1 Tumor Hypoxia

Gliomas encounter transient and chronic hypoxia as they outgrow their blood supply. The most well-studied cellular effect of hypoxia is slowed degradation of the hypoxia-inducible factor (HIF) protein (Semenza 2013; Semenza and Wang 1992). This protein is constitutively expressed and rapidly degraded in proteasomes in the presence of oxygen (Maxwell et al. 1999). When stabilized by hypoxia, HIF enhances the transcription of numerous genes to mitigate environmental stress, including glycolytic enzymes such as lactate dehydrogenase, enolase, and phosphofructokinase (Semenza et al. 1994). Genes involved in pluripotency, such as *SOX2*, *C-MYC*, *NONOG*, *OCT4*, *CD133*, and *Nestin*, have increased expression in hypoxic conditions (Bar 2011; Covello et al. 2006; Yun and Lin 2014), leading to increased

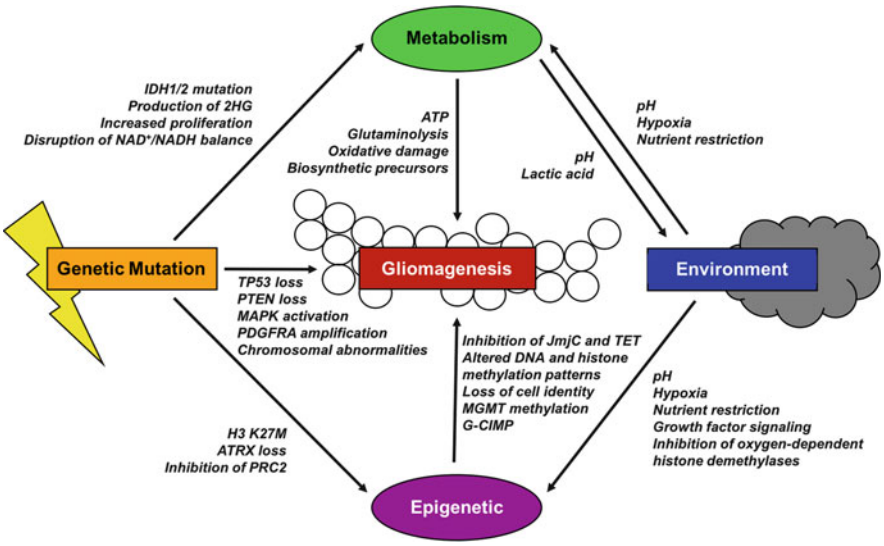

Fig. 18.2 Interaction between genome, epigenome, metabolism, and the environment in gliomagenesis

invasion (Joseph et al. 2015) and GSC resistance to chemotherapy and radiotherapy (Schwartz et al. 2011; Rohwer and Cramer 2011).

The effect of 2HG inhibition of α-KG-dependent dioxygenases in IDH-mutated tumors is similar to that of inactivation of these enzymes in the setting of hypoxia (Miller et al. 2017). Inhibition of TET family 5mC hydroxylases and JmjC domain-containing histone demethylases in both settings disrupts normal methylation patterns of DNA and histones, respectively, and thus epigenetic profiles can be similar between IDH mutants and hypoxic tumors (Miller et al. 2017). Either condition might contribute to the maintenance of GSCs (Miller et al. 2017).

18.4 Conclusion

The glioma genome and epigenome interact with derangements in metabolism and the microenvironment to enable gliomagenesis and maintenance of GSCs (Fig. 18.2). Understanding common epigenetic and metabolic features within this heterogeneous group may yield new treatment strategies.

References

Adam J, Yang M, Soga T, Pollard PJ (2014) Rare insights into cancer biology. Oncogene 33:2547–2556

Agnihotri S, Zadeh G (2016) Metabolic reprogramming in glioblastoma: the influence of cancer metabolism on epigenetics and unanswered questions. Neuro-Oncology 18:160–172

Ahmad F, Patrick S, Sheikh T, Sharma V, Pathak P, Malgulwar PB, Kumar A, Joshi SD, Sarkar C, Sen E (2017) Telomerase reverse transcriptase (Tert)—enhancer of Zeste homolog 2 (Ezh2) Network regulates lipid metabolism and DNA damage responses in glioblastoma. J Neurochem 143(6):671–683

Bak LK, Schousboe A, Waagepetersen HS (2006) The glutamate/Gaba-glutamine cycle: aspects of transport, neurotransmitter homeostasis and Ammonia transfer. J Neurochem 98:641–653

Bakhshinyan D, Adile AA, Qazi MA, Singh M, Kameda-Smith MM, Yelle N, Chokshi C, Venugopal C, Singh SK (2018) Introduction to cancer stem cells: past, present, and future. Methods Mol Biol 1692:1–16

Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, Shi Q, Mclendon RE, Bigner DD, Rich JN (2006) Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res 66:7843–7848

Bar EE (2011) Glioblastoma, cancer stem cells and hypoxia. Brain Pathol 21:119–129

Baysan M, Woolard K, Cam MC, Zhang W, Song H, Kotliarova S, Balamatsias D, Linkous A, Ahn S, Walling J, Belova GI, Fine HA (2017) Detailed longitudinal sampling of glioma stem cells in situ reveals Chr7 gain and Chr10 loss as repeated events in primary tumor formation and recurrence. Int J Cancer 141:2002–2013

Belanger M, Magistretti PJ (2009) The role of astroglia in neuroprotection. Dialogues Clin Neurosci 11:281–295

Belanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab 14:724–738

Belanich M, Randall T, Pastor MA, Kibitel JT, Alas LG, Dolan ME, Schold SC Jr, Gander M, Lejeune FJ, Li BF, White AB, Wasserman P, Citron ML, Yarosh DB (1996) Intracellular localization and intercellular heterogeneity of the human DNA repair protein O(6)-Methylguanine-DNA methyltransferase. Cancer Chemother Pharmacol 37:547–555

Bell RJ, Rube HT, Kreig A, Mancini A, Fouse SD, Nagarajan RP, Choi S, Hong C, He D, Pekmezci M, Wiencke JK, Wrensch MR, Chang SM, Walsh KM, Myong S, Song JS, Costello JF (2015) Cancer. The transcription factor Gabp selectively binds and activates the mutant tert promoter in cancer. Science 348:1036–1039

Bender S, Tang Y, Lindroth AM, Hovestadt V, Jones DT, Kool M, Zapatka M, Northcott PA, Sturm D, Wang W, Radlwimmer B, Hojfeldt JW, Truffaux N, Castel D, Schubert S, Ryzhova M, Seker-Cin H, Gronych J, Johann PD, Stark S, Meyer J, Milde T, Schuhmann M, Ebinger M, Monoranu CM, Ponnuswami A, Chen S, Jones C, Witt O, Collins VP, Von Deimling A, Jabado N, Puget S, Grill J, Helin K, Korshunov A, Lichter P, Monje M, Plass C, Cho YJ, Pfister SM (2013) Reduced H3k27me3 and DNA hypomethylation are major drivers of gene expression in K27m mutant pediatric high-grade gliomas. Cancer Cell 24:660–672

Bhat KPL, Balasubramaniyan V, Vaillant B, Ezhilarasan R, Hummelink K, Hollingsworth F, Wani K, Heathcock L, James JD, Goodman LD, Conroy S, Long L, Lelic N, Wang S, Gumin J, Raj D, Kodama Y, Raghunathan A, Olar A, Joshi K, Pelloski CE, Heimberger A, Kim SH, Cahill DP, Rao G, Den Dunnen WFA, Boddeke H, Phillips HS, Nakano I, Lang FF, Colman H, Sulman EP, Aldape K (2013) Mesenchymal differentiation mediated by Nf-Kappab promotes radiation resistance in glioblastoma. Cancer Cell 24:331–346

Boumezbeur F, Mason GF, De Graaf RA, Behar KL, Cline GW, Shulman GI, Rothman DL, Petersen KF (2010a) Altered brain mitochondrial metabolism in healthy aging as assessed by in vivo magnetic resonance spectroscopy. J Cereb Blood Flow Metab 30:211–221

Boumezbeur F, Petersen KF, Cline GW, Mason GF, Behar KL, Shulman GI, Rothman DL (2010b) The contribution of blood lactate to brain energy metabolism in humans measured by dynamic 13c nuclear magnetic resonance spectroscopy. J Neurosci 30:13983–13991

Bouzier-Sore AK, Voisin P, Bouchaud V, Bezancon E, Franconi JM, Pellerin L (2006) Competition between glucose and lactate as oxidative energy substrates in both neurons and astrocytes: a comparative NMR study. Eur J Neurosci 24:1687–1694

Brennan CW, Verhaak RG, Mckenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, Wu CJ, Genovese G, Shmulevich I, Barnholtz-Sloan J, Zou L, Vegesna R, Shukla SA, Ciriello G, Yung WK, Zhang W, Sougnez C, Mikkelsen T, Aldape K, Bigner DD, Van Meir EG, Prados M,

Sloan A, Black KL, Eschbacher J, Finocchiaro G, Friedman W, Andrews DW, Guha A, Iacocca M, O'neill BP, Foltz G, Myers J, Weisenberger DJ, Penny R, Kucherlapati R, Perou CM, Hayes DN, Gibbs R, Marra M, Mills GB, Lander E, Spellman P, Wilson R, Sander C, Weinstein J, Meyerson M, Gabriel S, Laird PW, Haussler D, Getz G, Chin L, Network TR (2013) The somatic genomic landscape of glioblastoma. Cell 155:462–477

Cabrini G, Fabbri E, Lo Nigro C, Dechecchi MC, Gambari R (2015) Regulation of expression of O6-Methylguanine-DNA methyltransferase and the treatment of glioblastoma (review). Int J Oncol 47:417–428

Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, Oh EY, Gaber MW, Finklestein D, Allen M, Frank A, Bayazitov IT, Zakharenko SS, Gajjar A, Davidoff A, Gilbertson RJ (2007) A perivascular niche for brain tumor stem cells. Cancer Cell 11:69–82

Castelo-Branco P, Choufani S, Mack S, Gallagher D, Zhang C, Lipman T, Zhukova N, Walker EJ, Martin D, Merino D, Wasserman JD, Elizabeth C, Alon N, Zhang L, Hovestadt V, Kool M, Jones DTW, Zadeh G, Croul S, Hawkins C, Hitzler J, Wang JCY, Baruchel S, Dirks PB, Malkin D, Pfister S, Taylor MD, Weksberg R, Tabori U (2013) Methylation of the Tert promoter and risk stratification of childhood brain tumours: an integrative genomic and molecular study. Lancet Oncol 14:534–542

Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC, Keith B (2006) Hif-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 20:557–570

Deberardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB (2007) Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci U S A 104:19345–19350

Dringen R (2000) Metabolism and functions of glutathione in brain. Prog Neurobiol 62:649–671

Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG (2000) Inactivation of the DNA-repair gene Mgmt and the clinical response of gliomas to alkylating agents. N Engl J Med 343:1350–1354

Ferris SP, Hofmann JW, Solomon DA, Perry A (2017) Characterization of gliomas: from morphology to molecules. Virchows Arch. 471(2):257–269

Flavahan WA, Wu Q, Hitomi M, Rahim N, Kim Y, Sloan AE, Weil RJ, Nakano I, Sarkaria JN, Stringer BW, Day BW, Li M, Lathia JD, Rich JN, Hjelmeland AB (2013) Brain tumor initiating cells adapt to restricted nutrition through preferential glucose uptake. Nat Neurosci 16:1373–1382

Fukumura D, Xu L, Chen Y, Gohongi T, Seed B, Jain RK (2001) Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. Cancer Res 61:6020–6024

Gallagher CN, Carpenter KL, Grice P, Howe DJ, Mason A, Timofeev I, Menon DK, Kirkpatrick PJ, Pickard JD, Sutherland GR, Hutchinson PJ (2009) The human brain utilizes lactate via the tricarboxylic acid cycle: a 13c-labelled microdialysis and high-resolution nuclear magnetic resonance study. Brain 132:2839–2849

Goffart N, Kroonen J, Di Valentin E, Dedobbeleer M, Denne A, Martinive P, Rogister B (2015) Adult mouse subventricular zones stimulate glioblastoma stem cells specific invasion through Cxcl12/Cxcr4 Signaling. Neuro-Oncology 17:81–94

Goffart N, Lombard A, Lallemand F, Kroonen J, Nassen J, Di Valentin E, Berendsen S, Dedobbeleer M, Willems E, Robe P, Bours V, Martin D, Martinive P, Maquet P, Rogister B (2017) Cxcl12 mediates glioblastoma resistance to radiotherapy in the subventricular zone. Neuro-Oncology 19:66–77

Gotze S, Wolter M, Reifenberger G, Muller O, Sievers S (2010) Frequent promoter hypermethylation of Wnt pathway inhibitor genes in malignant astrocytic gliomas. Int J Cancer 126:2584–2593

Gusyatiner O, Hegi ME (2017) Glioma epigenetics: from subclassification to novel treatment options. Semin Cancer Biol. 51:50–58

Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA (1999) Creation of human tumour cells with defined genetic elements. Nature 400:464–468

Halliday J, Helmy K, Pattwell SS, Pitter KL, Laplant Q, Ozawa T, Holland EC (2014) In vivo radiation response of proneural glioma characterized by protective P53 transcriptional program and proneural-mesenchymal shift. Proc Natl Acad Sci U S A 111:5248–5253

Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, Weller M, Herold-Mende C, Unterberg A, Jeuken JW, Wesseling P, Reifenberger G, Von Deimling A (2009) Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol 118:469–474

Hegi ME, Diserens AC, Gorlia T, Hamou MF, De Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R (2005) Mgmt gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997–1003

Helmlinger G, Yuan F, Dellian M, Jain RK (1997) Interstitial pH and pO_2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. Nat Med 3:177–182

Hjelmeland AB, Wu Q, Heddleston JM, Choudhary GS, Macswords J, Lathia JD, Mclendon R, Lindner D, Sloan A, Rich JN (2011) Acidic stress promotes a glioma stem cell phenotype. Cell Death Differ 18:829–840

Horiguchi K, Tomizawa Y, Tosaka M, Ishiuchi S, Kurihara H, Mori M, Saito N (2003) Epigenetic inactivation of Rassf1a candidate tumor suppressor gene at 3p21.3 in brain tumors. Oncogene 22:7862–7865

Houdova Megova M, Drabek J, Dwight Z, Trojanec R, Koudelakova V, Vrbkova J, Kalita O, Mlcochova S, Rabcanova M, Hajduch M (2017) Isocitrate dehydrogenase mutations are better prognostic marker than O6-Methylguanine-DNA methyltransferase promoter methylation in glioblastomas - a retrospective, single-Centre molecular genetics study of gliomas. Klin Onkol 30:361–371

Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA (2013) Highly recurrent Tert promoter mutations in human melanoma. Science 339:957–959

Itoh Y, Esaki T, Shimoji K, Cook M, Law MJ, Kaufman E, Sokoloff L (2003) Dichloroacetate effects on glucose and lactate oxidation by neurons and astroglia in vitro and on glucose utilization by brain in vivo. Proc Natl Acad Sci U S A 100:4879–4884

Jin X, Kim LJY, Wu Q, Wallace LC, Prager BC, Sanvoranart T, Gimple RC, Wang X, Mack SC, Miller TE, Huang P, Valentim CL, Zhou QG, Barnholtz-Sloan JS, Bao S, Sloan AE, Rich JN (2017) Targeting glioma stem cells through combined Bmi1 and Ezh2 inhibition. Nat Med 23:1352–1361

Jo SH, Lee SH, Chun HS, Lee SM, Koh HJ, Lee SE, Chun JS, Park JW, Huh TL (2002) Cellular defense against UVb-induced phototoxicity by cytosolic Nadp(+)-dependent isocitrate dehydrogenase. Biochem Biophys Res Commun 292:542–549

Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, Mclean CY, Fouse SD, Yamamoto S, Ueda H, Tatsuno K, Asthana S, Jalbert LE, Nelson SJ, Bollen AW, Gustafson WC, Charron E, Weiss WA, Smirnov IV, Song JS, Olshen AB, Cha S, Zhao Y, Moore RA, Mungall AJ, Jones SJM, Hirst M, Marra MA, Saito N, Aburatani H, Mukasa A, Berger MS, Chang SM, Taylor BS, Costello JF (2014) Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science 343:189–193

Joseph JV, Conroy S, Pavlov K, Sontakke P, Tomar T, Eggens-Meijer E, Balasubramaniyan V, Wagemakers M, Den Dunnen WF, Kruyt FA (2015) Hypoxia enhances migration and invasion in glioblastoma by promoting a mesenchymal shift mediated by the Hif1alpha-Zeb1 Axis. Cancer Lett 359:107–116

Kallinowski F, Vaupel P (1986) Concurrent measurements of O2 partial pressures and pH values in human mammary carcinoma. Xenotransplants. 200:609–621

Kato Y, Ozawa S, Miyamoto C, Maehata Y, Suzuki A, Maeda T, Baba Y (2013) Acidic extracellular microenvironment and cancer. Cancer Cell Int 13:89

Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA Jr, Friedman AH, Friedman H, Gallia GL, Giovanella BC, Grollman AP, He TC, He Y, Hruban RH, Jallo GI, Mandahl N, Meeker AK, Mertens F, Netto GJ, Rasheed BA, Riggins GJ, Rosenquist TA, Schiffman M, Shih Ie M, Theodorescu D, Torbenson MS, Velculescu VE, Wang TL, Wentzensen N, Wood LD, Zhang M, Mclendon RE, Bigner DD, Kinzler KW, Vogelstein B, Papadopoulos N, Yan H (2013) Tert promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci U S A 110:6021–6026

Koelsche C, Sahm F, Capper D, Reuss D, Sturm D, Jones DT, Kool M, Northcott PA, Wiestler B, Bohmer K, Meyer J, Mawrin C, Hartmann C, Mittelbronn M, Platten M, Brokinkel B, Seiz M, Herold-Mende C, Unterberg A, Schittenhelm J, Weller M, Pfister S, Wick W, Korshunov A, Von Deimling A (2013) Distribution of Tert promoter mutations in pediatric and adult tumors of the nervous system. Acta Neuropathol 126:907–915

Kokovay E, Goderie S, Wang Y, Lotz S, Lin G, Sun Y, Roysam B, Shen Q, Temple S (2010) Adult Svz lineage cells home to and leave the vascular niche via differential responses to Sdf1/Cxcr4 Signaling. Cell Stem Cell 7:163–173

Koschmann C, Calinescu AA, Nunez FJ, Mackay A, Fazal-Salom J, Thomas D, Mendez F, Kamran N, Dzaman M, Mulpuri L, Krasinkiewicz J, Doherty R, Lemons R, Brosnan-Cashman JA, Li Y, Roh S, Zhao L, Appelman H, Ferguson D, Gorbunova V, Meeker A, Jones C, Lowenstein PR, Castro MG (2016) Atrx loss promotes tumor growth and impairs nonhomologous end joining DNA repair in glioma. Sci Transl Med 8:328ra28

Lagadec C, Vlashi E, Della Donna L, Dekmezian C, Pajonk F (2012) Radiation-induced reprogramming of breast cancer cells. Stem Cells 30:833–844

Lambiv WL, Vassallo I, Delorenzi M, Shay T, Diserens AC, Misra A, Feuerstein B, Murat A, Migliavacca E, Hamou MF, Sciuscio D, Burger R, Domany E, Stupp R, Hegi ME (2011) The Wnt inhibitory factor 1 (Wif1) is targeted in glioblastoma and has a tumor suppressing function potentially by induction of senescence. Neuro-Oncology 13:736–747

Lebon V, Petersen KF, Cline GW, Shen J, Mason GF, Dufour S, Behar KL, Shulman GI, Rothman DL (2002) Astroglial contribution to brain energy metabolism in humans revealed by 13c nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism. J Neurosci 22:1523–1531

Lee SM, Koh H-J, Park D-C, Song BJ, Huh T-L, Park J-W (2002) Cytosolic Nadp + −dependent Isocitrate dehydrogenase status modulates oxidative damage to cells. Free Radic Biol Med 32:1185–1196

Leung JW, Ghosal G, Wang W, Shen X, Wang J, Li L, Chen J (2013) Alpha thalassemia/mental retardation syndrome X-linked gene product Atrx is required for proper replication restart and cellular resistance to replication stress. J Biol Chem 288:6342–6350

Lewis PW, Muller MM, Koletsky MS, Cordero F, Lin S, Banaszynski LA, Garcia BA, Muir TW, Becher OJ, Allis CD (2013) Inhibition of Prc2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. Science 340:857–861

Li Z, Bao S, Wu Q, Wang H, Eyler C, Sathornsumetee S, Shi Q, Cao Y, Lathia J, Mclendon RE, Hjelmeland AB, Rich JN (2009) Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell 15:501–513

Lim DA, Tramontin AD, Trevejo JM, Herrera DG, García-Verdugo JM, Alvarez-Buylla A (2000) Noggin antagonizes bmp signaling to create a niche for adult neurogenesis. Neuron 28:713–726

Lin B, Lee H, Yoon JG, Madan A, Wayner E, Tonning S, Hothi P, Schroeder B, Ulasov I, Foltz G, Hood L, Cobbs C (2015) Global analysis of H3k4me3 and H3k27me3 profiles in glioblastoma stem cells and identification of Slc17a7 as a bivalent tumor suppressor gene. Oncotarget 6:5369–5381

Louis DN, Perry A, Reifenberger G, Von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW (2016) The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol 131:803–820

Lunt SY, Vander Heiden MG (2011) Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 27:441–464

Martin GR, Jain RK (1994) Noninvasive measurement of interstitial pH profiles in Normal and neoplastic tissue using fluorescence ratio imaging microscopy. Cancer Res 54:5670–5674

Matsumoto K, Ema M (2014) Roles of VEGF-a signalling in development, regeneration, and tumours. J Biochem 156:1–10

Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ (1999) The tumour suppressor protein Vhl targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399:271–275

Mckenna MC (2007) The glutamate-glutamine cycle is not stoichiometric: fates of glutamate in brain. J Neurosci Res 85:3347–3358

Meacham CE, Morrison SJ (2013) Tumour heterogeneity and cancer cell plasticity. Nature 501:328–337

Miller JJ, Shih HA, Andronesi OC, Cahill DP (2017) Isocitrate dehydrogenase-mutant glioma: evolving clinical and therapeutic implications. Cancer 123:4535–4546

Mur P, Rodriguez De Lope A, Diaz-Crespo FJ, Hernandez-Iglesias T, Ribalta T, Fiano C, Garcia JF, Rey JA, Mollejo M, Melendez B (2015) Impact on prognosis of the regional distribution of Mgmt methylation with respect to the Cpg island methylator phenotype and age in glioma patients. J Neuro-Oncol 122:441–450

Nakano I (2014) Therapeutic potential of targeting glucose metabolism in glioma stem cells. Expert Opin Ther Targets 18:1233–1236

Nakano I (2015) Stem cell signature in glioblastoma: therapeutic development for a moving target. J Neurosurg 122:324–330

Nikbakht H, Panditharatna E, Mikael LG, Li R, Gayden T, Osmond M, Ho CY, Kambhampati M, Hwang EI, Faury D, Siu A, Papillon-Cavanagh S, Bechet D, Ligon KL, Ellezam B, Ingram WJ, Stinson C, Moore AS, Warren KE, Karamchandani J, Packer RJ, Jabado N, Majewski J, Nazarian J (2016) Spatial and temporal homogeneity of driver mutations in diffuse intrinsic pontine glioma. Nat Commun 7:11185

Nishioka K, Chuikov S, Sarma K, Erdjument-Bromage H, Allis CD, Tempst P, Reinberg D (2002) Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. Genes Dev 16:479–489

Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K, Cancer Genome Atlas Research N (2010) Identification of a Cpg island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 17:510–522

Ohba S, Mukherjee J, Johannessen TC, Mancini A, Chow TT, Wood M, Jones L, Mazor T, Marshall RE, Viswanath P, Walsh KM, Perry A, Bell RJ, Phillips JJ, Costello JF, Ronen SM, Pieper RO (2016) Mutant IDH1 expression drives Tert promoter reactivation as part of the cellular transformation process. Cancer Res 76:6680–6689

Ohgaki H, Kleihues P (2013) The definition of primary and secondary glioblastoma. Clin Cancer Res 19:764–772

Ottone C, Krusche B, Whitby A, Clements M, Quadrato G, Pitulescu ME, Adams RH, Parrinello S (2014) Direct cell-cell contact with the vascular niche maintains quiescent neural stem cells. Nat Cell Biol 16:1045–1056

Ozawa T, Riester M, Cheng YK, Huse JT, Squatrito M, Helmy K, Charles N, Michor F, Holland EC (2014) Most human non-Gcimp glioblastoma subtypes evolve from a common proneural-like precursor glioma. Cancer Cell 26:288–300

Paredes MF, James D, Gil-Perotin S, Kim H, Cotter JA, Ng C, Sandoval K, Rowitch DH, Xu D, Mcquillen PS, Garcia-Verdugo JM, Huang EJ, Alvarez-Buylla A (2016) Extensive migration of young neurons into the infant human frontal lobe. Science 354:pii: aaf7073

Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, Mclendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of human glioblastoma Multiforme. Science 321:1807–1812

Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, Louis DN, Rozenblatt-Rosen O, Suva ML, Regev A, Bernstein BE (2014) Single-cell RNA-Seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344:1396–1401

Pathania M, De Jay N, Maestro N, Harutyunyan AS, Nitarska J, Pahlavan P, Henderson S, Mikael LG, Richard-Londt A, Zhang Y, Costa JR, Hebert S, Khazaei S, Ibrahim NS, Herrero J, Riccio A, Albrecht S, Ketteler R, Brandner S, Kleinman CL, Jabado N, Salomoni P (2017) H3.3(K27m) cooperates with Trp53 loss and Pdgfra gain in mouse embryonic neural progenitor cells to induce invasive high-grade gliomas. Cancer Cell 32:684–700E9

Peifer M, Hertwig F, Roels F, Dreidax D, Gartlgruber M, Menon R, Kramer A, Roncaioli JL, Sand F, Heuckmann JM, Ikram F, Schmidt R, Ackermann S, Engesser A, Kahlert Y, Vogel W, Altmuller J, Nurnberg P, Thierry-Mieg J, Thierry-Mieg D, Mariappan A, Heynck S, Mariotti E, Henrich KO, Gloeckner C, Bosco G, Leuschner I, Schweiger MR, Savelyeva L, Watkins SC, Shao C, Bell E, Hofer T, Achter V, Lang U, Theissen J, Volland R, Saadati M, Eggert A, De Wilde B, Berthold F, Peng Z, Zhao C, Shi L, Ortmann M, Buttner R, Perner S, Hero B, Schramm A, Schulte JH, Herrmann C, O'sullivan RJ, Westermann F, Thomas RK, Fischer M (2015) Telomerase activation by genomic rearrangements in high-risk neuroblastoma. Nature 526:700–704

Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, Williams PM, Modrusan Z, Feuerstein BG, Aldape K (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9:157–173

Piao Y, Liang J, Holmes L, Henry V, Sulman E, De Groot JF (2013) Acquired resistance to anti-VEGF therapy in glioblastoma is associated with a mesenchymal transition. Clin Cancer Res 19:4392–4403

Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F, Vescovi AL (2006) Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 444:761–765

Prickaerts P, Adriaens ME, Beucken TVD, Koch E, Dubois L, Dahlmans VEH, Gits C, Evelo CTA, Chan-Seng-Yue M, Wouters BG, Voncken JW (2016) Hypoxia increases genome-wide bivalent epigenetic marking by specific gain of H3k27me3. Epigenetics Chromatin 9:46

Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414:105–111

Rohwer N, Cramer T (2011) Hypoxia-mediated drug resistance: novel insights on the functional interaction of Hifs and cell death pathways. Drug Resist Updat 14:191–201

Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai HH, Wong M, Gupta N, Berger MS, Huang E, Garcia-Verdugo JM, Rowitch DH, Alvarez-Buylla A (2011) Corridors of migrating neurons in the human brain and their decline during infancy. Nature 478:382–386

Schurr A, Miller JJ, Payne RS, Rigor BM (1999) An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. J Neurosci 19:34–39

Schwartz DL, Bankson J, Bidaut L, He Y, Williams R, Lemos R, Thitai AK, Oh J, Volgin A, Soghomonyan S, Yeh HH, Nishii R, Mukhopadhay U, Alauddin M, Mushkudiani I, Kuno N, Krishnan S, Bornman W, Lai SY, Powis G, Hazle J, Gelovani J (2011) Hif-1-dependent stromal adaptation to ischemia mediates in vivo tumor radiation resistance. Mol Cancer Res 9:259–270

Semenza GL (2013) Hif-1 mediates metabolic responses to Intratumoral hypoxia and oncogenic mutations. J Clin Invest 123:3664–3671

Semenza GL, Wang GL (1992) A nuclear factor induced by hypozia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 12:5447–5454

Semenza GL, Roth PH, Fang HM, Wang GL (1994) Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 269:23757–23763

Shiozawa Y, Pedersen EA, Havens AM, Jung Y, Mishra A, Joseph J, Kim JK, Patel LR, Ying C, Ziegler AM, Pienta MJ, Song J, Wang J, Loberg RD, Krebsbach PH, Pienta KJ, Taichman RS (2011) Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. J Clin Invest 121:1298–1312

Sinnaeve J, Mobley BC, Ihrie RA (2017) Space invaders: brain tumor exploitation of the stem cell niche. Am J Pathol. 188(1):29–38

Smith D, Pernet A, Hallett WA, Bingham E, Marsden PK, Amiel SA (2003) Lactate: a preferred fuel for human brain metabolism in vivo. J Cereb Blood Flow Metab 23:658–664

Sui A, Xu Y, Li Y, Hu Q, Wang Z, Zhang H, Yang J, Guo X, Zhao W (2017) The pharmacological role of histone demethylase Jmjd3 inhibitor Gsk-J4 on glioma cells. Oncotarget 8:68591–68598

Tano K, Shiota S, Collier J, Foote RS, Mitra S (1990) Isolation and structural characterization of a cDNA clone encoding the human DNA repair protein for O6-alkylguanine. Proc Natl Acad Sci U S A 87:686–690

Tardito S, Oudin A, Ahmed SU, Fack F, Keunen O, Zheng L, Miletic H, Sakariassen PO, Weinstock A, Wagner A, Lindsay SL, Hock AK, Barnett SC, Ruppin E, Morkve SH, Lund-Johansen M, Chalmers AJ, Bjerkvig R, Niclou SP, Gottlieb E (2015) Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine-restricted glioblastoma. Nat Cell Biol 17:1556–1568

Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS, Thompson CB, Kaufman A, Guryanova O, Levine R, Heguy A, Viale A, Morris LG, Huse JT, Mellinghoff IK, Chan TA (2012) IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 483:479–483

Turcan S, Makarov V, Taranda J, Wang Y, Fabius AWM, Wu W, Zheng Y, El-Amine N, Haddock S, Nanjangud G, Lekaye HC, Brennan C, Cross J, Huse JT, Kelleher NL, Osten P, Thompson CB, Chan TA (2017) Mutant-IDH1-dependent chromatin state reprogramming, reversibility, and persistence. Nat Genet. 50(1):62–72

Vander Heiden MG, Lunt SY, Dayton TL, Fiske BP, Israelsen WJ, Mattaini KR, Vokes NI, Stephanopoulos G, Cantley LC, Metallo CM, Locasale JW (2011) Metabolic pathway alterations that support cell proliferation. Cold Spring Harb Symp Quant Biol 76:325–334

Venneti S, Huse JT (2015) The evolving molecular genetics of low-grade glioma. Adv Anat Pathol 22:94–101

Venneti S, Thompson CB (2017) Metabolic reprogramming in brain tumors. Annu Rev Pathol 12:515–545

Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN, Cancer Genome Atlas Research N (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17:98–110

Warburg O (1956a) On respiratory impairment in cancer cells. Science 124:269–270

Warburg O (1956b) On the origin of cancer cells. Science 123:309–314

Watanabe T, Nobusawa S, Kleihues P, Ohgaki H (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol 174:1149–1153

Wilson JX (1997) Antioxidant defense of the brain: a role for astrocytes. Can J Physiol Pharmacol 75:1149–1163

Wise DR, Thompson CB (2010) Glutamine addiction: a new therapeutic target in cancer. Trends Biochem Sci 35:427–433

Wise DR, Deberardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, Mcmahon SB, Thompson CB (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A 105:18782–18787

Wright KD, Sabin ND, Cheuk D, Mcnall-Knapp R, Shurtleff SA, Gajjar A, Broniscer A (2015) Incidental diagnosis of diffuse intrinsic pontine glioma in children. Pediatr Blood Cancer 62:1081–1083

Xu L, Fukumura D, Jain RK (2002) Acidic extracellular pH induces vascular endothelial growth factor (Vegf) in human glioblastoma cells via Erk1/2 Mapk signaling pathway: mechanism of low pH-induced Vegf. J Biol Chem 277:11368–11374

Yan H, Parsons DW, Jin G, Mclendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765–773

Yin J, Park G, Kim TH, Hong JH, Kim YJ, Jin X, Kang S, Jung JE, Kim JY, Yun H, Lee JE, Kim M, Chung J, Kim H, Nakano I, Gwak HS, Yoo H, Yoo BC, Kim JH, Hur EM, Lee J, Lee SH, Park MJ, Park JB (2015) Pigment epithelium-derived factor (PEDF) expression induced by EGFRvIII promotes self-renewal and tumor progression of glioma stem cells. PLoS Biol 13: E1002152

Yu T, Wu Y, Hu Q, Zhang J, Nie E, Wu W, Wang X, Wang Y, Liu N (2017) CBX7 is a glioma prognostic marker and induces G1/S arrest via the silencing of CCNE1. Oncotarget 8:26637–26647

Yun Z, Lin Q (2014) Hypoxia and regulation of cancer cell stemness. Adv Exp Med Biol 772:41–53

Part IV

Myelin Pathology in the Peripheral Nervous System

Schwann Cell and the Pathogenesis of Charcot–Marie–Tooth Disease

19

Tatsufumi Murakami and Yoshihide Sunada

Abstract

Charcot–Marie–Tooth (CMT) disease is the most common hereditary neuropathy and genetically heterogeneous. CMT1 and CMTX are autosomal dominant and X-linked demyelinating neuropathies, respectively. CMT1A, CMT1B, and CMTX1 are the common forms of CMT, which are attributed to the genes encoding the myelin or gap junction proteins expressed in the myelinating Schwann cells. CMT4 is a rare autosomal recessive demyelinating neuropathy that usually shows an early-onset severe phenotype. Twelve genes have been described as CMT4, which encodes many kinds of proteins including mitochondrial proteins, phosphatases in the endosomal pathway, endocytic recycling proteins, and trafficking proteins. The genes responsible for CMT4 are expressed in Schwan cells and necessary for the development and maintenance in the peripheral nervous system. However, CMT1, CMT4, and CMTX1 are primarily demyelinating neuropathies, axonal degeneration is necessary for symptoms to develop. Schwann cell–axon interactions are impaired in the pathogenesis of demyelinating CMT.

Keywords

Charcot–Marie–Tooth disease · Demyelinating neuropathy · Peripheral nerve · Schwann cell

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19.1 Introduction

Charcot–Marie–Tooth disease (CMT), first described in 1886 by Charcot and Marie in France and Tooth in the UK, is the most common form of hereditary neuropathy, also referred to as the hereditary motor and sensory neuropathy (HMSN) (Murakami et al. 1996). The phenotype of CMT typically presents as muscle weakness and atrophy in the foot and legs followed by involvement of the hands. Distal sensory loss, and decreased or absent deep tendon reflexes are also recognized. Dejerine-Sottas syndrome (DSS) is a progressive hypertrophic interstitial neuropathy of childhood with marked nerve swelling and onion bulb formation. Congenital hypomyelinating neuropathy (CH) is a severe polyneuropathy with the early-infancy onset and thin myelin or absence of myelin on nerve biopsy.

CMT is genetically heterogeneous and shows autosomal dominant, X-linked, or autosomal recessive inheritance patterns (Pareyson and Marchesi 2009). Most CMT patients show an autosomal dominant inheritance pattern, and their CMT can be divided into two main categories. One is the demyelinating form of CMT (CMT1), which shows slowing motor nerve conduction velocity (NCV) and abnormality of myelin such as segmental demyelination, remyelination, and onion bulb formation. Another is the axonal form of CMT (CMT2), which shows relatively preserved motor NCV but a decrease in compound muscle action potential and axonal degeneration in the nerve. When the patients in a CMT family show various motor NCVs between CMT1 and CMT2, this CMT is considered a dominant intermediate form. Autosomal recessive demyelinating form of CMT is referred to as CMT4, whereas X-linked CMT is referred to as CMTX. DSS is referred to as CMT3, and most patients appear to be sporadic.

The genetic abnormality for demyelinating form of CMT was first identified as CMT1A duplication in patients in 1991 (Lupski et al. 1991; Raeymaekers et al. 1991). In the 2000s, responsible genes for axonal CMT were first found. Establishment of DNA tests for CMT has made it possible to diagnose sporadic or atypical cases. However, the causes of many CMT families remain unknown (Fig. 19.1). Next generation sequencing techniques have recently been developed and applied to DNA diagnosis for CMT (Lupski et al. 2010). Over 90 genes have been identified to date. Many causative genes may be discovered in the future.

Molecular and biological studies on CMT have revealed important pathways for developing, maintaining, and regenerating the human peripheral nervous system (Harel and Lupski 2014). CMT1 and CMT4 are mostly caused by mutations in Schwann cell-expressed genes (Brennan et al. 2015). In this chapter, we review the role of Schwann cells in the pathogenesis of demyelinating CMT.

19.2 CMT1A, CMT1B, and CMTX

CMT1A, CMT1B, and CMTX are common demyelinating forms of CMT (Fig. 19.1a) caused by mutations in peripheral myelin protein 22 kDa (PMP22), myelin protein zero (MPZ), and connexin 32 (Cx32), respectively (Table 19.1). All

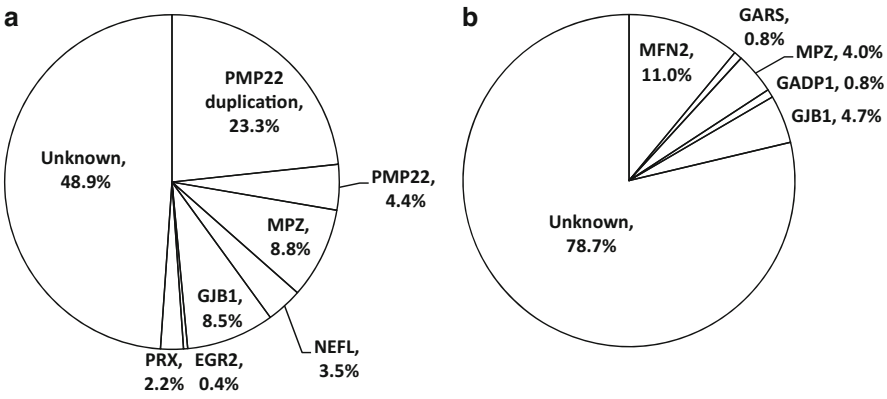

Fig. 19.1 DNA diagnosis of CMT in Japan [The graphs were made from data in Abe et al. (2011)]. (**a**) Gene mutations in demyelinating CMT (227 cases). LITAF, GADP1, MTMR2, MTMR13, DNM2, YARS: 0%. (**b**) Gene mutations in axonal CMT (127 cases). RAB7, NEFL, HSP27, HSP22, DNM2, YARS: 0%

Table 19.1 CMT1 subtypes

	Gene	Protein function	Protein localization	Additional features
CMT1A	*PMP22*	Myelin structure	Compact myelin	
CMT1B	*MPZ*	Myelin structure	Compact myelin	
CMT1C	*LITAF/ SIMPLE*	Endosome protein	Endosome	
CMT1D	*EGR2*	Transcription factor	Nucleoplasm	Cranial nerve involvement
CMT1E/ 2E	*PMP22*	Myelin structure	Compact myelin	Hearing loss
CMT1F	*NEFL*	Cytoskeleton	Axon	
CMT1G	*FBLN5*	Elastic fiber assembly	Extracellular matrix	Macular degeneration, hyperelastic skin

these genes are expressed in myelinating Schwann cells. Many studies have been performed clinically and basically in these CMTs.

19.2.1 CMT1A

CMT1A is the most common type of CMT and associated with duplication of 1.4 Mb region on Chromosome 17p11-12 encompassing PMP22 gene (Lupski et al. 1991; Patel et al. 1992; Raeymaekers et al. 1991). PMP22 is localized to compact myelin and attached myelin membranes. Three copies of the PMP22 gene

result in PMP22 overproduction, leading to demyelination. CMT1A duplication is formed as a de novo mutation by unequal crossing over during meiosis (Inoue et al. 2001; Reiter et al. 1996). CMT1A duplication is observed in 54% of demyelinating CMT in the USA, 57% in Italy, and 23% in Japan (Fig. 19.1) (Hayashi et al. 2013).

The phenotype of CMT1A ranges from typical CMT with childhood onset to mild muscle atrophy and weakness in aged patients. The sensory disturbances are mild. Nerve conduction study is a useful marker for diagnosis. Median motor NCV is usually below 38 m/s. Uniform slowing of NCV is measured between the nerves in the upper and lower extremities, distal and proximal parts of the same nerve, and right and left nerves. Conduction abnormalities are recognized by age of 2 years before the onset of symptoms. Clinical severity of CMT1A patients correlates with a decrease of the compound muscle action potential, suggesting that clinical symptoms are caused by secondary axonal degeneration.

PMP22 is a tetraspan glycoprotein, which is localized to the compact myelin of Schwann cells and comprises up to 5% of the total myelin protein. PMP22 interacts with other myelin proteins to maintain the myelin structure. Increased level of PMP22 mRNA and protein was reported in the sural nerve and skin biopsies (Katona et al. 2009). Excessive PMP22 protein might cause instability in compact myelin, leading to demyelination, remyelination, and onion bulb formation. Secondary axonal degeneration is correlated with clinical severity (Krajewski et al. 2000). Sural nerve segments from patients with CMT1A duplication were grafted into the cut ends of the sciatic nerve of nude mice. The nude mice axons showed an increase in axonal area and neurofilament density within the proximal part of the xenografts from CMT1A patients, and later distal axonal loss within them, indicating that the axonal loss might be caused by abnormalities in Schwann cell–axonal interactions (Sahenk et al. 1999).

Missense mutations in the PMP22 gene cause CMT1A and DSS. The clinical phenotype of patients with missense mutations in the PMP22 gene is more severe than that with CMT1A duplication. Trembler (Tr) and Tr^J mice are animal models for the hereditary demyelinating polyneuropathy caused by missense mutations in Pmp22. Both mutations in Tr and Tr^J mice were also reported in patients with DSS and CMT1A, respectively.

Cytoplasmic accumulation of PMP22 was reported in the Schwann cells of the sciatic nerve of patients with CMT1A duplication or PMP22 point mutations (Hanemann et al. 2000; Nishimura et al. 1996). Most PMP22 mutations alter the trafficking of the PMP22 in Schwann cells (D'Urso et al. 1998). Pmp22 aggregates were also observed in the Schwann cells of the sciatic nerve of Tr^J mice (Fortun et al. 2003; Ryan et al. 2002). PMP22 aggregates (or aggresomes) may be the first protective, but later toxic in the Schwann cells. Accumulation of PMP22 aggregates might induce endoplasmic reticulum (ER) stress, resulting in Schwann cell apoptosis and demyelination (Khajavi et al. 2007). More closely packed, less phosphorylated neurofilaments, and slow axonal transport were described in thinly myelinated axons in Tr mice, suggesting that myelinating Tr Schwann cells might cause these abnormalities in the axons (de Waegh et al. 1992).

Progesterone is synthesized in Schwann cells, which induces PMP22 expression and promotes myelin formation during regeneration. A progesterone antagonist, onapristone, has been shown to improve CMT1A phenotype in a rat model of CMT1A (Meyer zu Horste et al. 2007; Sereda et al. 2003). However, the drug has side effects and is not applicable to human patients.

Ascorbic acid (vitamin C) is known to be necessary to form basement membranes for in vitro myelin formation. Ascorbic acid treatment decreased the expression of PMP22 by reduced cAMP in the cells (Kaya et al. 2007) and ameliorated CMT1A phenotype in a mouse model of CMT1A (Passage et al. 2004). However, several randomized double-blind trials have shown no benefit in human CMT1A patients (Lewis et al. 2013; Pareyson et al. 2011).

Curcumin, found in the spice turmeric, improved the clinical and neuropathological phenotypes in Tr^J and CMT1B mice (Khajavi et al. 2007; Okamoto et al. 2013; Patzko et al. 2012). Curcumin relieved the ER stress by traversing the misfolded proteins from ER to plasma membranes, then promoting Schwann cell differentiation.

The phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K)-Akt signaling and Mek-Erk signaling pathways are related to the differentiation and proliferation of Schwann cells (Martini 2014; Ogata et al. 2004). In the peripheral nerves of Pmp22 overexpressing CMT1A rats, decreased PI3K-Akt activation and increased Mek-Erk activation were observed, suggesting an imbalance between the PI3-Akt and Mek-Erk pathways, which impaired differentiation of Schwann cells. Early treatment of neuregulin-1, activator of PI3K-Akt, restored CMT1A phenotype in the transgenic rats (Fledrich et al. 2014). Interestingly, a limited time window of the therapy was pointed out for long-term effect on axonal support in this study.

19.2.1.1 HNPP

Hereditary neuropathy with liability to pressure palsy (HNPP) is characterized by autosomal dominant repeated entrapment neuropathy and attributed to by the reciprocal 1.4-Mb deletion on Chromosome 17p11-12 containing PMP22 gene (Chance et al. 1993), or PMP22 nonsense/frameshift mutation (loss of function). Sural nerve biopsies from HNPP patients show segmental demyelination and remyelination with sausage-like focal thickening of the myelin sheaths (tomacula). Nerve conduction studies show conduction block and mild slowing of NCV in the patients. Thus, PMP22 expression is tightly regulated in the myelinated Schwann cells, and three copies of PMP22 cause CMT1A, while one copy HNPP. PMP22 gene is considered to be a dose-sensitive gene.

19.2.2 CMT1B

CMT1B is the second most common type of CMT1; the MPZ gene was identified as the causative gene in 1993 (Hayasaka et al. 1993). MPZ is a glycoprotein of 219 amino acids, expressed in myelinating Schwann cells. It forms tetramers, functions as a homophilic adhesion molecule, and is localized to compact myelin.

Fifty percent of myelin protein is the MPZ in the peripheral nerves. The protein consists of an immunoglobulin-like extracellular domain, a transmembrane domain, and a cytoplasmic domain. More than 200 mutations in the MPZ gene are associated with CMT1B, DSS, and CH (Brennan et al. 2015). Most mutations associated with hereditary neuropathies are localized in the extracellular domain of the protein. Gain of function effects or haploinsufficiency is considered as the molecular pathomechanism of the mutations (Grandis et al. 2008). Clinical manifestations of CMT1B show early-onset severe demyelinating neuropathy, or late-onset axonal and minimal demyelinating neuropathy (Shy et al. 2004). MPZ mutations in early-onset patients disrupt the tertiary structure of MPZ, resulting in myelin uncompaction and demyelination, whereas those in late-onset patients cause a more subtle change in the myelin structure, resulting in impairment of Schwann cell–axon interactions (Shy 2006). Misfolded mutated MPZ are retained in the ER and induce the unfolded protein response in the subset of mutated MPZ proteins (Grandis et al. 2008; Wrabetz et al. 2006). Ablation of the unfolded protein response-mediator transcription factor CHOP reduced demyelination in CMTB1 model mouse, suggesting that CHOP induces dysfunction in Schwann cells (Pennuto et al. 2008).

19.2.3 CMTX1

CMTX1 is the second most common CMT accounting for 10% of CMT cases. CMTX1 is caused by mutations in the GJB1 gene, encoding the gap junction protein Cx32 (Bergoffen et al. 1993). Cx32 is 283 amino acids and has four transmembrane domains. It is localized to the paranodal myelin loops and Schmidt-Lanterman incisures (non-compact myelin), and forms channels to exchange ions and small molecules (Balice-Gordon et al. 1998; Scherer et al. 1995a). Cx32 is also expressed in the oligodendrocytes of the central nervous system (CNS) (Scherer et al. 1995a). More than 400 mutations in GJB1 have been reported in this CMT (Kleopa et al. 2012). Male patients are more severely affected than female patients. Male patients often show "split hand syndrome," in which atrophy and weakness are more prominent in the abductor pollicis brevis muscle than in the abductor digiti minimi muscle. Males with CMTX1 usually show intermediate motor NCVs (25–35 m/s), and females present mildly slowed motor NCVs (>35 ms) (Saporta et al. 2011). Slowing of motor NCV is less uniform between nerves in CMTX1, and temporal dispersion may be seen (Gutierrez et al. 2000; Tabaraud et al. 1999). CNS involvement may be associated with CMTX1 clinically, electrophysiologically, or on brain MRI (Nicholson and Corbett 1996; Taylor et al. 2003). Nerve biopsy shows loss of myelinated fibers, regenerating axon clusters, and thin myelinating axons, suggesting prominent axonal degeneration (Senderek et al. 1999). Most mutations in GJB1 cause a loss of Cx32 function (Shy et al. 2007). Cx32-null mice developed a progressive demyelinating neuropathy at around 3 months of age with thin myelin sheaths, onion bulb formation, and enlarged periaxonal collars (Anzini et al. 1997; Scherer et al. 1998). Furthermore, it was found that axonal changes precede

demyelination in mice (Vavlitou et al. 2010). These mice were rescued by the expression of human Cx32 in myelinating Schwann cells (Scherer et al. 2005). Transplantation of nerve grafts from CMTX1 patients into the sciatic nerves of nude mice caused increased neurofilaments, depletion of microtubules, and increased vesicles and mitochondria in the nude mice axons, demonstrating the disturbed Schwann cell–axon interactions in CMTX1 (Sahenk and Chen 1998).

19.3 Additional Forms of CMT1

Subsequent studies revealed four causative genes for additional forms of CMT1 (Table 19.1). These CMT are rare, but each gene might have an important function in the peripheral nerves.

19.3.1 CMT1C: SIMPLE

CMT1C is caused by mutations in small integral membrane protein of lysosome/late endosome (SIMPLE) gene (Street et al. 2003), which is also called lipopolysaccharide-induced TNF-α factor (LITAF). SIMPLE is a 161 amino acid protein, having a C-terminal transmembrane domain. Pathogenic mutations are clustered around this domain (Bennett et al. 2004), and impair its insertion into the endosomal membrane, resulting in cytosolic aggregates in Schwann cells (Lee et al. 2011). Impaired proteasome and autophagy pathways may be associated with the pathogenesis of this demyelinating CMT (Lee et al. 2012). Recently, it has been shown that SIMPLE is related to the regulation of multivesicular bodies (MVBs), and mutated SIMPLE could cause MVB defects (Li et al. 2015).

19.3.2 CMT1D: EGR2

Mutations in the early growth response 2 (EGR2) gene have been identified in CMT1, DSS, and CH (Timmerman et al. 1999; Warner et al. 1998). EGR2 mutations occur in fewer than 1% of CMT patients (Vandenberghe et al. 2002). Cranial nerve abnormalities have also been described in the neuropathy (Pareyson et al. 2000). EGR2 is a zinc finger transcription factor in Schwann cells, which binds DNA and regulates the expression of myelin genes including MPZ and myelin basic protein genes. Dominant mutations are detected within the zinc finger domain of EGR2 (Warner et al. 1999). Homozygous mouse orthologue Krox20 knockout mice showed hypomyelination in the peripheral nerves, and impaired hindbrain development (Topilko et al. 1994).

19.3.3 CMT1F: NEFL

The neurofilament light (NEFL) chain is an intermediate neurofilament in the neuron. Mutations in the NEFL gene can cause the axonal form of CMT (CMT2E) (De Jonghe et al. 2001; Georgiou et al. 2002; Mersiyanova et al. 2000; Yoshihara et al. 2002). Mutations in the NEFL gene may also show early-onset severe phenotype with severely slowed NCV (Jordanova et al. 2003), which may be classified as CMT1 (CMT1F).

19.3.4 CMT1-FIBLN5 (CMT1G)

Fibulin-5 (FIBLN5) plays an essential role in elastic fiber assembly in the extracellular matrix, and the mutations have been described in cutis laxa (hyperextensibility of skin) or age-related macular degeneration (Yanagisawa et al. 2009). Missense mutations in FIBLN5 have been found in demyelinating CMT with cutis laxa or age-related macular degeneration (Auer-Grumbach et al. 2011). It remains unknown how extracellular matrix protein causes demyelinating neuropathy.

19.4 CMT4

CMT4 is a rare autosomal recessive demyelinating neuropathy, and usually shows an early-onset severe CMT phenotype. Twelve causative genes have been described as CMT4 to date (Table 19.2). The mutations in the genes are "loss of function" mutations. Interestingly, a number of responsible genes are related to the regulation of intracellular trafficking in Schwann cells.

19.4.1 CMT4A: GDAP1

CMT4A was first described and mapped to chromosome 8q in consanguineous Tunisian families (Ben Othmane et al. 1998). Mutations in ganglioside-induced differentiation-associated protein 1 (GDAP1) gene were identified in the families (Baxter et al. 2002). GDAP1 mutations were also identified in autosomal recessive axonal CMT with vocal cord palsy in Spanish families (Cuesta et al. 2002; Sevilla et al. 2003). Autosomal recessive CMT due to GDAP1 mutations presents demyelinating, axonal, or intermediate neuropathy electrophysiologically and pathologically (Nelis et al. 2002; Senderek et al. 2003a). The patients with these GDAP1 mutations show early-onset severe CMT phenotypes. Rare mutations in GDAP1 gene could cause autosomal dominant CMT (CMT2K) with a mild-to-moderate phenotype (Crimella et al. 2010; Sivera et al. 2010).

GDAP1 is expressed in both neurons and Schwann cells, located in the outer membrane of mitochondria, and regulates mitochondrial fragmentation (Niemann et al. 2005). GDAP1 induces mitochondrial fission, and its autosomal recessive

Table 19.2 CMT4 subtypes

	Gene	Protein function	Protein localization	Additional features
CMT4A	*GDAP1*	Mitochondrial function	Outer membrane of mitochondria	
CMT4B1	*MTMR2*	Phosphatase in endosomal pathway	Vesicles	Myelin outfoldings
CMT4B2	*SBF2/ MTMR13*	Phosphatase in endosomal pathway	Nucleoplasm	Myelin outfoldings, glaucoma
CMT4B3	*SBF1/ MTMR5*	Phosphatase in endosomal pathway	Nuclear bodies	Myelin outfoldings
CMT4C	*SH3TC2*	Endocytic recycling	Nucleoplasm, cytosol	Scoliosis
CMT4D	*NDRG1*	Trafficking	Cytosol	Hearing loss
CMT4E	*EGR2/ KROX20*	Transcription factor	Nucleoplasm	Arthrogryposis
CMT4F	*PRX*	Interaction with extracellular matrix	Plasma membrane	Marked sensory impairment
CMT4G	*HK1*	Hexokinase	Outer membrane of mitochondria	
CMT4H	*FGD4*	Activator of Cdc42, endosomal function	Actin filaments	Myelin outfoldings
CMT4J	*FIG4*	Phosphatase in endosomal pathway	Vesicles	
CMT4K	*SURF1*	COX synthesis	Inner membrane of mitochondria	Lactic acidosis

mutants reduce the fission activity, whereas autosomal dominant mutants impair the mitochondrial fusion (Niemann et al. 2009).

19.4.2 CMT4B

19.4.2.1 CMT4B1: MTMR2

CMT4B1 is an early-onset severe neuropathy with myelin outfoldings on nerve biopsy (Fig. 19.2) (Bolino et al. 1996), and has been reported in Italian, Saudi Arabian, English, Turkish, Algerian, and Japanese families (Murakami et al. 2013). Mutations in myotubularin-related 2 (MTMR2) cause CMT4B1 (Bolino et al. 2000). Myotubularin-related proteins are a broad family of phosphatase, and divide into catalytically active and inactive phosphatases (Bolis et al. 2007). Active myotubularin-related proteins including MTMR2 have the 3-phosphatase activity on PI(3)P and PI(3,5)P2. PI(3)P exists on early endosomes and the internal vesicles of MVBs. PI(3,5)P2 is necessary for the sorting membrane at the late endosome, suggesting a role of MTMR2 in intracellular trafficking and membrane homeostasis.

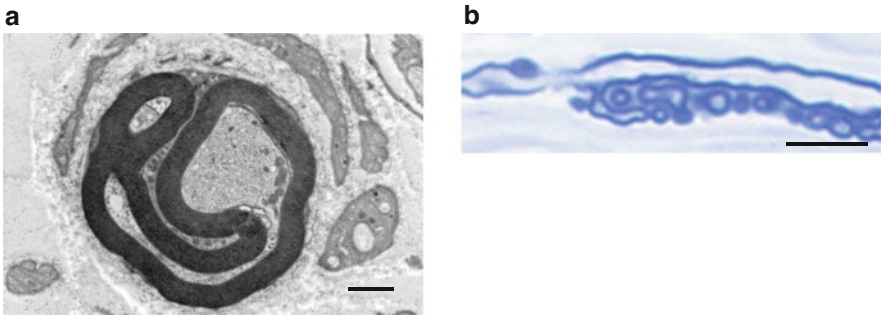

Fig. 19.2 Myelin outfoldings in the sural nerve from a CMT4B1 patient. (**a**) Electron micrograph of the sural nerve from a CMT4B1 patient. Typical myelin outfolding is observed. Bar = 2 μm (Murakami et al. 2014). (**b**) Toluidine blue staining of a longitudinal section of the sural nerve from a CMT4B1 patient. Myelin outfoldings are observed along the nerve fiber. Bar = 10 μm. The patient provided informed consent for presenting the nerve findings

Loss of Mtmr2 in Schwann cells causes CMT4B1 neuropathy with myelin outfoldings in a mouse model (Bolis et al. 2005).

19.4.2.2 CMT4B2: MTMR13

CMT4B2 is characterized by peripheral neuropathy, myelin outfoldings on nerve biopsy and early-onset glaucoma. Mutations in MTMR13 cause CMT4B2 (Azzedine et al. 2003; Hirano et al. 2004). MTMR13 is an inactive phosphatase, and MTMR13 and MTMR2 interact in the cultured Schwann cells and peripheral nerves (Berger et al. 2006; Bolis et al. 2007). MTMR2 activity is increased by heterotetramers in association with MTMR2 and MTMR13 homodimers (Berger et al. 2006). Mtmr13-deficent mice develop peripheral neuropathy with myelin outfoldings, and Mtmr2 levels were reduced in the sciatic nerves of the mice (Robinson et al. 2008).

19.4.2.3 CMT4B3: SBF1

Mutations in SET binding factor 1(SBF1), also known as MTMR5, were identified in Korean families with CMT4B3 (Nakhro et al. 2013). Myelin outfoldings were observed in the nerve biopsy of the patient. SBF1 is catalytically an inactive phosphatase, and its mutants might cause CMT4B phenotype as MTMR13 mutants do in CMT4B2.

19.4.3 CMT4C: SH3TC2

CMT4C is an early-onset severe demyelinating neuropathy with early-onset scoliosis (Gabreels-Festen et al. 1999). Cranial nerve involvements may be seen in patients. Nerve biopsy shows basal lamina onion bulbs. Mutations in the SH3TC2/KIAA1985 gene were identified in the CMT (Senderek et al. 2003b). Clinical

variation has been reported in age of onset, progression, and severity in the CMT phenotype (Azzedine et al. 2006; Gooding et al. 2005; Houlden et al. 2009). SH3TC is expressed in the nervous tissue, and localized to the plasma membrane and the perinuclear recycling compartment in Schwann cells (Roberts et al. 2010). SH3TC2 targets the intracellular recycling endosome via the small GTPase, Rab11, and the mutants are unable to associate with Rab11, resulting in loss of recycling endosome localization (Roberts et al. 2010). Sh3tc2 knockout mice developed hypomyelination, and wider nodal spaces in the node of Ranvier were observed in sural nerve biopsies from CMT4C patients (Arnaud et al. 2009). Neuregulin-1 (Nrg1)/ErbB signaling pathway controls myelination in the peripheral nervous system, and Sh3tc2 modulates ErbB2 receptor internalization on activation by Nrg1 (Gouttenoire et al. 2013). Mutations in Sh3tc2 in CMT4C might impair ErbB2 internalization, leading to a change in its signaling pathway (Gouttenoire et al. 2013).

19.4.4 CMT4D: NDRG1

CMT4D, also called HMSN-Lom, is an early-onset severe demyelinating neuropathy in the Roma/Gypsy population (Kalaydjieva et al. 1998). Deafness is an additional clinical feature. On nerve biopsy, demyelination, remyelination, onion bulb formation, and pleomorphic inclusion in adaxonal Schwann cell cytoplasm are observed (King et al. 1999). N-myc downstream-regulated gene (NDRG1) was identified as a responsible gene for CMT4D (Kalaydjieva et al. 2000). NDRG1 is highly expressed in Schwann cells, and has multiple functions. Pathogenic NDRG1 mutants might impair cellular trafficking in CMT4D (King et al. 2011).

19.4.5 CMT4F: PRX

CMT4F is characterized by early-onset slowly progressive neuropathy with marked sensory involvement, demyelination and onion bulb formation (Marchesi et al. 2010). Mutations in the periaxin (PRX) gene were found in CMT4F or recessive DSS (Boerkoel et al. 2001; Guilbot et al. 2001).

PRX is expressed by myelinating Schwann cells (Gillespie et al. 1994), and contains the PDZ domain. It has two isoforms, L-PRX and S-PRX. L-PRX is detected at adaxonal regions of the Schwann cells with the initial myelination, and then at the abaxonal, Schmidt-Lanterman incisure and paranode with the mature myelin sheath (Scherer et al. 1995b). PRX knockout mice show initial normal myelination, but develop a severe demyelinating neuropathy with allodynia and hyperalgesia, suggesting that PRX is necessary for the maintenance of the myelin sheaths (Gillespie et al. 2000). L-PRX interacts with dystrophin-related protein 2 (DRP2), and forms the L-PRX–DRP2–dystroglycan complex at the surface of Schwann cell plasma membrane (Sherman et al. 2001, 2012).

19.4.6 CMT4G: HK1

CMT4G, also called HMSN-Russe, is a rare severe demyelinating neuropathy found in the Gypsy population (Sevilla et al. 2013; Thomas et al. 2001). It is caused by a mutation in Hexokinase 1 (HK1) gene (Hantke et al. 2009). HK1 is ubiquitously expressed, and first catalyzes glucose. The molecular mechanism of this demyelinating neuropathy remains unknown.

19.4.7 CMT4H: FGD4

CMT4H is characterized by early-onset severe demyelinating neuropathy with scoliosis and myelin outfoldings in the nerve biopsy. Mutations in FGD4 encoding FGD1-related F-actin binding protein (frabin) have been identified in CMT4H families (Delague et al. 2007; Stendel et al. 2007). Frabin is a guanine nucleotide exchange factor for the Rho GTPase cell division cycle 42 (Cdc42), and couple the actin cytoskeleton with the plasma membrane (Nakanishi and Takai 2008). Rho GTPase signaling is critical for peripheral nerve myelination. Frabin knockout mice showed dysmyelination at the early stage and demyelination at the late stage with abnormal myelin structures including myelin outfoldings and infoldings, suggesting that the regulation of Cdc42 by frabin in Schwann cells is critical for nerve development and myelin maintenance (Horn et al. 2012).

19.4.8 CMT4J: FIG4

CMT4J was originally considered an early-onset severe demyelinating neuropathy, and mutations in FIG4 gene were identified in CMT4J patients (Chow et al. 2007). However, subsequent reports revealed that CMT4J displays variable onset and severity (Nicholson et al. 2011). Rapidly progressive asymmetric paralysis has been described in some cases (Cottenie et al. 2013; Zhang et al. 2008). The FIG4 gene encodes a PI(3,5)P2 5-phosphatase. Fig4-deficient pale mice show peripheral neuropathy, neurodegeneration in the brain and spinal cord, and diluted pigmentation (Chow et al. 2007). Many electron-dense organelles related to lysosome have been observed in motor neurons in mice (Katona et al. 2011). Fig4 motor neuron-specific loss developed neuronal and axonal degeneration in the knockout mice, and Fig4 Schwann cell-specific loss showed an endolysosomal trafficking impairment and demyelination in the knockout mice, suggesting that impaired trafficking in motor neurons and Schwann cells might cause CMT4J (Vaccari et al. 2015).

19.4.9 CMT4K: SURF1 Gene

CMT4K has been described in three patients and is characterized by a childhood-onset severe demyelinating neuropathy with lactic acidosis (Echaniz-Laguna et al. 2013).

SURF1 mutations were found in these patients. SURF1 is an assembly factor of complex IV (cytochrome c oxidase). Partial loss of complex IV was observed in muscle and fibroblasts from one patient. SURF mutations could also cause Leigh syndrome. Further clinical and basic studies are necessary to reveal the pathogenesis of this demyelinating neuropathy.

19.5 Conclusion

After the discovery of CMT1A duplication in CMT1A in 1991, many clinical and basic studies have revealed a number of causal genes for CMT and the molecular mechanisms of neuropathy. In this chapter, the growing knowledge of Schwann cells and demyelinating CMT have been reviewed. We hope that effective therapy for CMT will be developed based on these accumulated findings in the near future.

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References

Abe A, Numakura C, Kijima K, Hayashi M, Hashimoto T, Hayasaka K (2011) Molecular diagnosis and clinical onset of Charcot-Marie-Tooth disease in Japan. J Hum Genet 56:364–368

Anzini P, Neuberg DH, Schachner M, Nelles E, Willecke K, Zielasek J, Toyka KV, Suter U, Martini R (1997) Structural abnormalities and deficient maintenance of peripheral nerve myelin in mice lacking the gap junction protein connexin 32. J Neurosci 17:4545–4551

Arnaud E, Zenker J, de Preux Charles AS, Stendel C, Roos A, Medard JJ, Tricaud N, Kleine H, Luscher B, Weis J, Suter U, Senderek J, Chrast R (2009) SH3TC2/KIAA1985 protein is required for proper myelination and the integrity of the node of Ranvier in the peripheral nervous system. Proc Natl Acad Sci U S A 106:17528–17533

Auer-Grumbach M, Weger M, Fink-Puches R, Papic L, Frohlich E, Auer-Grumbach P, El Shabrawi-Caelen L, Schabhuttl M, Windpassinger C, Senderek J, Budka H, Trajanoski S, Janecke AR, Haas A, Metze D, Pieber TR, Guelly C (2011) Fibulin-5 mutations link inherited neuropathies, age-related macular degeneration and hyperelastic skin. Brain 134:1839–1852

Azzedine H, Bolino A, Taieb T, Birouk N, Di Duca M, Bouhouche A, Benamou S, Mrabet A, Hammadouche T, Chkili T, Gouider R, Ravazzolo R, Brice A, Laporte J, LeGuern E (2003) Mutations in MTMR13, a new pseudophosphatase homologue of MTMR2 and Sbf1, in two families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease associated with early-onset glaucoma. Am J Hum Genet 72:1141–1153

Azzedine H, Ravise N, Verny C, Gabreels-Festen A, Lammens M, Grid D, Vallat JM, Durosier G, Senderek J, Nouioua S, Hamadouche T, Bouhouche A, Guilbot A, Stendel C, Ruberg M, Brice A, Birouk N, Dubourg O, Tazir M, LeGuern E (2006) Spine deformities in Charcot-Marie-Tooth 4C caused by SH3TC2 gene mutations. Neurology 67:602–606

Balice-Gordon RJ, Bone LJ, Scherer SS (1998) Functional gap junctions in the Schwann cell myelin sheath. J Cell Biol 142:1095–1104

Baxter RV, Ben Othmane K, Rochelle JM, Stajich JE, Hulette C, Dew-Knight S, Hentati F, Ben Hamida M, Bel S, Stenger JE, Gilbert JR, Pericak-Vance MA, Vance JM (2002) Ganglioside-induced differentiation-associated protein-1 is mutant in Charcot-Marie-Tooth disease type 4A/8q21. Nat Genet 30:21–22

Ben Othmane K, Rochelle JM, Ben Hamida M, Slotterbeck B, Rao N, Hentati F, Pericak-Vance MA, Vance JM (1998) Fine localization of the CMT4A locus using a PAC contig and haplotype analysis. Neurogenetics 2:18–23

Bennett CL, Shirk AJ, Huynh HM, Street VA, Nelis E, Van Maldergem L, De Jonghe P, Jordanova A, Guergueltcheva V, Tournev I, Van Den Bergh P, Seeman P, Mazanec R, Prochazka T, Kremensky I, Haberlova J, Weiss MD, Timmerman V, Bird TD, Chance PF (2004) SIMPLE mutation in demyelinating neuropathy and distribution in sciatic nerve. Ann Neurol 55:713–720

Berger P, Berger I, Schaffitzel C, Tersar K, Volkmer B, Suter U (2006) Multi-level regulation of myotubularin-related protein-2 phosphatase activity by myotubularin-related protein-13/set-binding factor-2. Hum Mol Genet 15:569–579

Bergoffen J, Scherer SS, Wang S, Scott MO, Bone LJ, Paul DL, Chen K, Lensch MW, Chance PF, Fischbeck KH (1993) Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 262:2039–2042

Boerkoel CF, Takashima H, Stankiewicz P, Garcia CA, Leber SM, Rhee-Morris L, Lupski JR (2001) Periaxin mutations cause recessive Dejerine-Sottas neuropathy. Am J Hum Genet 68:325–333

Bolino A, Brancolini V, Bono F, Bruni A, Gambardella A, Romeo G, Quattrone A, Devoto M (1996) Localization of a gene responsible for autosomal recessive demyelinating neuropathy with focally folded myelin sheaths to chromosome 11q23 by homozygosity mapping and haplotype sharing. Hum Mol Genet 5:1051–1054

Bolino A, Muglia M, Conforti FL, LeGuern E, Salih MA, Georgiou DM, Christodoulou K, Hausmanowa-Petrusewicz I, Mandich P, Schenone A, Gambardella A, Bono F, Quattrone A, Devoto M, Monaco AP (2000) Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. Nat Genet 25:17–19

Bolis A, Coviello S, Bussini S, Dina G, Pardini C, Previtali SC, Malaguti M, Morana P, Del Carro U, Feltri ML, Quattrini A, Wrabetz L, Bolino A (2005) Loss of Mtmr2 phosphatase in Schwann cells but not in motor neurons causes Charcot-Marie-Tooth type 4B1 neuropathy with myelin outfoldings. J Neurosci 25:8567–8577

Bolis A, Zordan P, Coviello S, Bolino A (2007) Myotubularin-related (MTMR) phospholipid phosphatase proteins in the peripheral nervous system. Mol Neurobiol 35:308–316

Brennan KM, Bai Y, Shy ME (2015) Demyelinating CMT—what's known, what's new and what's in store? Neurosci Lett 596:14–26

Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, Swanson PD, Odelberg SJ, Disteche CM, Bird TD (1993) DNA deletion associated with hereditary neuropathy with liability to pressure palsies. Cell 72:143–151

Chow CY, Zhang Y, Dowling JJ, Jin N, Adamska M, Shiga K, Szigeti K, Shy ME, Li J, Zhang X, Lupski JR, Weisman LS, Meisler MH (2007) Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4J. Nature 448:68–72

Cottenie E, Menezes MP, Rossor AM, Morrow JM, Yousry TA, Dick DJ, Anderson JR, Jaunmuktane Z, Brandner S, Blake JC, Houlden H, Reilly MM (2013) Rapidly progressive asymmetrical weakness in Charcot-Marie-Tooth disease type 4J resembles chronic inflammatory demyelinating polyneuropathy. Neuromuscul Disord 23:399–403

Crimella C, Tonelli A, Airoldi G, Baschirotto C, D'Angelo MG, Bonato S, Losito L, Trabacca A, Bresolin N, Bassi MT (2010) The GST domain of GDAP1 is a frequent target of mutations in the dominant form of axonal Charcot Marie Tooth type 2K. J Med Genet 47:712–716

Cuesta A, Pedrola L, Sevilla T, Garcia-Planells J, Chumillas MJ, Mayordomo F, LeGuern E, Marin I, Vilchez JJ, Palau F (2002) The gene encoding ganglioside-induced differentiation-associated protein 1 is mutated in axonal Charcot-Marie-Tooth type 4A disease. Nat Genet 30:22–25

D'Urso D, Prior R, Greiner-Petter R, Gabreels-Festen AA, Muller HW (1998) Overloaded endoplasmic reticulum-Golgi compartments, a possible pathomechanism of peripheral neuropathies caused by mutations of the peripheral myelin protein PMP22. J Neurosci 18:731–740

De Jonghe P, Mersivanova I, Nelis E, Del Favero J, Martin JJ, Van Broeckhoven C, Evgrafov O, Timmerman V (2001) Further evidence that neurofilament light chain gene mutations can cause Charcot-Marie-Tooth disease type 2E. Ann Neurol 49:245–249

de Waegh SM, Lee VM, Brady ST (1992) Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating Schwann cells. Cell 68:451–463

Delague V, Jacquier A, Hamadouche T, Poitelon Y, Baudot C, Boccaccio I, Chouery E, Chaouch M, Kassouri N, Jabbour R, Grid D, Megarbane A, Haase G, Levy N (2007) Mutations in FGD4 encoding the Rho GDP/GTP exchange factor FRABIN cause autosomal recessive Charcot-Marie-Tooth type 4H. Am J Hum Genet 81:1–16

Echaniz-Laguna A, Ghezzi D, Chassagne M, Mayencon M, Padet S, Melchionda L, Rouvet I, Lannes B, Bozon D, Latour P, Zeviani M, Mousson de Camaret B (2013) SURF1 deficiency causes demyelinating Charcot-Marie-Tooth disease. Neurology 81:1523–1530

Fledrich R, Stassart RM, Klink A, Rasch LM, Prukop T, Haag L, Czesnik D, Kungl T, Abdelaal TA, Keric N, Stadelmann C, Bruck W, Nave KA, Sereda MW (2014) Soluble neuregulin-1 modulates disease pathogenesis in rodent models of Charcot-Marie-Tooth disease 1A. Nat Med 20:1055–1061

Fortun J, Dunn WA Jr, Joy S, Li J, Notterpek L (2003) Emerging role for autophagy in the removal of aggresomes in Schwann cells. J Neurosci 23:10672–10680

Gabreels-Festen A, van Beersum S, Eshuis L, LeGuern E, Gabreels F, van Engelen B, Mariman E (1999) Study on the gene and phenotypic characterisation of autosomal recessive demyelinating motor and sensory neuropathy (Charcot-Marie-Tooth disease) with a gene locus on chromosome 5q23-q33. J Neurol Neurosurg Psychiatry 66:569–574

Georgiou DM, Zidar J, Korosec M, Middleton LT, Kyriakides T, Christodoulou K (2002) A novel NF-L mutation Pro22Ser is associated with CMT2 in a large Slovenian family. Neurogenetics 4:93–96

Gillespie CS, Sherman DL, Blair GE, Brophy PJ (1994) Periaxin, a novel protein of myelinating Schwann cells with a possible role in axonal ensheathment. Neuron 12:497–508

Gillespie CS, Sherman DL, Fleetwood-Walker SM, Cottrell DF, Tait S, Garry EM, Wallace VC, Ure J, Griffiths IR, Smith A, Brophy PJ (2000) Peripheral demyelination and neuropathic pain behavior in periaxin-deficient mice. Neuron 26:523–531

Gooding R, Colomer J, King R, Angelicheva D, Marns L, Parman Y, Chandler D, Bertranpetit J, Kalaydjieva L (2005) A novel Gypsy founder mutation, p.Arg1109X in the CMT4C gene, causes variable peripheral neuropathy phenotypes. J Med Genet 42:e69

Gouttenoire EA, Lupo V, Calpena E, Bartesaghi L, Schupfer F, Medard JJ, Maurer F, Beckmann JS, Senderek J, Palau F, Espinos C, Chrast R (2013) Sh3tc2 deficiency affects neuregulin-1/ErbB signaling. Glia 61:1041–1051

Grandis M, Vigo T, Passalacqua M, Jain M, Scazzola S, La Padula V, Brucal M, Benvenuto F, Nobbio L, Cadoni A, Mancardi GL, Kamholz J, Shy ME, Schenone A (2008) Different cellular and molecular mechanisms for early and late-onset myelin protein zero mutations. Hum Mol Genet 17:1877–1889

Guilbot A, Williams A, Ravise N, Verny C, Brice A, Sherman DL, Brophy PJ, LeGuern E, Delague V, Bareil C, Megarbane A, Claustres M (2001) A mutation in periaxin is responsible for CMT4F, an autosomal recessive form of Charcot-Marie-Tooth disease. Hum Mol Genet 10:415–421

Gutierrez A, England JD, Sumner AJ, Ferer S, Warner LE, Lupski JR, Garcia CA (2000) Unusual electrophysiological findings in X-linked dominant Charcot-Marie-Tooth disease. Muscle Nerve 23:182–188

Hanemann CO, D'Urso D, Gabreels-Festen AA, Muller HW (2000) Mutation-dependent alteration in cellular distribution of peripheral myelin protein 22 in nerve biopsies from Charcot-Marie-Tooth type 1A. Brain 123(Pt 5):1001–1006

Hantke J, Chandler D, King R, Wanders RJ, Angelicheva D, Tournev I, McNamara E, Kwa M, Guergueltcheva V, Kaneva R, Baas F, Kalaydjieva L (2009) A mutation in an alternative

untranslated exon of hexokinase 1 associated with hereditary motor and sensory neuropathy—Russe (HMSNR). Eur J Hum Genet 17:1606–1614

Harel T, Lupski JR (2014) Charcot-Marie-Tooth disease and pathways to molecular based therapies. Clin Genet 86:422–431

Hayasaka K, Ohnishi A, Takada G, Fukushima Y, Murai Y (1993) Mutation of the myelin P0 gene in Charcot-Marie-tooth neuropathy type 1. Biochem Biophys Res Commun 194:1317–1322

Hayashi M, Abe A, Murakami T, Yamao S, Arai H, Hattori H, Iai M, Watanabe K, Oka N, Chida K, Kishikawa Y, Hayasaka K (2013) Molecular analysis of the genes causing recessive demyelinating Charcot-Marie-Tooth disease in Japan. J Hum Genet 58:273–278

Hirano R, Takashima H, Umehara F, Arimura H, Michizono K, Okamoto Y, Nakagawa M, Boerkoel CF, Lupski JR, Osame M, Arimura K (2004) SET binding factor 2 (SBF2) mutation causes CMT4B with juvenile onset glaucoma. Neurology 63:577–580

Horn M, Baumann R, Pereira JA, Sidiropoulos PN, Somandin C, Welzl H, Stendel C, Luhmann T, Wessig C, Toyka KV, Relvas JB, Senderek J, Suter U (2012) Myelin is dependent on the Charcot-Marie-Tooth Type 4H disease culprit protein FRABIN/FGD4 in Schwann cells. Brain 135:3567–3583

Houlden H, Laura M, Ginsberg L, Jungbluth H, Robb SA, Blake J, Robinson S, King RH, Reilly MM (2009) The phenotype of Charcot-Marie-Tooth disease type 4C due to SH3TC2 mutations and possible predisposition to an inflammatory neuropathy. Neuromuscul Disord 19:264–269

Inoue K, Dewar K, Katsanis N, Reiter LT, Lander ES, Devon KL, Wyman DW, Lupski JR, Birren B (2001) The 1.4-Mb CMT1A duplication/HNPP deletion genomic region reveals unique genome architectural features and provides insights into the recent evolution of new genes. Genome Res 11:1018–1033

Jordanova A, De Jonghe P, Boerkoel CF, Takashima H, De Vriendt E, Ceuterick C, Martin JJ, Butler IJ, Mancias P, Papasozomenos S, Terespolsky D, Potocki L, Brown CW, Shy M, Rita DA, Tournev I, Kremensky I, Lupski JR, Timmerman V (2003) Mutations in the neurofilament light chain gene (NEFL) cause early onset severe Charcot-Marie-Tooth disease. Brain 126:590–597

Kalaydjieva L, Nikolova A, Turnev I, Petrova J, Hristova A, Ishpekova B, Petkova I, Shmarov A, Stancheva S, Middleton L, Merlini L, Trogu A, Muddle JR, King RH, Thomas PK (1998) Hereditary motor and sensory neuropathy—Lom, a novel demyelinating neuropathy associated with deafness in gypsies. Clinical, electrophysiological and nerve biopsy findings. Brain 121 (Pt 3):399–408

Kalaydjieva L, Gresham D, Gooding R, Heather L, Baas F, de Jonge R, Blechschmidt K, Angelicheva D, Chandler D, Worsley P, Rosenthal A, King RH, Thomas PK (2000) N-myc downstream-regulated gene 1 is mutated in hereditary motor and sensory neuropathy-Lom. Am J Hum Genet 67:47–58

Katona I, Wu X, Feely SM, Sottile S, Siskind CE, Miller LJ, Shy ME, Li J (2009) PMP22 expression in dermal nerve myelin from patients with CMT1A. Brain 132:1734–1740

Katona I, Zhang X, Bai Y, Shy ME, Guo J, Yan Q, Hatfield J, Kupsky WJ, Li J (2011) Distinct pathogenic processes between Fig4-deficient motor and sensory neurons. Eur J Neurosci 33:1401–1410

Kaya F, Belin S, Bourgeois P, Micaleff J, Blin O, Fontes M (2007) Ascorbic acid inhibits PMP22 expression by reducing cAMP levels. Neuromuscul Disord 17:248–253

Khajavi M, Shiga K, Wiszniewski W, He F, Shaw CA, Yan J, Wensel TG, Snipes GJ, Lupski JR (2007) Oral curcumin mitigates the clinical and neuropathologic phenotype of the Trembler-J mouse: a potential therapy for inherited neuropathy. Am J Hum Genet 81:438–453

King RH, Tournev I, Colomer J, Merlini L, Kalaydjieva L, Thomas PK (1999) Ultrastructural changes in peripheral nerve in hereditary motor and sensory neuropathy-Lom. Neuropathol Appl Neurobiol 25:306–312

King RH, Chandler D, Lopaticki S, Huang D, Blake J, Muddle JR, Kilpatrick T, Nourallah M, Miyata T, Okuda T, Carter KW, Hunter M, Angelicheva D, Morahan G, Kalaydjieva L (2011) Ndrg1 in development and maintenance of the myelin sheath. Neurobiol Dis 42:368–380

Kleopa KA, Abrams CK, Scherer SS (2012) How do mutations in GJB1 cause X-linked Charcot-Marie-Tooth disease? Brain Res 1487:198–205

Krajewski KM, Lewis RA, Fuerst DR, Turansky C, Hinderer SR, Garbern J, Kamholz J, Shy ME (2000) Neurological dysfunction and axonal degeneration in Charcot-Marie-Tooth disease type 1A. Brain 123(Pt 7):1516–1527

Lee SM, Olzmann JA, Chin LS, Li L (2011) Mutations associated with Charcot-Marie-Tooth disease cause SIMPLE protein mislocalization and degradation by the proteasome and aggresome-autophagy pathways. J Cell Sci 124:3319–3331

Lee SM, Chin LS, Li L (2012) Protein misfolding and clearance in demyelinating peripheral neuropathies: therapeutic implications. Commun Integr Biol 5:107–110

Lewis RA, MP MD, Herrmann DN, Hoke A, Clawson LL, Siskind C, Feely SM, Miller LJ, Barohn RJ, Smith P, Luebbe E, Wu X, Shy ME, Muscle Study G (2013) High-dosage ascorbic acid treatment in Charcot-Marie-Tooth disease type 1A: results of a randomized, double-masked, controlled trial. JAMA Neurol 70:981–987

Li W, Zhu H, Zhao X, Brancho D, Liang Y, Zou Y, Bennett C, Chow CW (2015) Dysregulated Inflammatory Signaling upon Charcot-Marie-Tooth Type 1C Mutation of SIMPLE Protein. Mol Cell Biol 35:2464–2478

Lupski JR, de Oca-Luna RM, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, Saucedo-Cardenas-O, Barker DF, Killian JM, Garcia CA, Chakravarti A, Patel PI (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell 66:219–232

Lupski JR, Reid JG, Gonzaga-Jauregui C, Rio Deiros D, Chen DC, Nazareth L, Bainbridge M, Dinh H, Jing C, Wheeler DA, McGuire AL, Zhang F, Stankiewicz P, Halperin JJ, Yang C, Gehman C, Guo D, Irikat RK, Tom W, Fantin NJ, Muzny DM, Gibbs RA (2010) Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. N Engl J Med 362:1181–1191

Marchesi C, Milani M, Morbin M, Cesani M, Lauria G, Scaioli V, Piccolo G, Fabrizi GM, Cavallaro T, Taroni F, Pareyson D (2010) Four novel cases of periaxin-related neuropathy and review of the literature. Neurology 75:1830–1838

Martini R (2014) Neuregulin-1 alleviates Charcot-Marie-Tooth disease in rats. Nat Med 20:984–985

Mersiyanova IV, Perepelov AV, Polyakov AV, Sitnikov VF, Dadali EL, Oparin RB, Petrin AN, Evgrafov OV (2000) A new variant of Charcot-Marie-Tooth disease type 2 is probably the result of a mutation in the neurofilament-light gene. Am J Hum Genet 67:37–46

Meyer zu Horste G, Prukop T, Liebetanz D, Mobius W, Nave KA, Sereda MW (2007) Antiprogesterone therapy uncouples axonal loss from demyelination in a transgenic rat model of CMT1A neuropathy. Ann Neurol 61:61–72

Murakami T, Garcia CA, Reiter LT, Lupski JR (1996) Charcot-Marie-Tooth disease and related inherited neuropathies. Medicine (Baltimore) 75:233–250

Murakami T, Kutoku Y, Nishimura H, Hayashi M, Abe A, Hayasaka K, Sunada Y (2013) Mild phenotype of Charcot-Marie-Tooth disease type 4B1. J Neurol Sci 334:176–179

Murakami T, Kutoku Y, Nishimura H, Hayashi M, Abe A, Hayasaka K, Sunada Y (2014) Charcot-Marie-Tooth disease type 4B1 and myelin outfoldings. Peripher Nerv 25:52–58

Nakanishi H, Takai Y (2008) Frabin and other related Cdc42-specific guanine nucleotide exchange factors couple the actin cytoskeleton with the plasma membrane. J Cell Mol Med 12:1169–1176

Nakhro K, Park JM, Hong YB, Park JH, Nam SH, Yoon BR, Yoo JH, Koo H, Jung SC, Kim HL, Kim JY, Choi KG, Choi BO, Chung KW (2013) SET binding factor 1 (SBF1) mutation causes Charcot-Marie-Tooth disease type 4B3. Neurology 81:165–173

Nelis E, Erdem S, Van Den Bergh PY, Belpaire-Dethiou MC, Ceuterick C, Van Gerwen V, Cuesta A, Pedrola L, Palau F, Gabreels-Festen AA, Verellen C, Tan E, Demirci M, Van Broeckhoven C, De Jonghe P, Topaloglu H, Timmerman V (2002) Mutations in GDAP1: autosomal recessive CMT with demyelination and axonopathy. Neurology 59:1865–1872

Nicholson G, Corbett A (1996) Slowing of central conduction in X-linked Charcot-Marie-Tooth neuropathy shown by brain stem auditory evoked responses. J Neurol Neurosurg Psychiatry 61:43–46

Nicholson G, Lenk GM, Reddel SW, Grant AE, Towne CF, Ferguson CJ, Simpson E, Scheuerle A, Yasick M, Hoffman S, Blouin R, Brandt C, Coppola G, Biesecker LG, Batish SD, Meisler MH (2011) Distinctive genetic and clinical features of CMT4J: a severe neuropathy caused by mutations in the PI(3,5)P(2) phosphatase FIG4. Brain 134:1959–1971

Niemann A, Ruegg M, La Padula V, Schenone A, Suter U (2005) Ganglioside-induced differentiation associated protein 1 is a regulator of the mitochondrial network: new implications for Charcot-Marie-Tooth disease. J Cell Biol 170:1067–1078

Niemann A, Wagner KM, Ruegg M, Suter U (2009) GDAP1 mutations differ in their effects on mitochondrial dynamics and apoptosis depending on the mode of inheritance. Neurobiol Dis 36:509–520

Nishimura T, Yoshikawa H, Fujimura H, Sakoda S, Yanagihara T (1996) Accumulation of peripheral myelin protein 22 in onion bulbs and Schwann cells of biopsied nerves from patients with Charcot-Marie-Tooth disease type 1A. Acta Neuropathol 92:454–460

Ogata T, Iijima S, Hoshikawa S, Miura T, Yamamoto S, Oda H, Nakamura K, Tanaka S (2004) Opposing extracellular signal-regulated kinase and Akt pathways control Schwann cell myelination. J Neurosci 24:6724–6732

Okamoto Y, Pehlivan D, Wiszniewski W, Beck CR, Snipes GJ, Lupski JR, Khajavi M (2013) Curcumin facilitates a transitory cellular stress response in Trembler-J mice. Hum Mol Genet 22:4698–4705

Pareyson D, Marchesi C (2009) Diagnosis, natural history, and management of Charcot-Marie-Tooth disease. Lancet Neurol 8:654–667

Pareyson D, Taroni F, Botti S, Morbin M, Baratta S, Lauria G, Ciano C, Sghirlanzoni A (2000) Cranial nerve involvement in CMT disease type 1 due to early growth response 2 gene mutation. Neurology 54:1696–1698

Pareyson D, Reilly MM, Schenone A, Fabrizi GM, Cavallaro T, Santoro L, Vita G, Quattrone A, Padua L, Gemignani F, Visioli F, Laura M, Radice D, Calabrese D, Hughes RA, Solari A, Cmt T, Groups C-T (2011) Ascorbic acid in Charcot-Marie-Tooth disease type 1A (CMT-TRIAAL and CMT-TRAUK): a double-blind randomised trial. Lancet Neurol 10:320–328

Passage E, Norreel JC, Noack-Fraissignes P, Sanguedolce V, Pizant J, Thirion X, Robaglia-Schlupp A, Pellissier JF, Fontes M (2004) Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. Nat Med 10:396–401

Patel PI, Roa BB, Welcher AA, Schoener-Scott R, Trask BJ, Pentao L, Snipes GJ, Garcia CA, Francke U, Shooter EM, Lupski JR, Suter U (1992) The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A. Nat Genet 1:159–165

Patzko A, Bai Y, Saporta MA, Katona I, Wu X, Vizzuso D, Feltri ML, Wang S, Dillon LM, Kamholz J, Kirschner D, Sarkar FH, Wrabetz L, Shy ME (2012) Curcumin derivatives promote Schwann cell differentiation and improve neuropathy in R98C CMT1B mice. Brain 135:3551–3566

Pennuto M, Tinelli E, Malaguti M, Del Carro U, D'Antonio M, Ron D, Quattrini A, Feltri ML, Wrabetz L (2008) Ablation of the UPR-mediator CHOP restores motor function and reduces demyelination in Charcot-Marie-Tooth 1B mice. Neuron 57:393–405

Raeymaekers P, Timmerman V, Nelis E, De Jonghe P, Hoogendijk JE, Baas F, Barker DF, Martin JJ, De Visser M, Bolhuis PA et al (1991) Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT 1a). The HMSN Collaborative Research Group. Neuromuscul Disord 1:93–97

Reiter LT, Murakami T, Koeuth T, Pentao L, Muzny DM, Gibbs RA, Lupski JR (1996) A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. Nat Genet 12:288–297

Roberts RC, Peden AA, Buss F, Bright NA, Latouche M, Reilly MM, Kendrick-Jones J, Luzio JP (2010) Mistargeting of SH3TC2 away from the recycling endosome causes Charcot-Marie-Tooth disease type 4C. Hum Mol Genet 19:1009–1018

Robinson FL, Niesman IR, Beiswenger KK, Dixon JE (2008) Loss of the inactive myotubularin-related phosphatase Mtmr13 leads to a Charcot-Marie-Tooth 4B2-like peripheral neuropathy in mice. Proc Natl Acad Sci U S A 105:4916–4921

Ryan MC, Shooter EM, Notterpek L (2002) Aggresome formation in neuropathy models based on peripheral myelin protein 22 mutations. Neurobiol Dis 10:109–118

Sahenk Z, Chen L (1998) Abnormalities in the axonal cytoskeleton induced by a connexin32 mutation in nerve xenografts. J Neurosci Res 51(2):174–184

Sahenk Z, Chen L, Mendell JR (1999) Effects of PMP22 duplication and deletions on the axonal cytoskeleton. Ann Neurol 45:16–24

Saporta AS, Sottile SL, Miller LJ, Feely SM, Siskind CE, Shy ME (2011) Charcot-Marie-Tooth disease subtypes and genetic testing strategies. Ann Neurol 69:22–33

Scherer SS, Deschenes SM, Xu YT, Grinspan JB, Fischbeck KH, Paul DL (1995a) Connexin32 is a myelin-related protein in the PNS and CNS. J Neurosci 15:8281–8294

Scherer SS, Xu YT, Bannerman PG, Sherman DL, Brophy PJ (1995b) Periaxin expression in myelinating Schwann cells: modulation by axon-glial interactions and polarized localization during development. Development 121:4265–4273

Scherer SS, Xu YT, Nelles E, Fischbeck K, Willecke K, Bone LJ (1998) Connexin32-null mice develop demyelinating peripheral neuropathy. Glia 24:8–20

Scherer SS, Xu YT, Messing A, Willecke K, Fischbeck KH, Jeng LJ (2005) Transgenic expression of human connexin32 in myelinating Schwann cells prevents demyelination in connexin32-null mice. J Neurosci 25:1550–1559

Senderek J, Hermanns B, Bergmann C, Boroojerdi B, Bajbouj M, Hungs M, Ramaekers VT, Quasthoff S, Karch D, Schroder JM (1999) X-linked dominant Charcot-Marie-Tooth neuropathy: clinical, electrophysiological, and morphological phenotype in four families with different connexin32 mutations(1). J Neurol Sci 167:90–101

Senderek J, Bergmann C, Ramaekers VT, Nelis E, Bernert G, Makowski A, Zuchner S, De Jonghe P, Rudnik-Schoneborn S, Zerres K, Schroder JM (2003a) Mutations in the ganglioside-induced differentiation-associated protein-1 (GDAP1) gene in intermediate type autosomal recessive Charcot-Marie-Tooth neuropathy. Brain 126:642–649

Senderek J, Bergmann C, Stendel C, Kirfel J, Verpoorten N, De Jonghe P, Timmerman V, Chrast R, Verheijen MH, Lemke G, Battaloglu E, Parman Y, Erdem S, Tan E, Topaloglu H, Hahn A, Muller-Felber W, Rizzuto N, Fabrizi GM, Stuhrmann M, Rudnik-Schoneborn S, Zuchner S, Michael Schroder J, Buchheim E, Straub V, Klepper J, Huehne K, Rautenstrauss B, Buttner R, Nelis E, Zerres K (2003b) Mutations in a gene encoding a novel SH3/TPR domain protein cause autosomal recessive Charcot-Marie-Tooth type 4C neuropathy. Am J Hum Genet 73:1106–1119

Sereda MW, Meyer zu Horste G, Suter U, Uzma N, Nave KA (2003) Therapeutic administration of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A). Nat Med 9:1533–1537

Sevilla T, Cuesta A, Chumillas MJ, Mayordomo F, Pedrola L, Palau F, Vilchez JJ (2003) Clinical, electrophysiological and morphological findings of Charcot-Marie-Tooth neuropathy with vocal cord palsy and mutations in the GDAP1 gene. Brain 126:2023–2033

Sevilla T, Martinez-Rubio D, Marquez C, Paradas C, Colomer J, Jaijo T, Millan JM, Palau F, Espinos C (2013) Genetics of the Charcot-Marie-Tooth disease in the Spanish Gypsy population: the hereditary motor and sensory neuropathy-Russe in depth. Clin Genet 83:565–570

Sherman DL, Fabrizi C, Gillespie CS, Brophy PJ (2001) Specific disruption of a schwann cell dystrophin-related protein complex in a demyelinating neuropathy. Neuron 30:677–687

Sherman DL, Wu LM, Grove M, Gillespie CS, Brophy PJ (2012) Drp2 and periaxin form Cajal bands with dystroglycan but have distinct roles in Schwann cell growth. J Neurosci 32:9419–9428

Shy ME (2006) Peripheral neuropathies caused by mutations in the myelin protein zero. J Neurol Sci 242:55–66

Shy ME, Jani A, Krajewski K, Grandis M, Lewis RA, Li J, Shy RR, Balsamo J, Lilien J, Garbern JY, Kamholz J (2004) Phenotypic clustering in MPZ mutations. Brain 127:371–384

Shy ME, Siskind C, Swan ER, Krajewski KM, Doherty T, Fuerst DR, Ainsworth PJ, Lewis RA, Scherer SS, Hahn AF (2007) CMT1X phenotypes represent loss of GJB1 gene function. Neurology 68:849–855

Sivera R, Espinos C, Vilchez JJ, Mas F, Martinez-Rubio D, Chumillas MJ, Mayordomo F, Muelas N, Bataller L, Palau F, Sevilla T (2010) Phenotypical features of the p.R120W mutation in the GDAP1 gene causing autosomal dominant Charcot-Marie-Tooth disease. J Peripher Nerv Syst 15:334–344

Stendel C, Roos A, Deconinck T, Pereira J, Castagner F, Niemann A, Kirschner J, Korinthenberg R, Ketelsen UP, Battaloglu E, Parman Y, Nicholson G, Ouvrier R, Seeger J, De Jonghe P, Weis J, Kruttgen A, Rudnik-Schoneborn S, Bergmann C, Suter U, Zerres K, Timmerman V, Relvas JB, Senderek J (2007) Peripheral nerve demyelination caused by a mutant Rho GTPase guanine nucleotide exchange factor, frabin/FGD4. Am J Hum Genet 81:158–164

Street VA, Bennett CL, Goldy JD, Shirk AJ, Kleopa KA, Tempel BL, Lipe HP, Scherer SS, Bird TD, Chance PF (2003) Mutation of a putative protein degradation gene LITAF/SIMPLE in Charcot-Marie-Tooth disease 1C. Neurology 60:22–26

Tabaraud F, Lagrange E, Sindou P, Vandenberghe A, Levy N, Vallat JM (1999) Demyelinating X-linked Charcot-Marie-Tooth disease: unusual electrophysiological findings. Muscle Nerve 22:1442–1447

Taylor RA, Simon EM, Marks HG, Scherer SS (2003) The CNS phenotype of X-linked Charcot-Marie-Tooth disease: more than a peripheral problem. Neurology 61:1475–1478

Thomas PK, Kalaydjieva L, Youl B, Rogers T, Angelicheva D, King RH, Guergueltcheva V, Colomer J, Lupu C, Corches A, Popa G, Merlini L, Shmarov A, Muddle JR, Nourallah M, Tournev I (2001) Hereditary motor and sensory neuropathy-russe: new autosomal recessive neuropathy in Balkan Gypsies. Ann Neurol 50:452–457

Timmerman V, De Jonghe P, Ceuterick C, De Vriendt E, Lofgren A, Nelis E, Warner LE, Lupski JR, Martin JJ, Van Broeckhoven C (1999) Novel missense mutation in the early growth response 2 gene associated with Dejerine-Sottas syndrome phenotype. Neurology 52:1827–1832

Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi AB, Seitanidou T, Babinet C, Charnay P (1994) Krox-20 controls myelination in the peripheral nervous system. Nature 371:796–799

Vaccari I, Carbone A, Previtali SC, Mironova YA, Alberizzi V, Noseda R, Rivellini C, Bianchi F, Del Carro U, D'Antonio M, Lenk GM, Wrabetz L, Giger RJ, Meisler MH, Bolino A (2015) Loss of Fig4 in both Schwann cells and motor neurons contributes to CMT4J neuropathy. Hum Mol Genet 24:383–396

Vandenberghe N, Upadhyaya M, Gatignol A, Boutrand L, Boucherat M, Chazot G, Vandenberghe A, Latour P (2002) Frequency of mutations in the early growth response 2 gene associated with peripheral demyelinating neuropathies. J Med Genet 39:e81

Vavlitou N, Sargiannidou I, Markoullis K, Kyriacou K, Scherer SS, Kleopa KA (2010) Axonal pathology precedes demyelination in a mouse model of X-linked demyelinating/type I Charcot-Marie Tooth neuropathy. J Neuropathol Exp Neurol 69:945–958

Warner LE, Mancias P, Butler IJ, McDonald CM, Keppen L, Koob KG, Lupski JR (1998) Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. Nat Genet 18:382–384

Warner LE, Svaren J, Milbrandt J, Lupski JR (1999) Functional consequences of mutations in the early growth response 2 gene (EGR2) correlate with severity of human myelinopathies. Hum Mol Genet 8:1245–1251

Wrabetz L, D'Antonio M, Pennuto M, Dati G, Tinelli E, Fratta P, Previtali S, Imperiale D, Zielasek J, Toyka K, Avila RL, Kirschner DA, Messing A, Feltri ML, Quattrini A (2006) Different intracellular pathomechanisms produce diverse Myelin Protein Zero neuropathies in transgenic mice. J Neurosci 26:2358–2368

Yanagisawa H, Schluterman MK, Brekken RA (2009) Fibulin-5, an integrin-binding matricellular protein: its function in development and disease. J Cell Commun Signal 3:337–347

Yoshihara T, Yamamoto M, Hattori N, Misu K, Mori K, Koike H, Sobue G (2002) Identification of novel sequence variants in the neurofilament-light gene in a Japanese population: analysis of Charcot-Marie-Tooth disease patients and normal individuals. J Peripher Nerv Syst 7:221–224

Zhang X, Chow CY, Sahenk Z, Shy ME, Meisler MH, Li J (2008) Mutation of FIG4 causes a rapidly progressive, asymmetric neuronal degeneration. Brain 131:1990–2001

Guillain–Barré Syndrome

20

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Abstract

Guillain–Barré syndrome (GBS) is an acute immune-mediated polyradiculoneuropathy, and pathophysiologically classified into acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), and acute motor and sensory axonal neuropathy (AMSAN). The main pathophysiological mechanism is complement-mediated nerve injury caused by antibody–antigen interaction in the peripheral nerves. Antiglycolipid antibodies are most pathogenic factors in the development of GBS, but not found in 40% of patients with GBS. One of the principal target regions in GBS is the node of Ranvier where functional molecules including glycolipids are assembled. Nodal dysfunction induced by the immune response in nodal axolemma, termed "nodopathy," can electrophysiologically show reversible conduction failure, axonal degeneration, or segmental demyelination. To detect new target molecules in antiglycolipid antibody-negative GBS and to elucidate the pathophysiology in the subacute and the subsequent phases of the disorder are the next problems.

Keywords

Guillain–Barré syndrome · Glycolipid · Antibody · Complement · Neuropathy · Nodopathy

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20.1 Introduction

Guillain–Barré syndrome (GBS) is an acute immune-mediated polyradiculoneuropathy presenting typically symmetrical limb weakness and hyporeflexia, and a half of patients with GBS present with cranial nerve involvement. Several variants of GBS are known, which are defined based on the distribution of peripheral nerve lesions and symptoms. For example, Miller Fisher syndrome (MFS) is characterized by ophthalmoplegia, ataxia, and areflexia, and a pharyngeal-cervical-brachial (PCB) variant by swallowing disturbance, neck weakness, and proximal weakness of upper extremities. GBS is induced by some kind of immunological triggers, most of which are antecedent infections caused by pathogens such as *Campylobacter jejuni* (*C. jejuni*), *Haemophilus influenzae*, *Mycoplasma pneumoniae*, or *Cytomegalovirus*. In last two decades, experimental and clinical researches have indicated that humoral immunity plays a primary role in the pathogenesis of GBS. Autoantibodies to glycolipids, raised in 60% of patients with GBS, are main pathogenic factors. Most of the antiglycolipid antibodies are generated by immunoreaction against glycoconjugates in pathogens causing antecedent infections, the mechanism of which is called as "molecular mimicry theory" (Yuki et al. 2004; Yuki and Hartung 2012). Distribution of target antigens in the peripheral nervous system, as well as binding specificity of the antiglycolipid antibodies, defines variants of GBS; regional variants such as MFS or PCB and functional variants such as AIDP (acute inflammatory demyelinating polyneuropathy) or AMAN (acute motor axonal neuropathy).

Pathological features of GBS had been established as a primary demyelinating neuropathy, so-called AIDP before Feasby et al. (1986) proposed the concept of the axonal variant of GBS, AMAN that causes primary axonal dysfunction or degeneration. Subsequent clinicopathological studies confirmed that GBS is pathophysiologically divided into AIDP, AMAN, and AMSAN (acute motor and sensory axonal neuropathy) (Griffin et al. 1995, 1996a, b; Sobue et al. 1997). It has been recently proposed that immunoresponse at the nodal region plays a critical role in the pathophysiology of antiganglioside antibody-mediated GBS (Uncini and Kuwabara 2015). This type of neuropathy is called "Nodopathy." In this chapter, recent advances of studies on the pathophysiology of GBS are described, and emerging concepts such as the nodopathy are also explained.

20.2 Pathophysiology in GBS

20.2.1 AIDP, AMAN, and AMSAN

The pathology of AIDP is characteristic by demyelination, lymphocytic inflammation, variable degrees of axonal loss, and endoneurial edema (Griffin and Sheikh 2005). Spinal roots and nerve terminals are most vulnerable due to the relative deficiency of the blood–nerve barrier. Lymphocytes in the endoneurial space are characteristic in AIDP, but not necessarily concentrated in sites of demyelination. A

repertoire of the lymphocytes in the affected nerves is not constant and may be dependent upon the repertoire in the blood (Cornblath et al. 1990). Demyelination can be observed throughout the peripheral nerve fibers, the distribution of which is scattered and segmental. Ultrastructural analysis of demyelination in postmortem specimen suggests that penetration of Schwann cell basal lamina by macrophage and vesicular myelin degradation are a characteristic process of demyelination in AIDP (Griffin and Sheikh 2005). After the penetration of the basal lamina, macrophages strip the outer myelin lamellae at paranodes or internodes, invade the nerve fiber, and phagocyte myelin debris. Vesicular degeneration of myelin sheath, another process of demyelination, looks like soap bubbles and is found in the nerve fibers with deposition of immunoglobulin and complement activation products such as C3d (Hafer-Macko et al. 1996a). The vesicular changes are observed irrespective of the presence of macrophages. In response to myelin injury, the Schwann cells leave the basal lamina of the nerve fibers to generate a circlet of daughter Schwann cells around the demyelinated fiber. After the clearance of myelin, some daughter Schwann cells get into the basal lamina of the fiber, ensheath short segments of the fiber, and form short internodes within the preexisting internode (Griffin and Sheikh 2005). The newly formed short internodes dispersed in the previous internodes are specific markers indicative of earlier demyelination with remyelination (Griffin and Sheikh 2005).

AMAN and AMSAN are axonal forms of GBS, the pathological characteristics of which are deficient in lymphocytic inflammation and in demyelination (Feasby et al. 1986, 1993; McKhann et al. 1993). Nerve fibers that show marked inflammation and demyelination with a certain degree of axonal degeneration in the acute phase of GBS can cause severe axonal degeneration in the recovery phase, so-called the secondary axonal degeneration (Feasby et al. 1993). However, the pathophysiology of AMAN and AMSAN is characterized by primary axonal dysfunction, which progresses to axonal degeneration in the recovery phase or promptly recovers after immune-modulated treatment (Kuwabara et al. 1998a). In the former case, Wallerian-like degeneration of motor axons affecting the ventral roots without cellular infiltration predominates. In the latter case, reversible conduction failure at nodes of Ranvier and degeneration of motor nerve terminals and intramuscular axons are considered to cause motor axonal dysfunction without prominent pathological changes (Kuwabara et al. 1998b; Ho et al. 1997). When paranodal myelin is involved by a bystander effect of immune reaction at nodes or when axonal degeneration is severe, minor demyelination can be observed in the vicinity of injured axons.

20.2.2 Pathogenic Roles of Antiglycolipid Antibodies

As shown in autopsy studies, rabbit models of GBS immunized with ganglioside, and ex vivo studies in the mouse diaphragm, complement-mediated nerve injury is a predominant mechanism for acute neuropathy (Hafer-Macko et al. 1996a, b; Susuki et al. 2007; Halstead et al. 2008). On the other hand, a complement-independent

mechanism has been proposed. Apoptosis of large diameter cells in dorsal root ganglion are observed in rabbit models of sensory ataxic neuropathy sensitized with GD1b, without deposits of activated complements (Takada et al. 2008). IgG anti-GM1 antibodies complement independently caused alteration of lipid raft components in cultured cell membranes, leading to nerve cell damage (Ueda et al. 2010). Antiganglioside antibodies may directly influence the integrity of the rafts. In experiments using knockout mice with modified expression of inhibitory or activating Fc-gamma receptors (FcgR), transgenic mice with altered ganglioside and FcgR expression, and C5-deficient mutant mice, antiganglioside antibody-mediated axonal injury occurred without complement activation in transgenic mice expressing only activating FcgR (He et al. 2015). FcgR-mediated inflammation may play a role in GBS development. It is inferred from above observations that antiganglioside antibody-mediated neuropathy can independently be generated through an activating FcgR and the formation of immune complexes by antigen–antibody interaction in the nerve membranes.

20.2.3 Autonomic Involvement in GBS

Fifty years ago, autonomic nerve involvement was recognized as complications of GBS for the first time by detailed pathological studies of an autopsy case and clinical investigation of a patient with sympathetic hyperactivity (Matsuyama and Haymaker 1967; Mitchell and Meilman 1967). Dysautonomia is the major cause of death in GBS and recognized by blood pressure fluctuations and exaggerated drug responses, cardiac arrhythmias, gastrointestinal dysfunction, and bladder dysfunction (Zochodne 1994). Generally, dysautonomia is dominated by sympathetic hyperactivity during the acute phase and, on the other hand, during the recovery phase, parasympathetic failure is more evident. The first observation in autonomic nerve pathology in GBS patients was edema and inflammation in the superior cervical sympathetic ganglion (Haymaker and Kernohan 1949). Subsequently, disintegrating ganglion cells, perivascular mononuclear cells, and demyelination in the vagus were reported (Matsuyama and Haymaker 1967). Kanda et al. conducted a detailed pathological study of a GBS patient who had severe dysautonomia and died of sudden bradycardia, which exhibited myelin-destructive lesions in the sympathetic chains, slight lymphocytic infiltrations in intracardiac ganglia, and small fiber-predominant demyelination (Kanda et al. 1989). Taken together, demyelination, mononuclear cell infiltration, and inflammation are characteristic in the pathology of autonomic nerves in GBS.

There have been not a few pathophysiologic studies of dysautonomia in experimental allergic neuritis (EAN). In rodent models, perivenular inflammation, demyelination, and remyelination were observed in the vagus, and mild cell infiltration in sympathetic ganglia (Kalimo et al. 1982; Morey et al. 1985). Vagal neuropathy is an important feature (Solders et al. 1985).

20.3 Target Molecules in AIDP

In a pathological examination of autopsy cases of AIDP, there were mild internodal demyelination with sparse infiltration of lymphocytes and macrophages in a very early phase of the disease (Day 3), while in subsequent 5 or 6 days severe demyelination and widespread infiltration of lymphocytes and macrophages were observed (Hafer-Macko et al. 1996a). Acute monophasic EAN models produced by sensitization with a diversity of myelin protein antigens from whole proteins to synthetic peptides have revealed clinical and pathological similarities to AIDP (Fujioka 2018). Vigorous studies of such EAN, however, have not necessarily been conducive to identification of pathogenic factors and development of therapeutic approaches. Some cytokines and chemokines have been proved in EAN to play a crucial role in the upregulation of matrix metalloproteinases, which is essential for the breakdown of the blood–nerve barrier, but it is unclear whether the same cellular immune response occurs in AIDP patients who are not inoculated with myelin antigens.

Although there have been no evident biomarkers of AIDP, several studies suggest that IgG antibodies to myelin glycolipids such as galactocerebroside or LM1 play a pathogenic role in the development of AIDP (Samukawa et al. 2014, 2016; Yako et al. 1999; Kuwahara et al. 2011). Demyelination capability of anti-galactocerebroside antibodies is also explained by an experiment that rabbits immunized with galactocerebroside developed demyelinating neuropathy with multifocal conduction block (Saida et al. 1979). IgG antibodies to moesin, which is located on Schwann cell membrane in microvilli at nodes have been identified in sera from AIDP patients with antecedent cytomegalovirus infection (Sawai et al. 2014). Moesin is a member of the ezrin-radixin-moesin family of proteins and is associated with the rearrangement of plasma membrane flexibility. A pathogenic role of the anti-moesin antibody in demyelination remains unsettled.

20.4 Nodes of Ranvier as Target Regions for Immune Response in GBS: Nodopathy

The myelinated nerve fibers consist of a node, paranode, juxta-paranode, and internode. The nodes are short gaps (about 1 μm in length) between two adjacent myelin segments, where specialized, functional molecules such as ion channels, cell adhesion molecules, and scaffolding proteins are assembled and action potentials responsible for saltatory conduction are generated. Recent studies have revealed that the nodes of Ranvier are the main target regions in pathophysiology in GBS, especially AMAN (Susuki 2016). Autopsy studies and analyses of rabbit models sensitized with gangliosides have shown that deposits of activated complements and disruption of clusters of voltage-gated sodium channels (VGSCs) are observed only at nodes (Hafer-Macko et al. 1996b; Susuki et al. 2007, 2012). The nodes are often target regions primarily affected in immune-mediated neuropathy when the term "nodopathy" is used as described in recent review papers (Uncini and Kuwabara 2015; Uncini and Vallat 2018; Fehmi et al. 2018). In GBS, nodopathy is induced by

antibody binding to target molecules like gangliosides in the axonal membrane at nodes, where nodal lengthening, disruptions of nodal VGSCs, paranodal myelin detachment, and disorganized polarization resulting in inexcitable axolemma are observed to various degrees and lead to axonal conduction block. Therefore, nodopathy can show wide-ranging findings in the electrophysiological examination: spontaneous resolution (reversible conduction failure, RCF), axonal degeneration, or segmental demyelination, even in the same patient (Fig. 20.1). It is unknown what factors control and decide these findings in nodopathy, except for intra-axonal accumulation of calcium inducing axonal degeneration. There are two putative mechanisms of the calcium accumulation in the axolemma. First, depletion of ATP due to energy failure reduces the function of the sodium/potassium pump with an increase of axoplasmic sodium ions and axolemmal depolarization. As a result, persistent sodium channels are activated, and further sodium influx and accumulation occurs. And then, the activity of calcium/sodium exchanger is reversed, and calcium ions accumulate in the axoplasm. Second, antibody–antigen interaction in the axolemma induces activation of complement in the classical pathway and the formation of membrane attack complex (MAC). The MAC

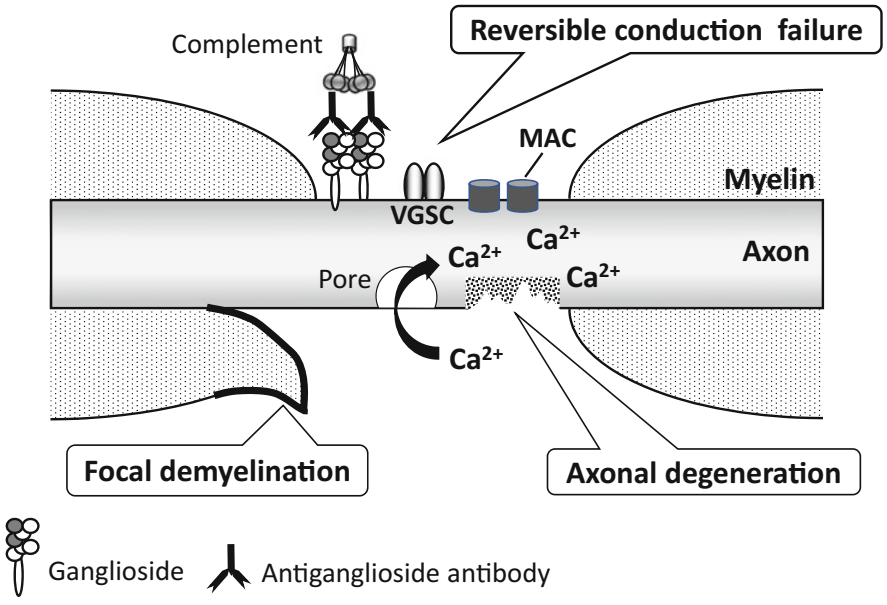

Fig. 20.1 Axonal conduction block at a node of Ranvier. Antibody–antigen interaction at nodes induces complement-mediated axonal conduction block (CB) at nodes, which is termed "nodopathy" and the main pathophysiology of GBS with antiganglioside antibodies. The axonal CB develops into reversible conduction failure, axonal degeneration, or focal demyelination by unknown factors. These conditions can simultaneously emerge in one patient. It is known that intra-axonal accumulation of calcium leads to axonal degeneration. *MAC* membrane attack complex, *VGSC* voltage-gated sodium channel

perforates the axolemma and makes a pore, through which calcium ions inflow and accumulate in the axoplasm.

RCF is a distinctive finding of nodopathy, described as prompt improvement of distal motor latency, conduction block, or temporal dispersion. The follow-up electrodiagnostic test at a peak or recovery phase of GBS will be required to confirm RCF. Peripheral neuropathies have been classified as primarily demyelinating or axonal before the new category of nodopathy emerges. The dichotomous classification will be revised to overcome the discrepancy between electrophysiological findings and location of lesions primarily affected.

20.5 Perspectives

The pathophysiology of GBS has been elucidated to a limited degree, especially in antiganglioside antibody-positive cases. Past studies suggest that complement-dependent nerve injury through antigen–antibody interaction is a predominant pathomechanism. Indeed, antiglycolipid antibodies are most pathogenic factors in the development of GBS, but approximately 40% of patients with GBS have no antibodies and few AIDP-related autoantibodies have been detected. Recent studies have shown that nodal or paranodal proteins also are candidates for target antigens in GBS. IgG antibodies to moesin located in microvilli were identified in acute sera from GBS patients following cytomegalovirus infection (Sawai et al. 2014). In addition, IgG antibodies to neurofascin 155 or contactin-associated protein 1 have been detected in GBS sera, although the frequency of those antibodies is extremely low (<5%) in GBS (Ng et al. 2012; Doppler et al. 2016). These nodal or paranodal proteins, as well as glycolipid complexes, should be searched as target antigens in GBS.

Recent basic research and therapeutic intervention study have shown that administration of complement inhibitor in an acute phase of GBS improves clinical outcome (Halstead et al. 2008; Misawa et al. 2018), probably because the humoral immune response is predominant mechanism early after the onset. In subacute stages of GBS, however, cytotoxic T cell responses can play an important role for myelin damage (Wanschitz et al. 2003). To elucidate the pathophysiology in the subacute, plateau, and recovery phases, as well as the mechanism of nerve repairing, will also lead to developing promising treatment in GBS.

References

Cornblath DR, Griffin DE, Welch D et al (1990) Quantitative analysis of endoneurial T-cells in human sural nerve biopsies. J Neuroimmunol 26:113–118

Doppler K, Appeltshauser L, Villmann C et al (2016) Auto-antibodies to contactin-associated protein 1 (Caspr) in two patients with painful inflammatory neuropathy. Brain 139:2617–2630

Feasby TE, Gilbert JJ, Brown WF et al (1986) An acute axonal form of Guillain-Barré polyneuropathy. Brain 109:1115–1126

Feasby TE, Hahn AF, Brown WF et al (1993) Severe axonal degeneration in acute Guillain-Barré syndrome: evidence of two different mechanisms? J Neurol Sci 116:185–192
Fehmi J, Scherer SS, Willison HJ et al (2018) Nodes, paranodes and neuropathies. J Neurol Neurosurg Psychiatry 89:61–71. https://doi.org/10.1136/jnnp-2016-315480
Fujioka T (2018) Experimental autoimmune neuritis. Clin Exp Neuroimmunol 9:84–92. https://doi.org/10.1111/cen3.12461
Griffin JW, Sheikh K (2005) The Guillain-Barré syndromes. In: Thomas PK, Dyck PJ (eds) Peripheral neuropathy, 4th edn. Elsevier Saunders, Philadelphia, PA, pp 2197–2219
Griffin JW, Li CY, Ho TW et al (1995) Guillain-Barré syndrome in northern China. The spectrum of neuropathological changes in clinically defined cases. Brain 118:577–595
Griffin JW, Li CY, Ho TW et al (1996a) Pathology of the motor sensory axonal Guillain-Barré syndrome. Ann Neurol 39:17–28. https://doi.org/10.1002/ana.410390105
Griffin JW, Li CY, Macko C et al (1996b) Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain-Barré syndrome. J Neurocytol 25:33–51
Hafer-Macko CE, Sheikh KA, Li CY et al (1996a) Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. Ann Neurol 39:625–635. https://doi.org/10.1002/ana.410390512
Hafer-Macko C, Hsieh ST, Li CY et al (1996b) Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. Ann Neurol 40:635–644. https://doi.org/10.1002/ana.410400414
Halstead SK, Zitman FM, Humphreys PD et al (2008) Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model. Brain 131:1197–1208. https://doi.org/10.1093/brain/awm316
Haymaker WE, Kernohan JW (1949) The Landry-Guillain-Barré syndrome; a clinicopathologic report of 50 fatal cases and a critique of the literature. Medicine 28:59–141
He L, Zhang G, Liu W et al (2015) Anti-ganglioside antibodies induce nodal and axonal injury via Fcc receptor-mediated inflammation. J Neurosci 35:6770–6785. https://doi.org/10.1523/JNEUROSCI.4926-14.2015
Ho TW, Hsieh ST, Nachamkin I et al (1997) Motor nerve terminal degeneration provides a potential mechanism for rapid recovery in acute motor axonal neuropathy after Campylobacter infection. Neurology 48:717–724
Kalimo H, Molnár GK, Saksa M et al (1982) Involvement of autonomic nervous system in experimental allergic neuritis. A light- and electron-microscopic study. J Neuroimmunol 2:9–19
Kanda T, Hayashi H, Tanabe H et al (1989) A fulminant case of Guillain-Barré syndrome: topographic and fibre size related analysis of demyelinating changes. J Neurol Neurosurg Psychiatry 52:857–864
Kuwabara S, Asahina M, Koga M et al (1998a) Two patterns of clinical recovery in Guillain-Barré syndrome with IgG anti-GM1 antibody. Neurology 51:1656–1660
Kuwabara S, Yuki N, Koga M et al (1998b) IgG anti-GM1 antibody is associated with reversible conduction failure and axonal degeneration in Guillain-Barré syndrome. Ann Neurol 44:202–208. https://doi.org/10.1002/ana.410440210
Kuwahara M, Suzuki S, Takada K et al (2011) Antibodies to LM1 and LM1-containing ganglioside complexes in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. J Neuroimmunol 239:87–90. https://doi.org/10.1016/j.jneuroim.2011.08.016
Matsuyama H, Haymaker W (1967) Distribution of lesions in the Landry-Guillain-Barré syndrome, with emphasis on involvement of the sympathetic system. Acta Neuropathol 8:230–241
McKhann GM, Cornblath DR, Griffin JW et al (1993) Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. Ann Neurol 33:333–342. https://doi.org/10.1002/ana.410330402
Misawa S, Kuwabara S, Sato Y et al (2018) Safety and efficacy of eculizumab in Guillain-Barré syndrome: a multicentre, double-blind, randomised phase 2 trial. Lancet Neurol 17:519–529. https://doi.org/10.1016/S1474-4422(18)30114-5
Mitchell PL, Meilman E (1967) The mechanism of hypertension in the Guillain-Barré syndrome. Am J Med 42:986–995

Morey MK, Wiley CA, Hughes RA et al (1985) Autonomic nerves in experimental allergic neuritis in the rat. Acta Neuropathol 67:75–80
Ng JK, Malotka J, Kawakami N et al (2012) Neurofascin as a target for autoantibodies in peripheral neuropathies. Neurology 79:2241–2248
Saida T, Saida K, Dorfman SH et al (1979) Experimental allergic neuritis induced by sensitization with galactocerebroside. Science 204:1103–1106
Samukawa M, Hamada Y, Kuwahara M et al (2014) Clinical features in Guillain-Barré syndrome with anti-Gal-C antibody. J Neurol Sci 337:55–60. https://doi.org/10.1016/j.jns.2013.11.016
Samukawa M, Kuwahara M, Morikawa M et al (2016) Electrophysiological assessment of Guillain-Barré syndrome with both Gal-C and ganglioside antibodies; tendency for demyelinating type. J Neuroimmunol 301:61–64. https://doi.org/10.1016/j.jneuroim.2016.10.006
Sawai S, Satoh M, Mori M et al (2014) Moesin is a possible target molecule for cytomegalovirus-related Guillain-Barré syndrome. Neurology 83:113–117. https://doi.org/10.1212/WNL.0000000000000566
Sobue G, Li M, Terao S et al (1997) Axonal pathology in Japanese Guillain-Barré syndrome: a study of 15 autopsied cases. Neurology 48:1694–1700
Solders G, Persson A, Kristensson K et al (1985) Autonomic dysfunction in experimental allergic neuritis. Acta Neurol Scand 72:18–25
Susuki K (2016) Node of Ranvier disruption in Guillain–Barré syndrome. Clin Exp Neuroimmunol 7:324–329. https://doi.org/10.1111/cen3.12338
Susuki K, Rasband MN, Tohyama K et al (2007) Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. J Neurosci 27:3956–3967. https://doi.org/10.1523/JNEUROSCI.4401-06.2007
Susuki K, Yuki N, Schafer DP et al (2012) Dysfunction of nodes of Ranvier: a mechanism for anti-ganglioside antibody-mediated neuropathies. Exp Neurol 233:534–542. https://doi.org/10.1016/j.expneurol.2011.11.039
Takada K, Shimizu J, Kusunoki S (2008) Apoptosis of primary sensory neurons in GD1b-induced sensory ataxic neuropathy. Exp Neurol 209:279–283. https://doi.org/10.1016/j.expneurol.2007.09.010
Ueda A, Shima S, Miyashita T et al (2010) Anti-GM1 antibodies affect the integrity of lipid rafts. Mol Cell Neurosci 45:355–362. https://doi.org/10.1016/j.mcn.2010.07.008
Uncini A, Kuwabara S (2015) Nodopathies of the peripheral nerve: an emerging concept. J Neurol Neurosurg Psychiatry 86:1186–1195. https://doi.org/10.1136/jnnp-2014-310097
Uncini A, Vallat JM (2018) Autoimmune nodo-paranodopathies of peripheral nerve: the concept is gaining ground. J Neurol Neurosurg Psychiatry 89:627–635. https://doi.org/10.1136/jnnp-2017-317192
Wanschitz J, Maier H, Lassmann H et al (2003) Distinct time pattern of complement activation and cytotoxic T cell response in Guillain-Barré syndrome. Brain 126:2034–2042. https://doi.org/10.1093/brain/awg207
Yako K, Kusunoki S, Kanazawa I (1999) Serum antibody against a peripheral nerve myelin ganglioside, LM1, in Guillain-Barré syndrome. J Neurol Sci 168:85–89
Yuki N, Hartung HP (2012) Guillain-Barré Syndrome. N Engl J Med 366:2294–2304. https://doi.org/10.1056/NEJMra1114525
Yuki N, Susuki K, Koga M et al (2004) Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barre syndrome. Proc Natl Acad Sci U S A 101:11404–11409. https://doi.org/10.1073/pnas.0402391101
Zochodne DW (1994) Autonomic involvement in Guillain-Barré syndrome: a review. Muscle Nerve 17:1145–1155. https://doi.org/10.1002/mus.880171004

Chronic Inflammatory Demyelinating Polyneuropathy

21

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Abstract

Chronic inflammatory demyelinating polyneuropathy (CIDP) is immune-mediated neuropathy defined by clinical progression for more than 2 months, and electrodiagnostic evidence of peripheral nerve demyelination. However, there are several clinical phenotypes, classified into "typical CIDP," and "atypical CIDP" such as "multifocal acquired demyelinating sensory and motor neuropathy (MADSAM)." Typical CIDP is a most common form, characterized by symmetric proximal and distal muscle weakness and motor-dominant manifestation. In typical CIDP, demyelination predominantly affects the distal nerve terminals and nerve roots, where the blood–nerve barrier is anatomically deficient. These features suggest antibody-mediated demyelination in typical CIDP. By contrast, MADSAM is characterized by multifocal demyelination in the nerve trunks, and such distribution of lesions results in multiple mononeuropathy or asymmetric polyneuropathy. In MADSAM, cellular immunity is likely to be involved in the breakdown of the blood–nerve barrier at the site of conduction block. Clinical features are probably determined by the distribution of demyelinative lesions and reflect the different immunopathogenesis of each CIDP subtype that would require different treatment strategy.

Keywords

Chronic inflammatory demyelinating polyneuropathy · Blood–nerve barrier · Demyelination · Nerve conduction

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21.1 Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an immune-mediated neuropathy and should be considered in any patient with progressive polyneuropathy with the progressive course for over 2 months. CIDP is a diagnostic term based on clinical symptoms and signs, electrophysiological evidence suggestive of demyelination, and laboratory tests (American Academy of Neurology 1991; Joint Task Force of the EFNS and the PNS 2005, 2010). CIDP is therefore likely to be not a single disease, but a heterogeneous disorder having a wide range of clinical phenotype, course, and response to immune treatments (Dyck et al. 1975; McCombe et al. 1987; Barohn et al. 1989; Simmons et al. 1993; Sghirlanzoni et al. 2000). The heterogeneity is partly due to a different definition of the disease. Classical CIDP, currently termed as "typical CIDP," is clinically defined as symmetric polyneuropathy with both proximal and distal muscle, and constitutes a uniform group of CIDP (Barohn et al. 1989; Mathey et al. 2015). However, there are further many clinical phenotypes, such as multifocal acquired demyelinating sensory and motor neuropathy (MADSAM), and demyelinating acquired distal symmetric neuropathy (DADS) could be included in CIDP (Saperstein et al. 1999).

In 2005, the Joint Task Force of the European Federation of Neurological Societies and the Peripheral Nerve Society (EFNS/PNS) classified CIDP into the clinical subtypes (Table 21.1); classical CIDP is renamed as "typical CIDP," and "atypical CIDP" included MADSAM or asymmetric CIDP, distal acquired demyelinating symmetric neuropathy (DADS), and pure motor or sensory CIDP (Joint Task Force of the EFNS and the PNS 2005). These conditions were still considered CIDP but have different features. In the criteria, multifocal motor neuropathy and demyelinating neuropathy with anti-myelin-associated glycoprotein antibody were excluded from CIDP (Table 21.1). Each CIDP subtype shares a common feature, acquired (presumably immune mediated) peripheral nerve demyelination, but the differences in phenotypes suggest that the pathophysiology and immunopathogenesis in each subtype

Table 21.1 Clinical subtypes of CIDP

A. Typical CIDP
Chronically progressive, stepwise, or recurrent symmetric proximal and distal weakness and sensory dysfunction of all extremities, developing over at least 2 months; cranial nerves may be affected; and absent or reduced tendon reflexes in all extremities
B. Atypical CIDP[a]
(1) Distal acquired demyelinating symmetric (DADS)
(2) Multifocal acquired demyelinating sensory and motor neuropathy (MADSAM) (Lewis-Sumner syndrome)
(3) Focal CIDP (involvement of the brachial or lumbosacral plexus or of one or more peripheral nerves in one upper or lower limb)
(4) Pure motor CIDP
(5) Pure sensory CIDP

Modified from EFNS/PNS guideline (Joint Task Force of the EFNS and the PNS. 2010)
[a]Multifocal motor neuropathy and anti-myelin-associated glycoprotein neuropathy are excluded

of CIDP are not identical. Previous studies have suggested that distribution patterns of demyelination determine the clinical phenotypes (Kuwabara et al. 2002).

Electrodiagnostic studies can provide important information about the distribution of demyelinating lesions. Clinical-electrophysiologic correlation in the subtypes of CIDP is summarized as below. Furthermore, reason(s) for proximal, as well as distal, muscle weakness, and for motor dominancy in typical CIDP are discussed.

21.2 Typical CIDP

21.2.1 Preferential Involvement of the Distal Nerve Terminals

Clinical features of typical CIDP are symmetric polyneuropathy, and involvement of both proximal and distal muscles. Generally, proximal muscle weakness is a rare manifestation of chronic polyneuropathy even in the advanced stage. On the other hand, when there is proximal as well as distal muscle weakness, typical CIDP is strongly suggested. What is the pathophysiology of such particular pattern of muscle weakness?

The distal nerve terminals and nerve roots, where the blood–nerve barrier is anatomically deficient (Olsson 1990), are preferentially involved in immune-mediated neuropathies (Bromberg and Albers 1993; Kuwabara 2007), and demyelination predominant in the most distal and proximal parts of the peripheral nerve are likely to be responsible for the "non-nerve length-dependent" muscle weakness in typical CIDP, because the short, as well as long peripheral nerves could be similarly involved in the nerve terminals and roots by immune attack.

In CIDP, the electrophysiologic examination must focus on the nerve conduction abnormalities seen in the distal nerve segments. Previous reports emphasized conduction block in the intermediate nerve trunk (e.g., in the forearm segment of the median or ulnar nerves); however, the distal nerve terminals are generally more severely involved than the nerve trunk in typical CIDP (Kuwabara et al. 2002). Figure 21.1a shows representative waveforms recorded in median motor nerve conduction studies in typical CIDP. Apparent conduction block (50% amplitude reduction) and nerve conduction slowing in the forearm segments is seen, but the most prominent abnormalities include severely decreased distal CMAP amplitude with markedly prolonged distal latency, indicating the extent of nerve conduction block is greater in the distal nerve terminals than in the intermediate nerve segments (nerve trunk). This pattern of nerve conduction abnormalities is very different from one in MADSAM neuropathy showing normal distal CMAP (Fig. 21.1b).

Another characteristic feature in typical CIDP is prolonged duration of distally evoked compound muscle action potential (CMAP), indicating temporal dispersion and thereby distal demyelination (Thaisetthawatkul et al. 2002; Isose et al. 2009; Stanton et al. 2006). In the revised electrophysiologic criteria proposed by the EFNS/PNS, prolonged duration of distal CMAP was newly added as evidence of nerve terminal demyelination (Joint Task Force of the EFNS and the PNS 2010). Figure 21.2 compares distal CMAPs in a number of demyelinating neuropathies; typical

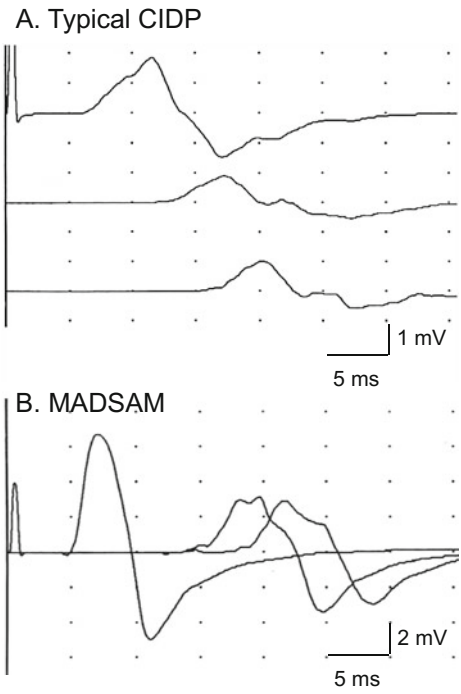

Fig. 21.1 Representative waveforms of median nerve conduction studies in a patient with typical CIDP (**a**), or multifocal acquired demyelinating sensory and motor neuropathy (MADSAM) (**b**). Reproduced from Kuwabara et al. (2015)

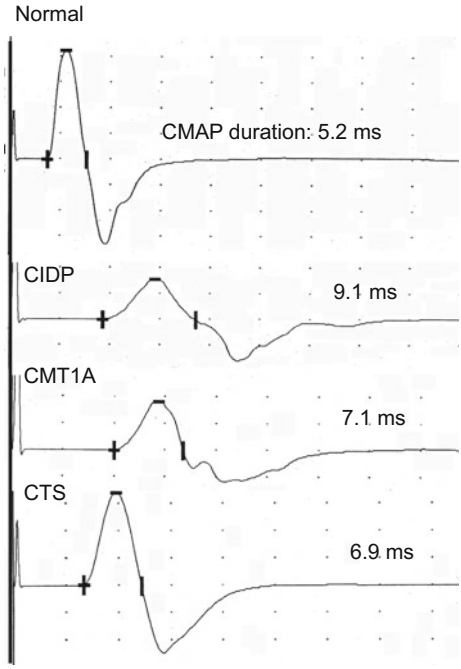

Fig. 21.2 Compound muscle action potentials elicited after median nerve stimulation at the wrist in a normal subject, and patients with CIDP, Charcot–Marie–Tooth disease type 1A (CMT1A), or carpal tunnel syndrome (CTS)

CIDP, Charcot–Marie–Tooth disease type 1A, and carpal tunnel syndrome. Whereas distal motor latencies are longer than normal in these neuropathies, prolongation of CMAP duration is most striking in typical CIDP, which suggests that demyelination occurs multifocally in the fasciculus variation within the nerve (Isose et al. 2009).

21.2.2 Nerve Root Involvement in Typical CIDP

In general, it is difficult to examine nerve conduction in the roots and proximal portions of the peripheral nerves when impulse transmission is severely impaired in the distal nerve segments. Analysis of F-waves is conventionally employed in electrophysiologic criteria of CIDP, but demyelination in the roots can be reliably evaluated only when nerve conduction in the nerve trunk and the distal portion is preserved. F-waves may be absent because of motor axonal loss, or to decreased excitability of anterior horn cells (Hara et al. 2010; Taniguchi et al. 2008). Magnetic stimulation to the nerve roots may be used for the detection of proximal demyelination in CIDP, but again, distal nerve lesion largely affects the results. Furthermore, it is not guaranteed whether stimulation is supramaximal or not.

The EFNS/PNS guideline recommends MRI of the spinal roots as a useful tool for the diagnosis of typical CIDP (Joint Task Force of the EFNS and the PNS 2010). MRI may demonstrate hypertrophy or abnormal contrast-enhancement of the nerves, suggestive of demyelination and supportive of a diagnosis of CIDP. Previous studies have shown symmetric nerve root hypertrophy on MRI in typical CIDP (Duggins et al. 1999; Kuwabara et al. 1997; Shibuya et al. 2015; Tazawa et al. 2008). It is notable that patterns of nerve enlargement are again different in typical CIDP and MADSAM, suggesting different distribution of demyelinating lesions. Typical CIDP has symmetric diffuse hypertrophy in the plexus to nerve roots, while multifocal nerve swelling is present at the sites of conduction block in MADSAM. Particularly, a recently developed reconstruction technique is extremely useful to evaluate peripheral nerve morphology (Shibuya et al. 2015) (Fig. 21.3). In the diagnosis of typical CIDP, nerve conduction studies and root MRI are complementary, respectively, evaluating demyelination in the distal nerve terminals and nerve roots.

21.2.3 Mechanisms for Motor Predominant Manifestation

Patients with immune-mediated neuropathy tend to have motor predominant sensory and motor impairment. There are several possible mechanisms for the motor predominancy; immunological, biophysical, and anatomical factors. In pure motor neuropathy such as multifocal motor neuropathy and axonal Guillain–Barré syndrome, the immunologically selective attack affects only motor axons (Yuki and Kuwabara 2007). Second, there are substantial differences in excitability in sensory and motor axons (Bostock et al. 1994; Bostock and Rothwell 1997), sensory axons have more prominent persistent sodium channels, and inward-rectifying current,

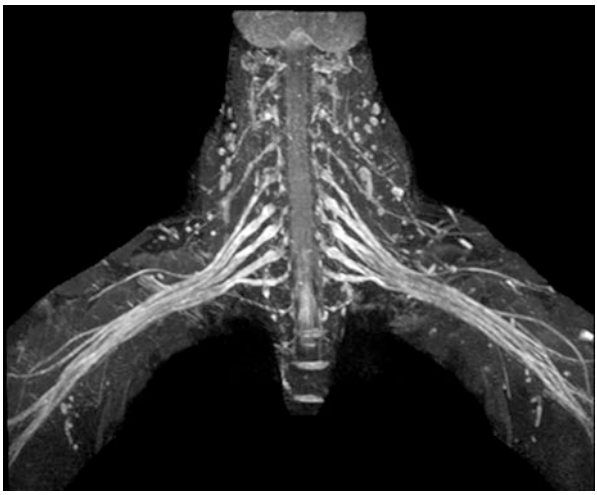

Fig. 21.3 Reconstruction MR neurography in a patient with typical CIDP. Note hypertrophy and high signal of the cervical roots. Reproduced from Shibuya et al. (2015)

activated in response to prolonged membrane hyperpolarization, are greater in sensory than motor axons. Excitability of motor axons is physiologically lower than that of sensory axons, resulting in more readily development of nerve conduction block.

Thirdly, there is a significant anatomical difference in motor and sensory axons; motor axons have branching near the neuromuscular junction, and this should lead to more susceptibility to nerve conduction block in motor axons by demyelination. Myelinated nerve fibers have salutatory conduction, and the safety factor for impulse transmission is expressed as the following formula:

$$\text{Safety factor} = \text{Driving current} / \text{Threshold (current required to open sodium channels)}$$

When an action potential is generated at a node of Ranvier, massive inward sodium currents spread to depolarize the next nodes, and this is termed as "driving current." When the driving current is greater than "threshold" at the next node, it results in another cycle of inward sodium currents (Kuwabara and Misawa 2004). Such salutatory conduction is made possible by the myelin that is an insulator. If myelin is disrupted, significant leak of driving currents in the internode leads to a decrease in the driving current, and if the safety factor becomes below unity, impulse transmission is blocked. This is a classical demyelinating conduction block.

If the motor and sensory axons suffer the same extent of demyelination, the difference significantly affects the number of blocked axons, and is likely to explain motor-dominant manifestation in typical CIDP in which the terminal axons with branching are preferentially involved (Fig. 21.4).

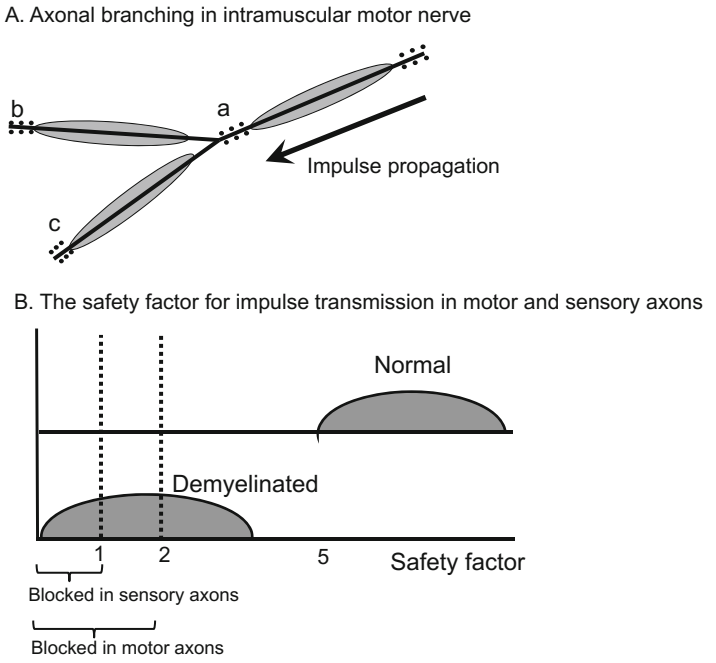

Fig. 21.4 (**A**) Axonal branching in intramuscular motor nerve. The safety factor for impulse transmission is halved because an action potential generated at the node "a" needs to depolarize the two distal nodes "b" and "c". (**B**) Schematic presentation of the safety factor in normal and demyelinated motor axons. For secure nerve conduction, the safety factor should be >2.0 for motor axons, because the safety factor is physiologically lowered by axonal branching as shown (**A**)

21.3 MADSAM

21.3.1 Multifocal Conduction Block in the Nerve Trunk

Multifocal acquired demyelinating sensory and motor neuropathy (MADSAM) is now considered as a variant of CIDP and is clinically defined by a mononeuropathy multiplex involving both motor and sensory axons. In this type of neuropathy, nerve conduction studies reveal multifocal conduction block and slowing in the nerve trunks (Saperstein et al. 1999). Patients with MADSAM are more refractory to immune-modulating treatments. Response to intravenous immunoglobulin treatment is not sufficiently good (Kuwabara et al. 2015).

Compared with typical CIDP patients, MADSAM patients tend to have longer disease duration and slower progression, and therefore they often develop asymmetric polyneuropathy during the long course of the disease. Electrophysiology of MADSAM is characterized by multifocal nerve conduction block in the intermediate nerve trunks, and distal CMAP amplitudes may be decreased due to Wallerian

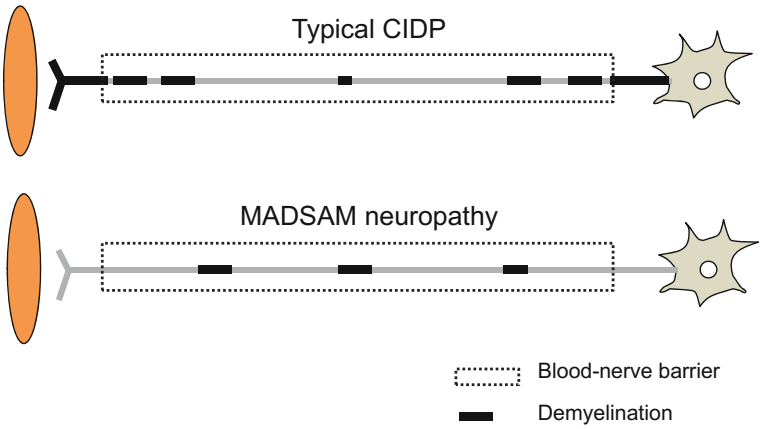

Fig. 21.5 Schematic presentation of the distribution of demyelinating lesions and the blood–nerve barrier in typical CIDP and multifocal acquired demyelinating sensory and motor (MADSAM) neuropathy. Reproduced from Kuwabara and Misawa (2011)

degeneration in the proximal nerve segments, particularly in those with long disease duration.

Figure 21.1b shows CMAP waveforms in MADSAM neuropathy. Nerve conduction studies reveal prominent conduction block in the forearm segment, suggesting focal demyelination at the site of conduction block in the forearm. The distribution of demyelinating lesions in multiple nerves should lead to multiple mononeuropathy clinically.

21.3.2 Breakdown of the Blood–Nerve Barrier

As described above, distribution patterns of demyelinating lesions are substantially different in typical CIDP and MADSAM neuropathy. Figure 21.5 shows schemata of the relationship between the blood–nerve barrier and demyelinative lesions in typical CIDP and MADSAM. In MADSAM, breakdown of the barrier is necessary for the development of nerve demyelination (Kuwabara and Misawa 2011). The process would be similar to one in multiple sclerosis; presumably activated lymphocytes attach and penetrate the endothelial cells, and invade nerve parenchyma, as in multiple sclerosis.

21.4 DADS and Other Subtypes of CIDP

Another subgroup of CIDP, termed as "demyelinating acquired distal symmetric neuropathy (DADS)" was reported (Katz et al. 2000). DADS is characterized by distal-dominant polyneuropathy type of CIDP. DADS patients are classified primarily according to the pattern of the neuropathy and secondarily according to the

presence and type of monoclonal protein (M-protein). Two-thirds of the patients with DADS neuropathy had IgM monoclonal gammopathy, and this specific combination predicted a poor response to immunomodulating therapy. Anti-myelin-associated glycoprotein (anti-MAG) antibodies were present in 67% of these patients.

It is now established anti-MAG antibodies are pathogenetic in this neuropathy. This disorder is mediated by IgM antibodies against MAG. Assuming the high molecular weight of IgM (approximately 800,000), the antibodies cannot access the nerve trunk because of the blood–nerve barrier and presumably bind to the distal nerve terminals, resulting in marked prolongation of distal latencies in nerve conduction studies (Kaku et al. 1994). DADS patients are usually refractory to immunoglobulin therapy, and corticosteroids, and currently rituximab is considered a promising agent for the disorder (Dalakas et al. 2009).

Other subtypes of CIDP include pure motor or sensory CIDP. Pure motor CIDP of the mononeuropathy multiplex type corresponds to multifocal motor neuropathy. Currently, it is still a matter of controversy whether pure motor symmetric CIDP and pure sensory CIDP really exist. Systematic future studies are required.

21.5 Conclusion

Currently, the concept of CIDP includes several clinical subtypes; typical CIDP and other variants. Clinical features are likely to be determined by the distribution of demyelinative lesions and the blood–nerve barrier. The different phenotypes would reflect the different immunopathogenesis. Among the CIDP spectrum, typical CIDP and MADSAM are the major subtypes, and their pathophysiology appears to be distinct. In typical CIDP, the distal nerve terminals and possibly the nerve roots, where the blood–nerve barrier is anatomically deficient, are preferentially affected, raising the possibility of antibody-mediated demyelination, whereas cellular immunity with the breakdown of the barrier may be important in MADSAM neuropathy.

References

Ad Hoc Subcommittee for the American Academy of Neurology AIDS Task Force (1991) Research criteria for chronic inflammatory demyelinating polyneuropathy (CIDP). Neurology 41:617–618

Barohn RJ et al (1989) Chronic inflammatory demyelinating polyradiculoneuropathy. Clinical characteristics, course, and recommendations for diagnostic criteria. Arch Neurol 46:878–884

Bostock H, Rothwell JC (1997) Latent addition in motor and sensory fibres of human peripheral nerve. J Physiol 498:277–294

Bostock H et al (1994) Differences in behaviour of sensory and motor axons following release of ischaemia. Brain 117:225–234

Bromberg MB, Albers JW (1993) Patterns of sensory nerve conduction abnormalities in demyelinating and axonal peripheral nerve disorders. Muscle Nerve 16:262–266

Dalakas MC et al (2009) Placebo-controlled trial of rituximab in IgM anti-myelin-associated glycoprotein antibody demyelinating neuropathy. Ann Neurol 65:286–293
Duggins AJ et al (1999) Spinal root and plexus hypertrophy in chronic inflammatory demyelinating polyneuropathy. Brain 122:1383–1390
Dyck PJ et al (1975) Chronic inflammatory demyelinating polyradiculoneuropathy. Mayo Clin Proc 50:621–637
Hara M et al (2010) Effect of motor imagery and voluntary muscle contraction on the F wave. Muscle Nerve 42:208–212
Isose S et al (2009) Utility of the distal compound muscle action potential duration for diagnosis of demyelinating neuropathies. J Peripher Nerv Syst 14:151–158
Joint Task Force of the EFNS and the PNS. European Federation of Neurological Societies/ Peripheral Nerve Society Guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy. (2005). Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. J Peripher Nerv Syst 10:220–228 and Eur J Neurol 13:326–332
Joint Task Force of the EFNS and the PNS. European Federation of Neurological Societies/ Peripheral Nerve Society Guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy (2010) Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society – First Revision. J Peripher Nerv Syst 15:1–9
Kaku DA et al (1994) Distal accentuation of conduction slowing in polyneuropathy associated with antibodies to myelin-associated glycoprotein and sulphated glucuronyl paragloboside. Brain 117:941–947
Katz JS et al (2000) Distal acquired demyelinating symmetric neuropathy. Neurology 54:615–620
Kuwabara S (2007) The blood-nerve barrier and sensory nerve conduction. Clin Neurophysiol 118:1901–1902
Kuwabara S, Misawa S (2004) Axonal ionic pathophysiology in human peripheral neuropathy and motor neuron disease. Curr Neurovasc Res 1:373–379
Kuwabara S, Misawa S (2011) Chronic inflammatory demyelinating polyneuropathy: clinical subtypes and their correlation with electrophysiology. Clin Exp Neuroimmunol 2:41–48
Kuwabara S et al (1997) Magnetic resonance imaging at the demyelinative foci in chronic inflammatory demyelinating polyneuropathy. Neurology 48:874–877
Kuwabara S et al (2002) Distribution patterns of demyelination correlate with clinical profiles in chronic inflammatory demyelinating polyneuropathy. J Neurol Neurosurg Psychiatry 72:37–42
Kuwabara S et al (2015) Different electrophysiological profiles and treatment response in 'typical' and 'atypical' chronic inflammatory demyelinating polyneuropathy. J Neurol Neurosurg Psychiatry 86:1054–1059
Mathey EK et al (2015) Chronic inflammatory demyelinating polyradiculoneuropathy: from pathology to phenotype. J Neurol Neurosurg Psychiatry 86:973–985
McCombe PA et al (1987) Chronic inflammatory demyelinating polyradiculoneuropathy. Brain 110:1617–1630
Olsson Y (1990) Microenviroment of the peripheral nervous system under normal and pathological conditions. Crit Rev Neurobiol 5:265–311
Saperstein DS et al (1999) Multifocal acquired demyelinating sensory and motor neuropathy: the Lewis-Sumner syndrome. Muscle Nerve 22:560–566
Sghirlanzoni A et al (2000) Chronic inflammatory demyelinating polyradiculoneuropathy: long-term course and treatment of 60 patients. Neurol Sci 21:31–37
Shibuya K et al (2015) Reconstruction magnetic resonance neurography in chronic inflammatory demyelinating polyneuropathy. Ann Neurol 77:333–337
Simmons Z et al (1993) Presentation and initial clinical course in patients with chronic inflammatory demyelinating polyradiculoneuropathy: comparison of patients without and with monoclonal gammopathy. Neurology 43:2202–2209

Stanton M et al (2006) Dispersion of compound muscle action potential in hereditary neuropathies and chronic inflammatory demyelinating polyneuropathy. Muscle Nerve 34:417–422
Taniguchi S et al (2008) Effect of motion imagery to counter rest-induced suppression of F-wave as a measure of anterior horn cell excitability. Clin Neurophysiol 119:1346–1352
Tazawa K et al (2008) Spinal nerve root hypertrophy on MRI: clinical significance in the diagnosis of chronic inflammatory demyelinating polyradiculoneuropathy. Intern Med 47:2019–2024
Thaisetthawatkul P et al (2002) Dispersion of distal compound muscle action potential as a diagnostic criterion for chronic inflammatory demyelinating polyneuropathy. Neurology 59:1526–1532
Yuki N, Kuwabara S (2007) Axonal Guillain-Barré syndrome: carbohydrate mimicry and pathophysiology. J Peripher Nerv Syst 12:238–249

Schwann Cells as Crucial Players in Diabetic Neuropathy

22

Keiko Naruse

Abstract

Schwann cells maintain peripheral nerve structure and function by ensheathment of unmyelinated axons, myelination of myelinated axons, and secretion of neurotrophic factors, and these cells also play a crucial role in the pathogenic mechanisms of diabetic neuropathy. A decrease in unmyelinated and small myelinated axons appeared earlier than a decrease in large myelinated fibers in diabetic neuropathy. Electron microscopic studies of human diabetic neuropathy demonstrated edematous cell cytoplasm, aggregates of glycogen particles, and hyperplasia of the surrounding basal lamina in Schwann cells. Diabetic conditions also induces metabolic disorders, such as polyol pathway hyperactivity, activation of protein kinase C, and increased advanced glycosylation end products in Schwann cells, followed by the depletion of neurotrophic factor production.

Cell transplantation using progenitor or stem cells is expected to cure diabetic neuropathy. Many studies demonstrated that the paracrine effect of abundant secreted factors from transplanted stem cells was crucial for the success of cell transplantation in diabetic neuropathy. Transplantation of progenitor or stem cells in diabetic animal models ameliorated impaired nerve conduction velocity, nerve blood flow, sensory disorders, and intraepidermal nerve fiber density, with an increase of myelin thickness. The supernatant from cultured dental pulp stem cells increased the proliferation and production of myelin-related protein in Schwann cells, suggesting that Schwann cells is the main target of cell transplantation for diabetic neuropathy.

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Keywords
Diabetes · Diabetic neuropathy · Schwann cell · Myelination · Neurotrophic factor · Cell transplantation

22.1 Introduction

The onset and progression of diabetic neuropathy are fundamentally linked to metabolic disorders and blood flow impediments resulting from chronic hyperglycemia, with immunological dysfunction and aging as additional contributing factors. Schwann cells, which are the most numerous glia of peripheral nerves (Jessen and Mirsky 2005), maintain peripheral nerve structure and function by ensheathment of unmyelinated axons, myelination of myelinated axons, and secretion of neurotrophic factors, and these cells also play a crucial role in the pathogenic mechanisms of diabetic neuropathy (Bunge 1993; Sharghi-Namini et al. 2006; Mizisin 2014).

Painful diabetic neuropathy is currently treated with neurotransmission blockers that produce relatively few adverse reactions. Although the options for symptomatic treatment of diabetic neuropathy have increased, therapeutic strategies targeting the cause of diabetic neuropathy are lacking. A particular need exists for radical treatments in cases where diabetic neuropathy has progressed. Cell therapy is being investigated as a possible method of addressing the underlying cause of diabetic neuropathy (Naruse et al. 2005; Han et al. 2016; Omi et al. 2017). Previous studies demonstrated that Schwann cells are key players in cell therapy for diabetic neuropathy.

In this chapter, the role of Schwann cells in the pathogenesis and the progression of diabetic neuropathy will be discussed, and then the potency of Schwann cells as a target of cell therapy for diabetic neuropathy will be demonstrated.

22.2 Pathology of Diabetic Neuropathy

Earlier studies demonstrated that progressive centripetal axon degeneration begins distally and is observed predominantly in the sensory diabetic neuropathy. A decrease in unmyelinated and small myelinated axons appeared earlier than a decrease in large myelinated fibers (Bischoff 1980; Said et al. 1983; Malik et al. 2005). Teased-fibers of sural nerve biopsies from patients with mild diabetic neuropathy revealed paranodal abnormalities, segmental demyelination, and remyelination without axonal degeneration, suggesting that Schwann cell abnormalities precede axonal degeneration (Malik et al. 2005). Decreases in myelinated fiber density and occupancy were observed in both large and small fibers as the disease progressed (Ohnishi et al. 1983; Sima et al. 1988). Nerve biopsies from middle-aged diabetic patients exhibited early primary distal axonal atrophy and degeneration and destruction of myelin sheaths (Yagihashi and Matsunaga 1979).

Vascular abnormalities, including basement membrane thickness, hyperplasia of endothelial cells, degeneration of pericytes, and arteriovenous shunt, were observed in diabetic neuropathy, which suggest ischemic damage in diabetic neuropathy (Malik et al. 1989; Yasuda and Dyck 1987; Tesfaye et al. 1993). Pathological, morphometric, and teased-fiber studies of sural nerves strongly suggested that microvascular pathological abnormalities were associated with nerve damage (Dyck et al. 1986; Malik et al. 2005).

22.3 Schwann Cells in Diabetic Neuropathy

22.3.1 Pathological Abnormalities of Schwann Cells in Diabetic Neuropathy

Schwann cells and axons are closely interdependent. Schwann cells maintain peripheral nerve structure and function by ensheathment of unmyelinated axons, myelination of myelinated axons, and secretion of neurotrophic factors (Bunge 1993; Sharghi-Namini et al. 2006). It is reported that axonal dwindling is associated with deranged myelin sheaths (Yagihashi and Matsunaga 1979). Morphological studies of sural nerves in diabetic neuropathy showed segmental demyelination and remyelination, which suggests a primary abnormality of Schwann cells (Thomas and Lascelles 1965; Sima et al. 1988).

Electron microscopic studies of human diabetic neuropathy demonstrated edematous cell cytoplasm, aggregates of glycogen particles, and hyperplasia of surrounding basal lamina in Schwann cells (Yagihashi and Matsunaga 1979; Bischoff 1980).

Although there is controversy about the apparent relationship between demyelination and axonal degeneration, many studies consistently demonstrate that Schwann cells are the key players in diabetic neuropathy.

22.3.2 Metabolic Abnormalities of Schwann Cells in Diabetic Neuropathy

Aldose reductase, a key enzyme of the polyol pathway, is localized in Schwann cells (Kern and Engerman 1982). High glucose conditions significantly increased metabolites of the polyol pathway, such as sorbitol and fructose, in cultured schwannoma cells (Kamiya et al. 2003). Animal studies indicated the effects of an aldose reductase inhibitor in myelinated nerve fiber size and fiber occupancy (Kern and Engerman 1982). In addition, a 12-month, randomized, placebo-controlled, double-blind trial of an aldose reductase inhibitor for diabetic neuropathy revealed an increase in regenerating myelinated nerve fibers and improvement of paranodal demyelination, segmental demyelination, and myelin wrinkling (Sima et al. 1988). Since aldose reductase is localized in Schwann cells, endoneurial vascular endothelial cells and pericytes in peripheral nerves, aldose reductase inhibitors may interact

not only in Schwann cells but also in endoneurial microvessels. Furthermore, a recent study revealed that high glucose-induced Schwann cell dedifferentiation into immature cells as a result of sorbitol accumulation (Hao et al. 2015). In addition, the polyol pathway hyperactivity during high glucose conditions induced protein kinase C (PKC)-α activation, but not PKC-β activation, in rat schwannoma cells (Kamiya et al. 2003). Diabetic rats showed suppressed PKC activity of endoneurial membrane fraction with decreased PKC-α expression (Yamagishi et al. 2008).

The formation of advanced glycosylation end products (AGEs) is one of the classical pathogenic pathways of diabetic complications. AGE formation was observed in myelin and axonal cytoskeletal proteins (Vlassara et al. 1983; Ryle et al. 1997). Study of a spontaneously immortalized adult Fischer rat Schwann cell line IFRS1 demonstrated high glucose significantly increased expression of AGE-binding proteins, such as galectin-3 and receptor for AGE (RAGE) (Tsukamoto et al. 2015).

22.3.3 Decreased Neurotrophic Factors from Schwann Cells in Diabetic Neuropathy

Schwann cells secrete abundant neurotrophic factors, such as nerve growth factor (NGF) and neurotrophin-3 (NT-3). Experimental animal studies revealed decreased nerve growth factor production with the metabolic disorders in Schwann cells under diabetic conditions. Cultured Schwann cells from both type 1 and type 2 diabetic mice showed lower production of NGF and NT-3 compared to cultured Schwann cells from normal mice (Dey et al. 2013). High glucose significantly reduced NGF secretion from cultured immortalized mouse Schwann cells, which reduced the response on neurite outgrowth (Tosaki et al. 2008) (Fig. 22.1). Diabetic rats showed decreased NGF in sciatic nerves, which was recovered by treatment of the metabolic

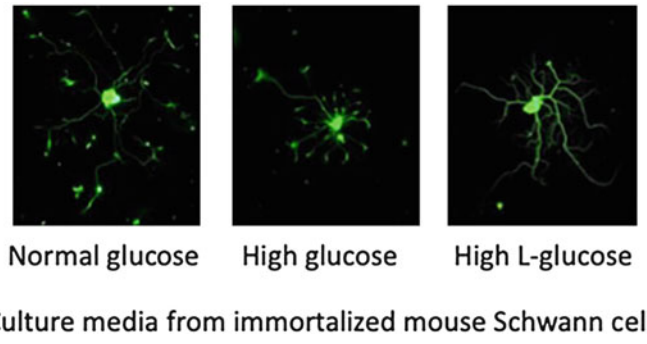

Fig. 22.1 Culture media from immortalized mouse Schwann cells under high glucose conditions showed lower efficacy on neurite outgrowth from dorsal ganglion neurons. Normal glucose: 5.5 mM D-glucose, high glucose: 30 mM D-glucose, high L-glucose: 5.5 mM D-glucose + 24.5 mM L-glucose. Reproduced from Tosaki et al. (2008) *Experimental Neurology* 213: 381–387, with permission from Elsevier

disorder using an aldose reductase inhibitor or oxidative stress inhibitor (Ohi et al. 1998; Obrosova et al. 2001). Although NGF administration improved sensory disorders in diabetic rats, a clinical trial with recombinant human NGF failed in phase III (Apfel et al. 2000).

22.4 Cell Transplantation for Diabetic Neuropathy

22.4.1 Progenitor/Stem Cell Transplantation in Regenerative Medicine

Although cell transplantation is most advanced in the field of hematological disease, cell therapy approaches have also been investigated for many non-hematological diseases. The conditions targeted include ischemic and neurodegenerative disorders; investigations have focused on the use of precursor cells and stem cells, and these therapies are already being applied in clinical use (Perin et al. 2012; Makkar et al. 2012). Initially, researchers expected angiogenesis to occur as a result of the direct differentiation of grafted cells. However, subsequent researches revealed that very few grafted cells survive at the site of transplantation. Current thinking highlights the abundant secretion of cytokines, including angiogenic factors, which exhibit effective angiogenic and cytoprotective actions at the site of transplantation. On the other hand, a contrasting result has been reported that found long-term graft survival in the sciatic nerve after transplantation of endothelial progenitor cells and MSCs along the sciatic nerve (Han et al. 2016; Jeong et al. 2009). Because epineurium and perineurium consist of a membrane and vasa nervorum, they may provide a scaffold and nutrition to the transplanted cells. Further research is required to reveal the survival of grafted cells in the epineurium following cellular transplantation along peripheral nerves.

22.4.2 Therapeutic Efficacy of Progenitor/Stem Cell Transplantation for Diabetic Neuropathy

Cell transplantation for diabetic neuropathy has largely been conducted in animal studies involving a wide range of precursor and stem cells, such as endothelial progenitor cells (EPCs) (Naruse et al. 2005; Jeong et al. 2009), peripheral blood and bone marrow mononuclear cells (Hasegawa et al. 2006; Naruse et al. 2011), mesenchymal stem cells (MSCs) (Shibata et al. 2008; Xia et al. 2015; Han et al. 2016), dental pulp stem cells (DPSCs) (Hata et al. 2015), embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells (Himeno et al. 2013, 2015; Okawa et al. 2013) (Fig. 22.2). Cells have been transplanted into hind-limb skeletal muscles in murine models of diabetes. These transplantations have significantly increased motor and sensory nerve conduction velocity and blood flow in the sciatic nerve in diabetic animals (Fig. 22.3). Stem cell or bone marrow-derived mononuclear cell transplantation ameliorated sensory disorders, such as hypoalgesia and hyperalgesia,

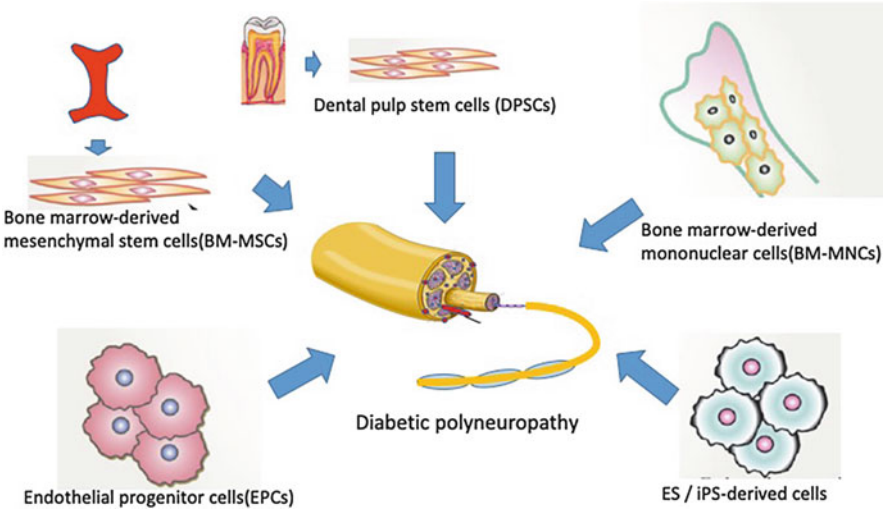

Fig. 22.2 Cell therapy for diabetic neuropathy: proposed cells for transplantation

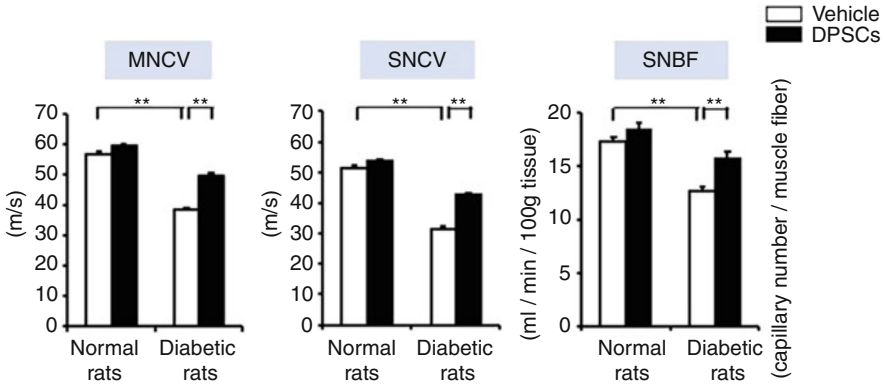

Fig. 22.3 Effects of dental pulp stem cell transplantation on sciatic motor nerve conduction velocity (MNCV), sciatic sensory nerve conduction velocity (SNCV), and sciatic nerve blood flow (SNBF). Diabetic rats showed significant reductions in MNCV, SNCV, and SNBF. DPSC transplantation improved these factors to almost the same level as those of normal rats. $**P < 0.01$

in diabetic neuropathy (Naruse et al. 2011; Okawa et al. 2013; Himeno et al. 2015; Omi et al. 2017). Decreased intraepidermal nerve fiber density of the skin in diabetic neuropathy was recovered by transplantation of stem cells (Okawa et al. 2013; Hata et al. 2015).

One study of long-term diabetic neuropathy in rats with a 52-week duration of diabetes demonstrated that the transplantation of dental pulp stem cells, a type of mesenchymal stem cell, improved nerve conduction velocity, nerve blood flow, sensory disorders, and intraepidermal nerve fiber density of the skin, as well as

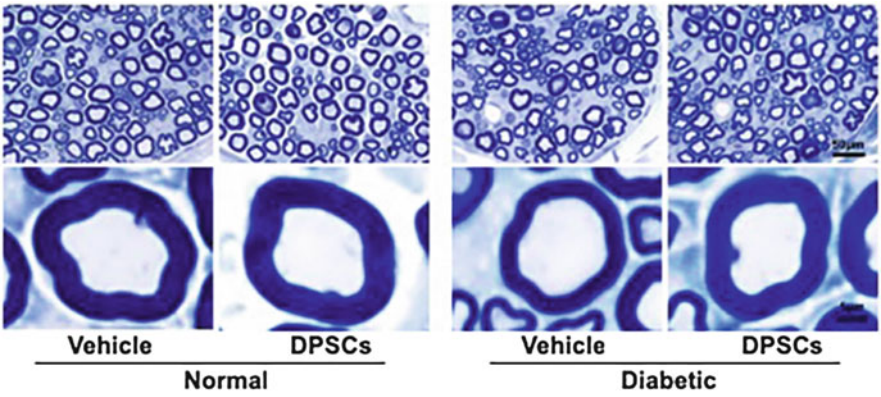

Fig. 22.4 Cross-sections of the sural nerves of rats. Myelin thickness was lower in diabetic rats compared to that in normal rats. The transplantation of dental pulp stem cells (DPSCs) significantly increased myelin thickness and circularity. Reproduced from Omi et al. (2017) *Stem Cell Research and Therapy* 8: 279, with permission from Springer

peripheral nerve morphology, suggesting that stem cell transplantation will be the preferable therapy for diabetic neuropathy (Fig. 22.4) (Omi et al. 2017).

22.4.3 Schwann Cells as a Therapeutic Target of Stem Cell Transplantation for Diabetic Neuropathy

Several reports demonstrated that the transplanted stem cells differentiated into Schwann-like cells in the transplant sites (Okawa et al. 2013). The differentiation into vascular endothelial cells and vascular smooth muscle cells was also observed following the transplantation of EPCs, iPS-derived neural crest-like cells, ES cell-derived angioblasts, and DPSCs (Okawa et al. 2013; Hata et al. 2015; Himeno et al. 2015). However, the rate of the differentiation into Schwann cells was very low compared to the number of transplanted stem cells regardless of the decrease in the number of residual transplanted stem cells over time.

The main therapeutic efficacy of cell transplantation for diabetic neuropathy is primarily due to the paracrine effects of abundant secreted factors by transplanted stem cells. Progenitor cells or stem cells, such as EPCs, MSCs, and DPSCs, produce abundant angiogenic factors, neurotrophic factors, and immunomodulatory factors even after transplantation (Shibata et al. 2008; Hata et al. 2015; Omi et al. 2017).

Studies of cultured Schwann cells revealed that supernatants from cultured DPSCs increased the proliferation of Schwan cells and the production of myelin-related protein, MP0, which is crucial for Schwann cell development and peripheral myelin formation (Figs. 22.5 and 22.6) (Omi et al. 2017; Sango et al. 2011; Takaku et al. 2018). The transplantation of EPCs, MSCs, DPSCs, and iPS-derived neural crest-like cells increased the expression of neurotrophic factors, such as NGF, NT-3, and brain-derived neurotrophic factor (BDNF) in neurons, which may increase the

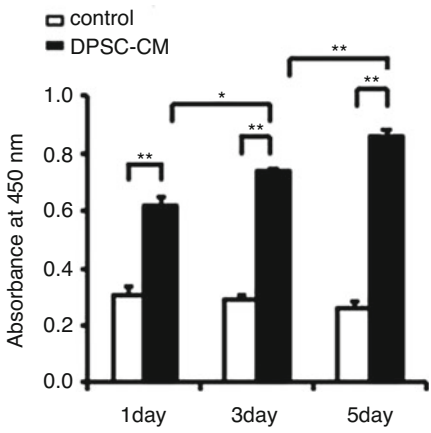

Fig. 22.5 Conditioned media from cultured dental pulp stem cells (DPSC-CM) significantly increased Schwann cell viability. Reproduced from Omi et al. (2017) *Stem Cell Research and Therapy* 8: 279, with permission from Springer

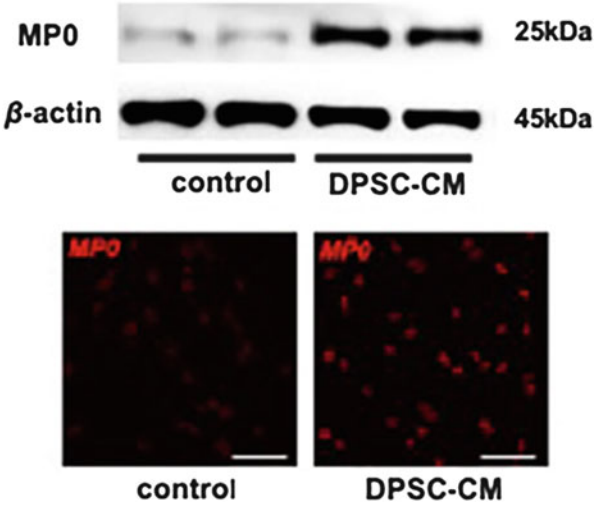

Fig. 22.6 Incubation with conditioned media from cultured dental pulp stem cells (DPSC-CM) significantly increased the protein expression of myelin protein zero (MP0), which is a protein related to the myelination of Schwann cells. Reproduced from Omi et al. (2017) *Stem Cell Research and Therapy* 8:279, with permission from Springer

myelin area and thickness of peripheral nerves (Han et al. 2016; Omi et al. 2017). These results strongly suggest that improvement of Schwann cell viability is crucial in stem cell transplantation for diabetic neuropathy.

22.4.4 Challenges for the Application of Cell Transplantation Therapy to Diabetic Neuropathy

Studies of diabetic patients and animals revealed that EPCs and MSCs exhibited impaired function and reduced counts, similar to mature cells (Loomans et al. 2004; Nakamura et al. 2011; Jin et al. 2010). Age is also a major factor associated with cellular dysfunction (Jin et al. 2010). This dysfunction is naturally reflected in the

effects of cell transplantation (Kondo et al. 2015). Based on the assumption that many cell therapy recipients are elderly DM patients, methods of overcoming the problem of cell dysfunction are desirable.

iPS cells are produced by reprogramming mature cells into an undifferentiated state, and cellular reprogramming to reverse the effects of age should be anticipated. We previously applied a grafting technique with differentiated cells reprogrammed into transplantable iPS cells to establish iPS-derived cells from geriatric mouse cells. We found that transplantation of these cells ameliorated diabetic neuropathy, and anticipate bringing this approach into clinical use (Himeno et al. 2013, 2015; Okawa et al. 2013).

The use of DPSCs is another technique to overcome the problem of cell dysfunction. DPSCs could be cultured after isolation from deciduous dentition and/or teeth extracted at a young age (e.g., the third molars) and cryopreserved until they are needed (Hata et al. 2015). Thawed and recultured DPSCs could potentially be used for cell therapy in patients with DM or other conditions because the autologous cells originated from a younger age prior to disease onset.

The safety and efficacy of cell therapy were demonstrated in clinical research using bone marrow and peripheral blood mononuclear cells in autologous cell grafting (Strauer et al. 2002; Tateishi-Yuyama et al. 2002). The less mature MSCs may raise concerns about tumorigenesis and accelerated tumor propagation. Hematological cell therapy applications involve cell grafting after eradication of cancer cells with radiation therapy, and the satisfactory results that can be achieved with this technique demonstrate that patients should undergo full physical screening to establish their tumor-free status when receiving cell therapy.

22.5 Conclusion

Axons and Schwann cells closely interact with each other. Pathological and metabolic studies of Schwann cells demonstrated that Schwann cell is a key player in diabetic neuropathy. Future studies should focus on the recovery of Schwann cells as a therapy for diabetic neuropathy.

Stem cell transplantation is a potential therapy for diabetic neuropathy. Animal studies demonstrated that the transplantation of progenitor or stem cells ameliorated impaired nerve conduction velocity, nerve blood flow, sensory disorder, and intraepidermal nerve fiber density with the increase of myelin thickness, which suggested that the impairment of Schwann cells in diabetic neuropathy would be a good candidate for stem cell transplantation.

References

Apfel SC, Schwartz S, Adornato BT, Freeman R, Biton V, Rendell M, Vinik A, Giuliani M, Stevens JC, Barbano R, Dyck PJ (2000) Efficacy and safety of recombinant human nerve growth factor in patients with diabetic polyneuropathy: a randomized controlled trial. rhNGF Clinical Investigator Group. JAMA 284:2215–2221

Bischoff A (1980) Morphology of diabetic neuropathy. Horm Metab Res Suppl 9:18–28

Bunge RP (1993) Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration. Curr Opin Neurobiol 3:805–809

Dey I, Midha N, Singh G, Forsyth A, Walsh SK, Singh B, Kumar R, Toth C, Midha R (2013) Diabetic Schwann cells suffer from nerve growth factor and neurotrophin-3 underproduction and poor associability with axons. Glia 61:1990–1999

Dyck PJ, Lais A, Karnes JL, O'Brien P, Rizza R (1986) Fiber loss is primary and multifocal in sural nerves in diabetic polyneuropathy. Ann Neurol 19:425–439

Han JW, Choi D, Lee MY, Huh YH, Yoon YS (2016) Bone marrow-derived mesenchymal stem cells improve diabetic neuropathy by direct modulation of both angiogenesis and myelination in peripheral nerves. Cell Transplant 25:313–326

Hao W, Tashiro S, Hasegawa T, Sato Y, Kobayashi T, Tando T, Katsuyama E, Fujie A, Watanabe R, Morita M, Miyamoto K, Morioka H, Nakamura M, Matsumoto M, Amizuka N, Toyama Y, Miyamoto T (2015) Hyperglycemia promotes Schwann cell de-differentiation and de-myelination via sorbitol accumulation and igf1 protein down-regulation. J Biol Chem 290:17106–17115

Hasegawa T, Kosaki A, Shimizu K, Matsubara H, Mori Y, Masaki H, Toyoda N, Inoue-Shibata M, Nishikawa M, Iwasaka T (2006) Amelioration of diabetic peripheral neuropathy by implantation of hematopoietic mononuclear cells in streptozotocin-induced diabetic rats. Exp Neurol 199:274–280

Hata M, Omi M, Kobayashi Y, Nakamura N, Tosaki T, Miyabe M, Kojima N, Kubo K, Ozawa S, Maeda H, Tanaka Y, Matsubara T, Naruse K (2015) Transplantation of cultured dental pulp stem cells into the skeletal muscles ameliorated diabetic polyneuropathy: therapeutic plausibility of freshly isolated and cryopreserved dental pulp stem cells. Stem Cell Res Ther 6:162

Himeno T, Kamiya H, Naruse K, Cheng Z, Ito S, Kondo M, Okawa T, Fujiya A, Kato J, Suzuki H (2013) Mesenchymal stem cell-like cells derived from mouse induced pluripotent stem cells ameliorate diabetic polyneuropathy in mice. Biomed Res Int 2013:259187

Himeno T, Kamiya H, Naruse K, Cheng Z, Ito S, Shibata T, Kondo M, Kato J, Okawa T, Fujiya A (2015) Angioblast derived from ES cells construct blood vessels and ameliorate diabetic polyneuropathy in mice. J Diabetes Res 2015:257230

Jeong JO, Kim MO, Kim H, Lee MY, Kim SW, Ii M, Lee JU, Lee J, Choi YJ, Cho HJ, Lee N, Silver M, Wecker A, Kim DW, Yoon YS (2009) Dual angiogenic and neurotrophic effects of bone marrow-derived endothelial progenitor cells on diabetic neuropathy. Circulation 119:699–708

Jessen KR, Mirsky R (2005) The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci 6:671–682

Jin P, Zhang X, Wu Y, Li L, Yin Q, Zheng L, Zhang H, Sun C (2010) Streptozotocin-induced diabetic rat-derived bone marrow mesenchymal stem cells have impaired abilities in proliferation, paracrine, antiapoptosis, and myogenic differentiation. Transplant Proc 42(7):2745–2752

Kamiya H, Nakamura J, Hamada Y, Nakashima E, Naruse K, Kato K, Yasuda Y, Hotta N (2003) Polyol pathway and protein kinase C activity of rat Schwannoma cells. Diabetes Metab Res Rev 19:131–139

Kern TS, Engerman RL (1982) Immunohistochemical distribution of aldose reductase. Histochem J 14:507–515

Kondo M, Kamiya H, Himeno T, Naruse K, Nakashima E, Watarai A, Shibata T, Tosaki T, Kato J, Okawa T (2015) Therapeutic efficacy of bone marrow-derived mononuclear cells in diabetic polyneuropathy is impaired with aging or diabetes. J Diabetes Investig 6:140–149

Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ (2004) Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes 53:195–199

Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marbán L, Mendizabal A, Johnston PV (2012) Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet 379:895–904

Malik RA, Newrick PG, Sharma AK, Jennings A, Ah-See AK, Mayhew TM, Jakubowski J, Boulton AJ, Ward JD (1989) Microangiopathy in human diabetic neuropathy: relationship between capillary abnormalities and the severity of neuropathy. Diabetologia 32:92–102

Malik RA, Tesfaye S, Newrick PG, Walker D, Rajbhandari SM, Siddique I, Sharma AK, Boulton AJ, King RH, Thomas PK, Ward JD (2005) Sural nerve pathology in diabetic patients with minimal but progressive neuropathy. Diabetologia 48:578–585

Mizisin AP (2014) Mechanisms of diabetic neuropathy: Schwann cells. Handb Clin Neurol 126:401–428

Nakamura N, Naruse K, Kobayashi Y, Matsuki T, Hamada Y, Nakashima E, Kamiya H, Hata M, Nishikawa T, Enomoto A, Takahashi M, Murohara T, Matsubara T, Oiso Y, Nakamura J (2011) High glucose impairs the proliferation and increases the apoptosis of endothelial progenitor cells by suppression of Akt. J Diabetes Investig 2:262–270

Naruse K, Hamada Y, Nakashima E, Kato K, Mizubayashi R, Kamiya H, Yuzawa Y, Matsuo S, Murohara T, Matsubara T, Oiso Y, Nakamura J (2005) Therapeutic neovascularization using cord blood-derived endothelial progenitor cells for diabetic neuropathy. Diabetes 54:1823–1828

Naruse K, Sato J, Funakubo M, Hata M, Nakamura N, Kobayashi Y, Kamiya H, Shibata T, Kondo M, Himeno T, Matsubara T, Oiso Y, Nakamura J (2011) Transplantation of bone marrow-derived mononuclear cells improves mechanical hyperalgesia, cold allodynia and nerve function in diabetic neuropathy. PLoS One 6:e27458

Obrosova IG, Fathallah L, Stevens MJ (2001) Taurine counteracts oxidative stress and nerve growth factor deficit in early experimental diabetic neuropathy. Exp Neurol 172:211–219

Ohi T, Saita K, Furukawa S, Ohta M, Hayashi K, Matsukura S (1998) Therapeutic effects of aldose reductase inhibitor on experimental diabetic neuropathy through synthesis/secretion of nerve growth factor. Exp Neurol 151:215–220

Ohnishi A, Harada M, Tateishi J, Ogata J, Kawanami S (1983) Segmental demyelination and remyelination in lumbar spinal roots of patients dying with diabetes mellitus. Ann Neurol 13:541–548

Okawa T, Kamiya H, Himeno T, Kato J, Seino Y, Fujiya A, Kondo M, Tsunekawa S, Naruse K, Hamada Y, Ozaki N, Cheng Z, Kito T, Suzuki H, Ito S, Oiso Y, Nakamura J, Isobe KI (2013) Transplantation of neural crest-like cells derived from induced pluripotent stem cells improves diabetic polyneuropathy in mice. Cell Transplant 22:1767–1783

Omi M, Hata M, Nakamura N, Miyabe M, Ozawa S, Nukada H, Tsukamoto M, Sango K, Himeno T, Kamiya H, Nakamura J, Takebe J, Matsubara T, Naruse K (2017) Transplantation of dental pulp stem cells improves long-term diabetic polyneuropathy together with improvement of nerve morphometrical evaluation. Stem Cell Res Ther 8:279

Perin EC, Willerson JT, Pepine CJ, Henry TD, Ellis SG, Zhao DX, Silva GV, Lai D, Thomas JD, Kronenberg MW (2012) Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. JAMA 307:1717–1726

Ryle C, Leow CK, Donaghy M (1997) Nonenzymatic glycation of peripheral and central nervous system proteins in experimental diabetes mellitus. Muscle Nerve 20:577–584

Said G, Slama G, Selva J (1983) Progressive centripetal degeneration of axons in small fibre diabetic polyneuropathy. Brain 106(Pt 4):791–807

Sango K, Yanagisawa H, Kawakami E, Takaku S, Ajiki K, Watabe K (2011) Spontaneously immortalized Schwann cells from adult Fischer rat as a valuable tool for exploring neuron-Schwann cell interactions. J Neurosci Res 89:898–908

Sharghi-Namini S, Turmaine M, Meier C, Sahni V, Umehara F, Jessen KR, Mirsky R (2006) The structural and functional integrity of peripheral nerves depends on the glial-derived signal desert hedgehog. J Neurosci 26:6364–6376

Shibata T, Naruse K, Kamiya H, Kozakae M, Kondo M, Yasuda Y, Nakamura N, Ota K, Tosaki T, Matsuki T, Nakashima E, Hamada Y, Oiso Y, Nakamura J (2008) Transplantation of bone marrow-derived mesenchymal stem cells improves diabetic polyneuropathy in rats. Diabetes 57:3099–3107

Sima AA, Bril V, Nathaniel V, McEwen TA, Brown MB, Lattimer SA, Greene DA (1988) Regeneration and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy treated with sorbinil. N Engl J Med 319:548–555

Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P (2002) Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 106:1913–1918

Takaku S, Yako H, Niimi N, Akamine T, Kawanami D, Utsunomiya K, Sango K (2018) Establishment of a myelinating co-culture system with a motor neuron-like cell line NSC-34 and an adult rat Schwann cell line IFRS1. Histochem Cell Biol 149:537–543

Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T (2002) Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 360:427–435

Tesfaye S, Harris N, Jakubowski JJ, Mody C, Wilson RM, Rennie IG, Ward JD (1993) Impaired blood flow and arterio-venous shunting in human diabetic neuropathy: a novel technique of nerve photography and fluorescein angiography. Diabetologia 36:1266–1274

Thomas PK, Lascelles RG (1965) Schwann-cell abnormalities in diabetic neuropathy. Lancet 1:1355–1357

Tosaki T, Kamiya H, Yasuda Y, Naruse K, Kato K, Kozakae M, Nakamura N, Shibata T, Hamada Y, Nakashima E, Oiso Y, Nakamura J (2008) Reduced NGF secretion by Schwann cells under the high glucose condition decreases neurite outgrowth of DRG neurons. Exp Neurol 213:381–387

Tsukamoto M, Sango K, Niimi N, Yanagisawa H, Watabe K, Utsunomiya K (2015) Upregulation of galectin-3 in immortalized Schwann cells IFRS1 under diabetic conditions. Neurosci Res 92:80–85

Vlassara H, Brownlee M, Cerami A (1983) Excessive nonenzymatic glycosylation of peripheral and central nervous system myelin components in diabetic rats. Diabetes 32:670–674

Xia N, Xu JM, Zhao N, Zhao QS, Li M, Cheng ZF (2015) Human mesenchymal stem cells improve the neurodegeneration of femoral nerve in a diabetic foot ulceration rats. Neurosci Lett 597:84–89

Yagihashi S, Matsunaga M (1979) Ultrastructural pathology of peripheral nerves in patients with diabetic neuropathy. Tohoku J Exp Med 129:357–366

Yamagishi S, Ogasawara S, Mizukami H, Yajima N, Wada R, Sugawara A, Yagihashi S (2008) Correction of protein kinase C activity and macrophage migration in peripheral nerve by pioglitazone, peroxisome proliferator activated-gamma-ligand, in insulin-deficient diabetic rats. J Neurochem 104:491–499

Yasuda H, Dyck PJ (1987) Abnormalities of endoneurial microvessels and sural nerve pathology in diabetic neuropathy. Neurology 37:20–28

Drug-Induced Demyelinating Neuropathies 23

Naoko Niimi, Shizuka Takaku, Hideji Yako, and Kazunori Sango

Abstract

A large variety of drugs have been reported to cause peripheral neuropathies as dose-limiting adverse effects; however, most of them primarily affect axons and/or neuronal cell bodies rather than Schwann cells and/or myelin sheaths. In this chapter, we focus on the drugs that seem to elicit the neuropathies with schwannopathy and/or myelinopathy-predominant phenotypes, such as amiodarone, dichloroacetate, and tumor necrosis factor-α antagonists. Although the pathogenesis of demyelination induced by these drugs remain largely obscure, the recent in vivo and in vitro studies have implicated the involvement of metabolic abnormalities and impaired autophagy in Schwann cells and immune system disorders in the disruption of neuron–Schwann cell contact and interactions.

Keywords

Schwann cells · Myelinopathy · Amiodarone · Dichloroacetate · Tumor necrosis factor-α antagonists

23.1 Introduction

The most common causes of peripheral neuropathies are genetic mutations (e.g., Charcot–Marie–Tooth diseases), immune system disorders (e.g., Guillain–Barré syndrome (GBS), and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)), and systemic diseases (e.g., diabetic neuropathy). However, it is important to bear in mind that a large variety of substances, especially pharmaceutical and

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industrial agents, can be peripheral nerve toxicants (London and Albers 2007). Exposure to some environmental agents, such as arsenic and hexacarbons, has been implicated as a cause of peripheral neuropathies resembling GBS (Rao et al. 2014; Little and Albers 2015). In this chapter, however, we focus on the pharmacotherapy-induced neuropathies, which are usually intractable and dose-limiting adverse effects of medications (Manji 2013; Bilbao and Schmidt 2015). Although numerous drugs are thought to exhibit undesirable effects on the peripheral nervous system (PNS), there is not much evidence of a link between the administration of them and the development of neuropathies.

In accordance with the site of lesion in the PNS, drug-induced neuropathies can be classified into axonopathy, neuronopathy, and myelinopathy (Han and Smith 2013). In axonopathy and neuronopathy, the primary targets of neurotoxicity are axons and neuronal cell bodies, respectively. Because of lacking an effective blood–brain barrier (Manji 2013) and loose blood–nerve barrier caused by fenestrated capillaries (Crowell and Gwathmey 2017), primary sensory neurons in dorsal root ganglia (DRG) are more vulnerable to circulating drugs than spinal cord motor neurons. In addition, the sensory axons are more severely affected by the drugs than motor axons. Myelinopathy, less common than axonopathy and neuronopathy, results from the injury to Schwann cells and/or myelin structure by direct or immune-mediated cytotoxicity of the drugs (Stubgen 2011).

The optimum use of in vivo and in vitro experimental models is essential for the development of effective mechanism-based therapies against drug-induced neuropathies. Although various animal models have been developed (Hoke 2012), several critical issues against animal use (e.g., ethical problems, irrelevance to human diseases, and use of inappropriate outcome measures) remain a matter of debate. In addition to these animal models, culture of neurons and Schwann cells has been utilized for the study of drug-induced neuropathies (Marmiroli et al. 2012); in most of the previous studies, however, these cells were obtained by primary culture with embryonic or neonatal animals. Because some biological properties of these cells change with maturation and aging, culture systems of neurons and/or Schwann cells derived from adult animals appear to mimic peripheral nerve degeneration and regeneration better than those from immature animals (Sango et al. 2006). A spontaneously immortalized Schwann cell line IFRS1 established from long-term cultures of adult Fischer rat peripheral nerves (Sango et al. 2011) exhibits high proliferative activity and retains distinct Schwann cell phenotypes, such as spindle-shaped morphology with expression of glial cell markers (S100, glial fibrillary acidic protein, and p75 low-affinity neurotrophin receptor), synthesis and secretion of neurotrophic factors and cytokines, and fundamental ability to myelinate neurites in cocultures with adult rat DRG neurons (Sango et al. 2011), nerve growth factor-primed PC12 cells (Sango et al. 2012), and NSC-34 motor neuron-like cells (Takaku et al. 2018). Consequently, IFRS1 cells and their cocultures with neurons can be useful tools for exploring the pathogenesis of drug-induced schwannopathy and myelinopathy (Niimi et al. 2016).

In addition to summarizing the pathogenic mechanisms of drug-induced demyelinating neuropathies, we introduce recent studies using these culture models.

23.2 Axonopathy and Neuronopathy

Although these kinds of neuropathies are beyond the scope of this chapter, we briefly describe the representative drugs leading to the injury of axons and/or neuronal cell bodies.

23.2.1 Axonopathy

Chemotherapy-induced peripheral neuropathy (CIPN) is a major dose-limiting adverse effect of various types of cancer chemotherapy. A majority of the CIPN appears to primarily result from axonal injury, and vinca alkaloids (e.g., vinblastine and vincristine) have been shown to inhibit microtubule polymerization in the axons, thereby leading to axonal transport disorders and axonal degeneration (Kerckhove et al. 2017). Vincristine exhibited direct toxicity on growing neurites, but not cell bodies of embryonic DRG explants maintained in compartmentalized chambers (Silva et al. 2006). Consistently, deleterious effects of vincristine on the axonal transport in cultured adult mouse DRG neurons have been reported (Van Helleputte et al. 2018).

23.2.2 Neuronopathy

Oxaliplatin, a platinum-based agent that suppresses DNA replication and transcription, is widely used for the treatment of colorectal cancers; however, its potent neurotoxicity causes almost all the patients acute and/or chronic neuropathies (Argyriou 2015). It is notable that DRG neurons are the most common target of oxaliplatin and other platinum-based compounds, and a considerable number of in vivo and in vitro studies have been made on the mechanisms of oxaliplatin-induced DRG neuronal cell injury and death (e.g., nuclear and mitochondrial DNA damage, ion channel disorders, and oxidative stress) (Ta et al. 2006; Joseph et al. 2008; Schmitt et al. 2018). Consistent with those studies, we observed the oxaliplatin-induced death of cultured adult rat DRG neurons in a dose-dependent manner (Takaku et al., unpublished data).

23.3 Myelinopathy

23.3.1 Overview

Among a large variety of drugs that have been reported to cause demyelinating neuropathies (Stubgen 2011; Bilbao and Schmidt 2015), we draw special attention to *amiodarone*, *dichloroacetate*, and *tumor necrosis factor (TNF)-α antagonists*. These drugs are widely used in developed countries, but the incidents of peripheral neuropathies seem to be overshadowed by their clinical efficacy and/or potential

benefits. If the clinicians do not recognize that the patients take these drugs, toxic neuropathies associated with them may be misdiagnosed as neuropathies due to inflammatory or systemic diseases.

23.3.2 Amiodarone

Amiodarone hydrochloride (AMD) is a cationic amphiphilic agent commonly prescribed for patients with refractory or life-threatening arrhythmias. The primary action mechanism of AMD is to prolong the cardiac action potential and repolarization time via altering the function of diverse membrane proteins (e.g., ion channels, ion exchangers, and adrenergic receptors) (Rusinova et al. 2015). Because of its long elimination half-life, AMD has a number of adverse effects, including corneal microdeposits, pulmonary toxicity, liver and thyroid dysfunction, and peripheral neuropathy (Orr and Ahlskog 2009; Punnam et al. 2010). Despite a lower incidence of peripheral neuropathy than other abnormalities, exposure to long-term and/or high-dose AMD therapy increases the risk of its onset. The typical neurologic symptoms, such as paresis and weakness in the lower extremities, develop a few weeks to months after the AMD prescription, and the recovery can be slow following its cessation. Histological analyses with peripheral nerve specimens of the AMD-induced neuropathy revealed demyelination-predominant pathology with some axonal loss (Pulipaka et al. 2002). Characteristic lysosomal inclusions in Schwann cells and vessels detected by electron microscopy can help distinguish the disease from immune-mediated demyelinating neuropathies, such as GBS and CIDP (London and Albers 2007).

Although the pathogenesis of AMD neurotoxicity remains largely unclear, AMD and its metabolites (desethylamiodarone and bis-desethylamiodarone) are likely to inhibit lysosomal phospholipases and induce accumulation of phospholipids and other substances in the lysosomes of Schwann cells (Bilbao and Schmidt 2015), leading to the disruption of myelin structure and function. Other amphiphilic agents, such as chloroquine and perhexiline, have also been reported to cause demyelinating neuropathy with the formation of lysosomal inclusion bodies in Schwann cells (Fardeau et al. 1979; Tegner et al. 1988).

Only a few attempts have been made at the AMD neurotoxicity in vitro. Han et al. (2009) reported that treatment with 10 μM AMD reduced the viability of human neural stem cells through the TNF-related signaling pathway, whereas Pomponio et al. (2015) observed dose-dependent (0.35 μM < 1.25 μM < 10 μM) cytotoxicity of AMD with the formation of its major oxidative metabolite (mono-N-desethylamiodarone) in embryonic mouse brain neurons. The concentrations of AMD in these studies were close to those in the therapeutic plasma range of AMD (0.7–3.7 μM) (Vassallo and Trohman 2007). In our recent study (Niimi et al. 2016), treatment of IFRS1 Schwann cells with AMD (1, 5, and 10 μM) induced dose- and time-dependent cell death, and intracellular storage of phospholipids and G_{M2} and G_{M3} gangliosides. By electron microscopy, intracytoplasmic inclusion bodies with pleomorphic shapes were evident in AMD-treated IFRS1 cells (Fig. 23.1). AMD

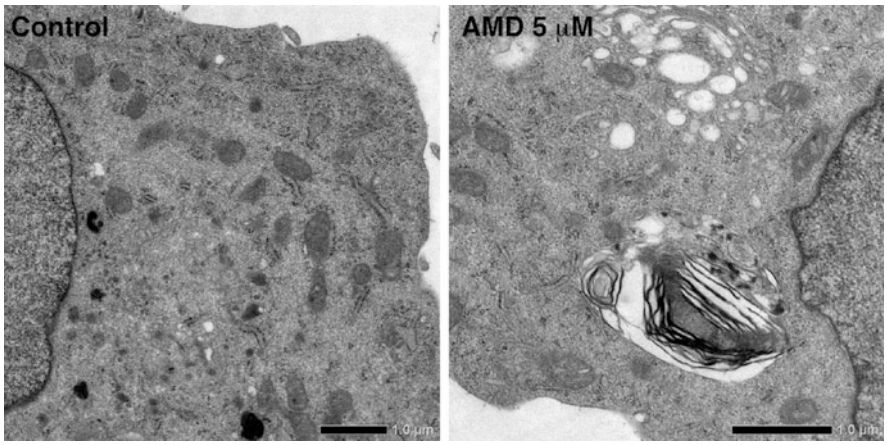

Fig. 23.1 By electron microscopy, intracytoplasmic inclusion bodies with pleomorphic shapes are evident in IFRS1 cells maintained for 24 h under exposure to 5 μM AMD (*right*), but not in the cells under AMD-free conditions [Control] (*left*)

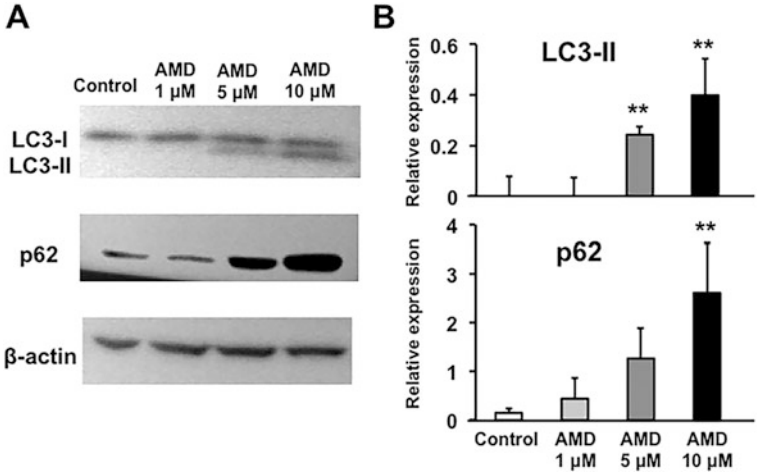

Fig. 23.2 The representative picture of the Western blot (**a**) and quantitative data (**b**) indicate that AMD induces upregulation of LC3-II and p62 in a dose-dependent manner. The molecular sizes of LC3-I, LC3-II, p62, and β-actin are approximately 18 kDa, 16 kDa, 60 kDa, and 42 kDa, respectively. Values represent the mean ± SD of 3 samples. $^{*}P < 0.05$ as compared with [Control] by Kruskal–Wallis test followed by the Steel test

also enhanced nuclear factor E2-related factor (Nrf2) DNA-binding activity, upregulated the expression of autophagy markers LC3-II and p62 (Fig. 23.2), and induced the phosphorylation of p62. A possible connection among these changes in the process of IFRS1 cell injury and death was closely examined. Under normal conditions, Nrf2 is negatively regulated by interaction with the ubiquitin ligase

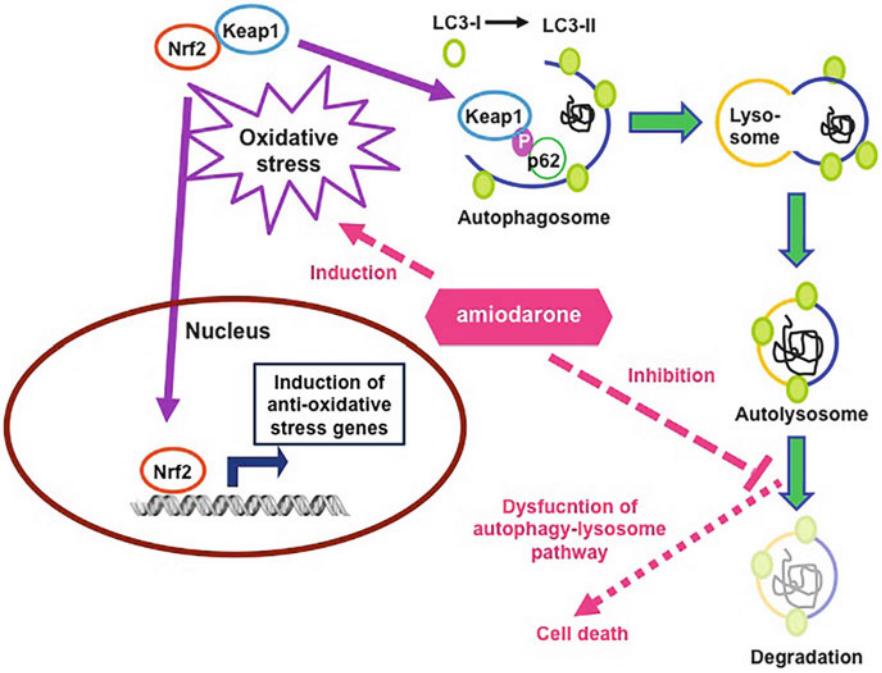

Fig. 23.3 Oxidative stress and dysfunction of autophagy-lysosome pathway are involved in AMD-induced IFRS1 cell death. Reproduced from Niimi et al. (2017) *Medical Research Archives* 5:2 (The publisher grants us the right to reuse the figure in other publications)

adaptor, Keap1, in the cytoplasm. When the cells are exposed to AMD, Nrf2 appears to be released from Keap1 and translocated to nuclei where it upregulates the expression of antioxidative stress genes (Ma 2013). In addition, the upregulation and phosphorylation of p62 enhance p62's-binding affinity for Keap1 (Ichimura et al. 2013). The Keap1–phosphorylated p62 complexes augment the formation of autophagosomes, which combine with lysosomes to form autolysosomes, and eventually, autolysosomes are degraded by proteases (Hung et al. 2013). However, the diminished activities of lysosomal enzymes under exposure to the high dose (10 μM) of AMD might impair the degradation of autolysosomes (Niimi et al. 2017) (Fig. 23.3). Furthermore, treatment of the DRG neuron–IFRS1 and PC12–IFRS1 coculture with AMD induced detachment of IFRS1 cells from neurite networks in a time- and dose-dependent manner (Fig. 23.4). These findings suggest that the impaired autophagy-lysosome pathway in Schwann cells is involved in AMD-induced demyelinating neuropathy. Lower survival ratios and far greater lipid accumulation in IFRS1 cells than PC12 cells under AMD exposure suggest that the former are much more vulnerable to the drug compared with the latter, and may account for Schwann cell injury-dominant morphological changes in AMD-induced neuropathy. In contrast, neurons seem to be more sensitive to AMD insults with respect to glial cells in reaggregating embryonic rat brain cell

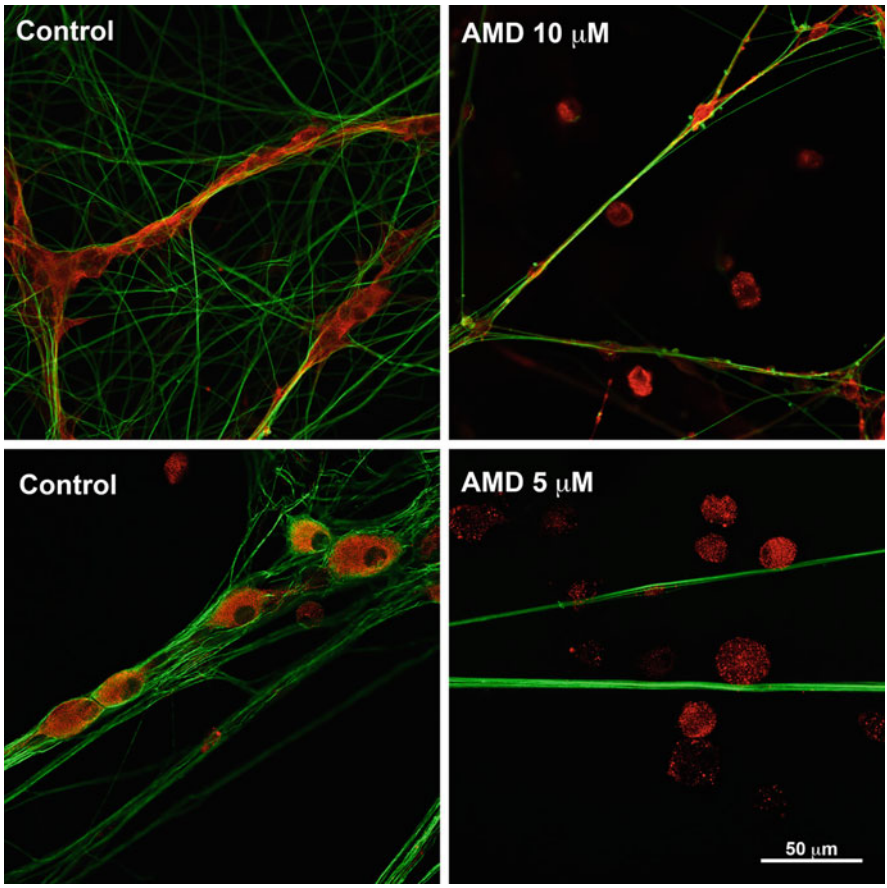

Fig. 23.4 The representative immunofluorescence micrographs indicate that AMD induces demyelination-like changes. P0 (*red, upper panels*)- and MBP (*red, lower panels*)-immunoreactive Schwann cells are surrounding βIII tubulin-immunoreactive neurites (*green*) in DRG neuron-IFRS1 coculture in the absence of AMD ([Control]), whereas most of them are detached under exposure to [AMD-10 μM] for 24 h or [AMD-5 μM] for 48 h. Reproduced from Niimi et al. (2016) *European Journal of Neuroscience* 44:1723–1733, with permission from John Wiley & Sons publications

culture (Pomponio et al. 2015). It remains unclear why the same drug exhibits more potent toxicity to neurons than glial cells in the brain and to Schwann cells than neurons in the PNS.

23.3.3 Dichloroacetate

Dichloroacetate (DCA) has been used as an investigational drug for the treatment of a large variety of metabolic disorders, especially inherited mitochondrial diseases, and more recently, cancer (James and Stacpoole 2016). Its therapeutic utility is based

on the pharmacological property of inhibiting mitochondrial pyruvate dehydrogenase kinase and subsequent activation of pyruvate dehydrogenase complex and oxidative phosphorylation. DCA is recognized as a potent lactate-lowering agent in clinical use for the patients with congenital and acquired lactic acidosis, whereas it increases the production of reactive oxygen species by activation of the mitochondrial respiratory chain and induces selective apoptosis of cancer cells. Despite those merits, chronic DCA treatment leads to the development of peripheral neuropathy in both experimental animals (Calcutt et al. 2009) and patients with mitochondrial diseases (Spruijt et al. 2001; Kaufmann et al. 2006) and melanoma (Brandsma et al. 2010). DCA-induced peripheral neuropathy is characterized as tingling or painful sensations and numbness in the lower extremities and muscle weakness, and appears to be the major treatment-limiting factor (James et al. 2017).

By using a coculture system with primary cultured DRG neurons and Schwann cells, Felitsyn et al. (2007) observed that exposure to DCA diminished the expression of myelin proteins, such as myelin basic protein, myelin protein zero, myelin-associated glycoprotein, and peripheral myelin protein 22, in a dose- and duration-dependent manner. Because discontinuation of DCA treatment in the middle of coculture partially restored the expression of these proteins, DCA-induced demyelination might be reversible. They also reported that DRG neurons were more vulnerable to DCA as compared with Schwann cells, suggesting that DCA-induced demyelination is attributable to its toxicity to neurons, rather than Schwann cells. In our ongoing study, DCA dose-dependently induced demyelination-like changes in the coculture system with adult rat DRG neurons and IFRS1 cells (Takaku et al., unpublished data). However, the precise mechanisms accounting for the DCA-induced demyelination remain unclear, and we are focusing on the direct DCA toxicity to DRG neurons and IFRS1 cells, respectively (e.g., oxidative stress, deficient autophagy, and alteration of the differentiation state of Schwann cells). DCA neurotoxicity was also investigated with animal models, but the findings from these in vivo studies do not support the demyelination observed in the coculture studies. Katz et al. (1981) reported that prolonged exposure of DCA to rats and dogs induced demyelination in the CNS, but not in the PNS. Calcutt et al. (2009) suggested that the primary target of DCA is axons, but DCA might affect axons through disrupting the metabolic function of Schwann cells. Further studies are needed for the detection of the main DCA target cells in the PNS.

23.3.4 TNF-α Antagonists

TNF-α antagonists (e.g., infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol) have been utilized widely for the treatment of patients with a variety of immune-mediated inflammatory disorders, such as rheumatoid arthritis, psoriasis, ankylosing spondylitis, and inflammatory bowel disease (Crohn's disease and ulcerative colitis) (Kalliolias and Ivashkiv 2016). Several adverse effects of these agents have been reported, including infections, lupus-like syndrome, vasculitis, and demyelinating disorders in the central and peripheral nervous systems.

Although the incidence of demyelinating peripheral neuropathy seems to be lower than that of other adverse effects, the clinicians should be aware of the risk of its development during the treatment of patients with these agents (Tristano 2010; Bosch et al. 2011). According to a large number of case reports, patients receiving the drugs developed various types of immune-mediated neuropathies, such as GBS (Silburn et al. 2008), Miller Fischer syndrome (Ratnarajan et al. 2015), CIDP (Richez et al. 2005), and multifocal motor neuropathy with conduction block (Fernandez-Menendez et al. 2015).

Although the pathogenesis of demyelinating neuropathy associated with TNF-α antagonists remains largely unknown, the drugs appear to affect the myelin structure and function via immune-mediated cytotoxicity rather than direct Schwann cell injury (Stubgen 2011). TNF-α has been shown to facilitate the immune reactions in the PNS (Hall et al. 2000), and the serum TNF-α levels are elevated in patients with some of the immune-mediated neuropathies (Misawa et al. 2001). These findings suggest a pro-inflammatory role of TNF-α in those neuropathies and seem to contradict the evidence that blocking of TNF-α triggered the immune attack against peripheral nerve myelin. One of the possible explanations for such discrepant phenomena is the dual functions of TNF-α in the inflammatory responses; it is recognized as a cytokine with not only pro-inflammatory but also immunosuppressive properties. Accumulating evidence reveals the inhibitory effects of TNF-α on T cell reactivity to autoantigens and T cell receptor signaling (Stubgen 2008; Chen and Oppenheim 2011), and the findings that the inhibition of TNF-α activity by its neutralizing antibodies augmented T cell proliferative responses and cytokine production (Cope et al. 1997) suggest that anti-TNF-α treatment might stimulate myelin-specific T cell reactivity, thereby leading to the development of immune-mediated neuropathies. In addition to disturbing the equilibrium of the immune system, TNF-α antagonists could trigger vascular inflammation through the formation of immune complexes that deposit in the blood vessels, which results in the activation of the complement-mediated immunoreactions (Jarrett et al. 2003).

A few studies have described TNF-α toxicity against Schwann cells and peripheral myelin sheath (Hall et al. 2000), whereas the direct effects of TNF-α antagonists on them remain unknown. Etanercept exhibited protective effects on the sciatic axonal injury, but not myelin sheath damage, of a rat neuropathy model induced by neonatal hypoxic-ischemic injury (Buyukakilli et al. 2014).

23.4 Conclusion

Among the drug-induced neuropathies, the incidence of myelinopathy-predominant type is much lower than that of axonopathy- and neuronopathy-predominant types. However, clinicians must be aware of the risks of the neuropathies when they prescribe the patients with these drugs. Much remains to be done to elucidate the precise mechanisms of demyelinating neuropathies associated with the drugs described in this chapter (AMD, DCA, and TNF-α antagonists), and our coculture

systems can be beneficial tools for investigating the neuron–Schwann cell interactions under those pathological conditions.

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References

Argyriou AA (2015) Updates on oxaliplatin-induced peripheral neurotoxicity (OXAIPN). Toxics 3 (2):187–197. https://doi.org/10.3390/toxics3020187

Bilbao JM, Schmidt RE (2015) Biopsy diagnosis of peripheral neuropathy. Springer, Cham. ISBN:978-3-319-07311-8

Bosch X, Saiz A, Ramos-Casals M (2011) Monoclonal antibody therapy-associated neurological disorders. Nat Rev Neurol 7(3):165–172. https://doi.org/10.1038/nrneurol.2011.1

Brandsma D, Dorlo TP, Haanen JH, Beijnen JH, Boogerd W (2010) Severe encephalopathy and polyneuropathy induced by dichloroacetate. J Neurol 257(12):2099–2100. https://doi.org/10.1007/s00415-010-5654-9

Buyukakilli B, Atici A, Balli E, Ozkan A, Gurgul S, Tasdelen B, Dagtekin O (2014) Effects of a tumor necrosis factor-alpha inhibitor (etanercept) on the sciatic nerve in a hypoxic ischemia-induced neonatal rat model. Adv Clin Exp Med 23(5):705–713

Calcutt NA, Lopez VL, Bautista AD, Mizisin LM, Torres BR, Shroads AL, Mizisin AP, Stacpoole PW (2009) Peripheral neuropathy in rats exposed to dichloroacetate. J Neuropathol Exp Neurol 68(9):985–993. https://doi.org/10.1097/NEN.0b013e3181b40217

Chen X, Oppenheim JJ (2011) Contrasting effects of TNF and anti-TNF on the activation of effector T cells and regulatory T cells in autoimmunity. FEBS Lett 585(23):3611–3618. https://doi.org/10.1016/j.febslet.2011.04.025

Cope AP, Liblau RS, Yang XD, Congia M, Laudanna C, Schreiber RD, Probert L, Kollias G, McDevitt HO (1997) Chronic tumor necrosis factor alters T cell responses by attenuating T cell receptor signaling. J Exp Med 185(9):1573–1584

Crowell A, Gwathmey KG (2017) Sensory neuronopathies. Curr Neurol Neurosci Rep 17(10):79. https://doi.org/10.1007/s11910-017-0784-4

Fardeau M, Tome FM, Simon P (1979) Muscle and nerve changes induced by perhexiline maleate in man and mice. Muscle Nerve 2(1):24–36. https://doi.org/10.1002/mus.880020105

Felitsyn N, Stacpoole PW, Notterpek L (2007) Dichloroacetate causes reversible demyelination in vitro: potential mechanism for its neuropathic effect. J Neurochem 100(2):429–436. https://doi.org/10.1111/j.1471-4159.2006.04248.x

Fernandez-Menendez S, Gonzalez Nafria N, Redondo-Robles L, Sierra-Ausin M, Garcia-Santiago-R, Saponaro-Gonzalez A (2015) Multifocal-motor-neuropathy-like disease associated with infliximab treatment in a patient with Crohn's disease. J Neurol Sci 349(1–2):246–248. https://doi.org/10.1016/j.jns.2015.01.003

Hall SM, Redford EJ, Smith KJ (2000) Tumour necrosis factor-alpha has few morphological effects within the dorsal columns of the spinal cord, in contrast to its effects in the peripheral nervous system. J Neuroimmunol 106(1–2):130–136

Han Y, Smith MT (2013) Pathobiology of cancer chemotherapy-induced peripheral neuropathy (CIPN). Front Pharmacol 4:156. https://doi.org/10.3389/fphar.2013.00156

Han Y, Miller A, Mangada J, Liu Y, Swistowski A, Zhan M, Rao MS, Zeng X (2009) Identification by automated screening of a small molecule that selectively eliminates neural stem cells derived from hESCs but not dopamine neurons. PLoS One 4(9):e7155. https://doi.org/10.1371/journal.pone.0007155

Hoke A (2012) Animal models of peripheral neuropathies. Neurotherapeutics 9(2):262–269. https://doi.org/10.1007/s13311-012-0116-y

Hung YH, Chen LM, Yang JY, Yang WY (2013) Spatiotemporally controlled induction of autophagy-mediated lysosome turnover. Nat Commun 4:2111. https://doi.org/10.1038/ncomms3111

Ichimura Y, Waguri S, Sou YS, Kageyama S, Hasegawa J, Ishimura R, Saito T, Yang Y, Kouno T, Fukutomi T, Hoshii T, Hirao A, Takagi K, Mizushima T, Motohashi H, Lee MS, Yoshimori T, Tanaka K, Yamamoto M, Komatsu M (2013) Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. Mol Cell 51(5):618–631. https://doi.org/10.1016/j.molcel.2013.08.003

James MO, Stacpoole PW (2016) Pharmacogenetic considerations with dichloroacetate dosing. Pharmacogenomics 17(7):743–753. https://doi.org/10.2217/pgs-2015-0012

James MO, Jahn SC, Zhong G, Smeltz MG, Hu Z, Stacpoole PW (2017) Therapeutic applications of dichloroacetate and the role of glutathione transferase zeta-1. Pharmacol Ther 170:166–180. https://doi.org/10.1016/j.pharmthera.2016.10.018

Jarrett SJ, Cunnane G, Conaghan PG, Bingham SJ, Buch MH, Quinn MA, Emery P (2003) Anti-tumor necrosis factor-alpha therapy-induced vasculitis: case series. J Rheumatol 30 (10):2287–2291

Joseph EK, Chen X, Bogen O, Levine JD (2008) Oxaliplatin acts on IB4-positive nociceptors to induce an oxidative stress-dependent acute painful peripheral neuropathy. J Pain 9(5):463–472. https://doi.org/10.1016/j.jpain.2008.01.335

Kalliolias GD, Ivashkiv LB (2016) TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nat Rev Rheumatol 12(1):49–62. https://doi.org/10.1038/nrrheum.2015.169

Katz R, Tai CN, Diener RM, McConnell RF, Semonick DE (1981) Dichloroacetate, sodium: 3-month oral toxicity studies in rats and dogs. Toxicol Appl Pharmacol 57(2):273–287

Kaufmann P, Engelstad K, Wei Y, Jhung S, Sano MC, Shungu DC, Millar WS, Hong X, Gooch CL, Mao X, Pascual JM, Hirano M, Stacpoole PW, DiMauro S, De Vivo DC (2006) Dichloroacetate causes toxic neuropathy in MELAS: a randomized, controlled clinical trial. Neurology 66 (3):324–330. https://doi.org/10.1212/01.wnl.0000196641.05913.27

Kerckhove N, Collin A, Conde S, Chaleteix C, Pezet D, Balayssac D (2017) Long-term effects, pathophysiological mechanisms, and risk factors of chemotherapy-induced peripheral neuropathies: a comprehensive literature review. Front Pharmacol 8:86. https://doi.org/10.3389/fphar.2017.00086

Little AA, Albers JW (2015) Clinical description of toxic neuropathies. Handb Clin Neurol 131:253–296. https://doi.org/10.1016/b978-0-444-62627-1.00015-9

London Z, Albers JW (2007) Toxic neuropathies associated with pharmaceutic and industrial agents. Neurol Clin 25(1):257–276. https://doi.org/10.1016/j.ncl.2006.10.001

Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Manji H (2013) Drug-induced neuropathies. Handb Clin Neurol 115:729–742. https://doi.org/10.1016/b978-0-444-52902-2.00042-4

Marmiroli P, Nicolini G, Miloso M, Scuteri A, Cavaletti G (2012) The fundamental role of morphology in experimental neurotoxicology: the example of chemotherapy-induced peripheral neurotoxicity. Ital J Anat Embryol 117(2):75–97

Misawa S, Kuwabara S, Mori M, Kawaguchi N, Yoshiyama Y, Hattori T (2001) Serum levels of tumor necrosis factor-alpha in chronic inflammatory demyelinating polyneuropathy. Neurology 56(5):666–669

Niimi N, Yako H, Tsukamoto M, Takaku S, Yamauchi J, Kawakami E, Yanagisawa H, Watabe K, Utsunomiya K, Sango K (2016) Involvement of oxidative stress and impaired lysosomal degradation in amiodarone-induced schwannopathy. Eur J Neurosci 44(1):1723–1733. https://doi.org/10.1111/ejn.13268

Niimi N, Takaku S, Yako H, Sango K (2017) Immortalized Schwann cells IFRS1 as a new strategic tool for the study of myelination and demyelination. Med Res Arch 5(2). https://journals.kei.org/index.php/mra/article/view/1028

Orr CF, Ahlskog JE (2009) Frequency, characteristics, and risk factors for amiodarone neurotoxicity. Arch Neurol 66(7):865–869. https://doi.org/10.1001/archneurol.2009.96

Pomponio G, Zurich MG, Schultz L, Weiss DG, Romanelli L, Gramowski-Voss A, Di Consiglio E, Testai E (2015) Amiodarone biokinetics, the formation of its major oxidative metabolite and neurotoxicity after acute and repeated exposure of brain cell cultures. Toxicol In Vitro 30(1 Pt A):192–202. https://doi.org/10.1016/j.tiv.2015.01.012

Pulipaka U, Lacomis D, Omalu B (2002) Amiodarone-induced neuromyopathy: three cases and a review of the literature. J Clin Neuromuscul Dis 3(3):97–105

Punnam SR, Goyal SK, Kotaru VP, Pachika AR, Abela GS, Thakur RK (2010) Amiodarone - a 'broad spectrum' antiarrhythmic drug. Cardiovasc Hematol Disord Drug Targets 10(1):73–81

Rao DB, Jortner BS, Sills RC (2014) Animal models of peripheral neuropathy due to environmental toxicants. ILAR J 54(3):315–323. https://doi.org/10.1093/ilar/ilt058

Ratnarajan G, Thompson A, Dodridge C, Parry A, Elston J (2015) Novel variant of Miller Fisher syndrome occurring with tumor necrosis factor alpha antagonist therapy. JAMA Neurol 72 (11):1377–1378. https://doi.org/10.1001/jamaneurol.2015.2251

Richez C, Blanco P, Lagueny A, Schaeverbeke T, Dehais J (2005) Neuropathy resembling CIDP in patients receiving tumor necrosis factor-alpha blockers. Neurology 64(8):1468–1470. https://doi.org/10.1212/01.wnl.0000158681.29117.8b

Rusinova R, Koeppe RE 2nd, Andersen OS (2015) A general mechanism for drug promiscuity: studies with amiodarone and other antiarrhythmics. J Gen Physiol 146(6):463–475. https://doi.org/10.1085/jgp.201511470

Sango K, Saito H, Takano M, Tokashiki A, Inoue S, Horie H (2006) Cultured adult animal neurons and Schwann cells give us new insights into diabetic neuropathy. Curr Diabetes Rev 2 (2):169–183

Sango K, Yanagisawa H, Kawakami E, Takaku S, Ajiki K, Watabe K (2011) Spontaneously immortalized Schwann cells from adult Fischer rat as a valuable tool for exploring neuron-Schwann cell interactions. J Neurosci Res 89(6):898–908. https://doi.org/10.1002/jnr.22605

Sango K, Kawakami E, Yanagisawa H, Takaku S, Tsukamoto M, Utsunomiya K, Watabe K (2012) Myelination in coculture of established neuronal and Schwann cell lines. Histochem Cell Biol 137(6):829–839. https://doi.org/10.1007/s00418-012-0934-3

Schmitt LI, Leo M, Kleinschnitz C, Hagenacker T (2018) Oxaliplatin modulates the characteristics of voltage-gated calcium channels and action potentials in small dorsal root ganglion neurons of rats. Mol Neurobiol 55(12):8842–8855. https://doi.org/10.1007/s12035-018-1029-5. [Epub ahead of print]

Silburn S, McIvor E, McEntegart A, Wilson H (2008) Guillain-Barre syndrome in a patient receiving anti-tumour necrosis factor alpha for rheumatoid arthritis: a case report and discussion of literature. Ann Rheum Dis 67(4):575–576. https://doi.org/10.1136/ard.2005.043208

Silva A, Wang Q, Wang M, Ravula SK, Glass JD (2006) Evidence for direct axonal toxicity in vincristine neuropathy. J Peripher Nerv Syst 11(3):211–216. https://doi.org/10.1111/j.1529-8027.2006.0090.x

Spruijt L, Naviaux RK, McGowan KA, Nyhan WL, Sheean G, Haas RH, Barshop BA (2001) Nerve conduction changes in patients with mitochondrial diseases treated with dichloroacetate. Muscle Nerve 24(7):916–924

Stubgen JP (2008) Tumor necrosis factor-alpha antagonists and neuropathy. Muscle Nerve 37 (3):281–292. https://doi.org/10.1002/mus.20924

Stubgen JP (2011) Drug-induced dysimmune demyelinating neuropathies. J Neurol Sci 307 (1–2):1–8. https://doi.org/10.1016/j.jns.2011.05.010

Ta LE, Espeset L, Podratz J, Windebank AJ (2006) Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum-DNA binding. Neurotoxicology 27 (6):992–1002. https://doi.org/10.1016/j.neuro.2006.04.010

Takaku S, Yako H, Niimi N, Akamine T, Kawanami D, Utsunomiya K, Sango K (2018) Establishment of a myelinating co-culture system with a motor neuron-like cell line NSC-34 and an adult rat Schwann cell line IFRS1. Histochem Cell Biol 149(5):537–543. https://doi.org/10.1007/s00418-018-1649-x

Tegner R, Tome FM, Godeau P, Lhermitte F, Fardeau M (1988) Morphological study of peripheral nerve changes induced by chloroquine treatment. Acta Neuropathol 75(3):253–260

Tristano AG (2010) Neurological adverse events associated with anti-tumor necrosis factor alpha treatment. J Neurol 257(9):1421–1431. https://doi.org/10.1007/s00415-010-5591-7

Van Helleputte L, Kater M, Cook DP, Eykens C, Rossaert E, Haeck W, Jaspers T, Geens N, Vanden Berghe P, Gysemans C, Mathieu C, Robberecht W, Van Damme P, Cavaletti G, Jarpe M, Van Den Bosch L (2018) Inhibition of histone deacetylase 6 (HDAC6) protects against vincristine-induced peripheral neuropathies and inhibits tumor growth. Neurobiol Dis 111:59–69. https://doi.org/10.1016/j.nbd.2017.11.011

Vassallo P, Trohman RG (2007) Prescribing amiodarone: an evidence-based review of clinical indications. JAMA 298(11):1312–1322. https://doi.org/10.1001/jama.298.11.1312

Transthyretin Amyloid Neuropathy: The Schwann Cell Hypothesis

24

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Abstract

Transthyretin (TTR)-familial amyloid polyneuropathy (FAP) is a systemic amyloidosis caused by mutations in the TTR gene. Typically, patients initially present with sensory and autonomic symptoms, which can lead to sensory dominant polyneuropathy and autonomic neuropathy. Mutations in TTR cause the tetrameric protein to dissociate and form amyloid deposits in the peripheral nervous system, most prominently in dorsal root ganglia (DRG), autonomic ganglia, and nerve trunks. Teased fiber studies have shown that segmental demyelination and axonal degeneration preferentially occur in the proximal and distal regions of the peripheral nerves, respectively. Nevertheless, it remains unknown why genetic variants of TTR lead to neurodegeneration in the peripheral nervous system. Recent studies in our laboratory have uncovered an important role for Schwann cells in the disease progression of FAP. In this review, we summarize findings implicating Schwann cells in FAP, and provide evidence that DRG may serve as the initial site of lesion formation in the disease.

Keywords

Amyloid · Dorsal root ganglia · Familial amyloid polyneuropathy · Peripheral nerve · Schwann cell · Transthyretin

24.1 Introduction

Familial amyloid polyneuropathy (FAP) is an autosomal dominant peripheral nerve disorder related to systemic amyloidosis and characterized by sensory dominant polyneuropathy and autonomic neuropathy (Andrade 1952). First described in

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Portugal by Andrade in 1952, FAP was initially identified in patients in endemic foci within Portugal, Sweden, and Japan. In FAP patients, extracellular deposits of amyloid fibrils are observed in systemic organs, particularly in the peripheral nervous system. The result is a progressive disease course that is ultimately fatal within 10–20 years. Transthyretin (TTR) was identified as a precursor protein of the amyloid fibrils in FAP patients (Costa et al. 1978), and a methionine substitution for valine at codon 30 of TTR was first demonstrated as an amyloidogenic variant (Mita et al. 1984; Murakami et al. 1995). This mutation—V30 M—is the most common variant and is considered the prototypical variant of the disease. As TTR is primarily produced in the liver (as well as the choroid plexus), liver transplantation was viewed as a potentially promising therapeutic approach. In 1990, the first liver transplantation was performed in two FAP patients, and this therapy successfully inhibited the progression of the disease (Holmgren et al. 1991; Planté-Bordeneuve and Said 2011).

The mature TTR monomer is a 127-amino acid peptide composed of eight ß strands and two ß sheets. TTR exists as a homotetramer that, in the blood, forms a complex with retinol-binding protein and facilitates the transport of retinol (vitamin A). The TTR tetramer also transports thyroxine in the cerebral spinal fluid, using a central thyroxine-binding pocket. To date, more than one hundred TTR variants have been reported in FAP patients. These variants consist of single amino acid substitutions distributed evenly across the protein sequence, and most (though not all) of these TTR mutations are pathogenic (Connors et al. 2003). Pathogenic TTR variants destabilize the formation of the homotetramer, leading to tetramer dissociation and the aggregation of TTR monomers into amyloid fibrils (Colon and Kelly 1992; Sekijima et al. 2005). TTR tetramer stabilizers, developed based on TTR structural studies, have subsequently been shown to slow the progression of FAP (Planté-Bordeneuve and Said 2011).

In sural nerve biopsies, TTR amyloid deposits can be seen in the endoneurium (in contact with Schwann cells), the subperineurium and around the endoneurial vessels. Axonal degeneration and, less frequently, segmental demyelination are typically observed in teased fiber studies (Dyck and Lambert 1969; Said et al. 1984; Planté-Bordeneuve and Said 2011). Postmortem studies have shown marked amyloid deposition in the dorsal root ganglia (DRG), nerve trunk and autonomic ganglia (Hanyu et al. 1989; Sobue et al. 1990; Toyooka et al. 1995). Differences in nerve fiber damage were observed at different regions along the peripheral nerve, with segmental demyelination occurring at the proximal region of the nerve (proximal median nerve and sciatic nerve) and axonal degeneration occurring at the distal region (distal median nerve and sural nerve) (Hanyu et al. 1989; Sobue et al. 1990; Toyooka et al. 1995). Nerve conduction studies have shown a reduced amplitude or a complete absence of sensory action potentials, and reduced amplitude of compound action potentials. These observations are primarily indicative of dying-back neuropathy (Luís 1978).

The mechanism underlying neurodegeneration in FAP remains largely unknown. Several hypotheses have been put forward, including mechanical compression of the nerves by amyloid fibrils, nerve ischemia and TTR toxicity (Sousa and Saraiva 2003;

Murakami and Sunada 2014). It is thought that amyloid physically compresses peripheral nerves, resulting in Wallerian degeneration (Koike et al. 2016; Said et al. 1984). Nerve ischemia, in turn, may be caused by perivascular amyloid. It was reported that non-fibrillar TTR aggregates are toxic in vitro and present in FAP nerves (Sousa et al. 2001). At advanced stages of the disease, several or all of these mechanisms may be involved, but it remains unknown what mechanism ultimately causes sensory polyneuropathy and autonomic neuropathy at the onset of FAP. Recent studies, including several conducted in our laboratory, suggest a key role for Schwann cells in the early stages of FAP. In this chapter, we review the growing evidence that Schwann cells contribute to early FAP pathogenesis.

24.2 TTR Gene Expression in Schwann Cells of the Peripheral Nervous System

Several lines of evidence suggest that TTR is expressed in Schwann cells. The TTR gene is significantly expressed in DRG of human, mouse, and rat (Murakami et al. 2008). In humans, TTR mRNA is 20- and 30-fold more abundant than in mouse and rat, respectively (Murakami et al. 2008). TTR mRNA was also detected in the human intercostal nerve (Murakami and Sunada 2014) and in the mouse sciatic nerve (Murakami et al. 2010).

To identify the cell types that express the TTR gene in mouse DRG, our laboratory performed laser microdissection analysis. This is a useful approach to assessing TTR expression, as in situ hybridization lacks the sensitivity to detect the minimal amounts of TTR mRNA in the DRG. TTR mRNA was detected in the basal and middle parts of DRG where Schwann cells and satellite Schwann cells exist, respectively (Murakami et al. 2008). Mouse TTR mRNA was also found in a primary Schwann cell culture derived from mouse neonatal sciatic nerves, and in the mouse Schwann cell line IMS32 derived from DRG (Murakami et al. 2010). Human TTR mRNA was detected in DRG of FAP transgenic mice expressing human TTR gene in a mouse TTR-null background. Furthermore, human TTR mRNA and protein was identified in a primary Schwann cell culture derived from the DRG of FAP transgenic mice.

Recently, it was demonstrated that V30 M TTR deposits occur in the ligated sciatic nerves of FAP transgenic mice. Importantly, these TTR deposits are not derived from blood but from Schwann cells (Goncalves et al. 2014b). It was further reported that, in FAP transgenic mice, human TTR mRNA is present in satellite Schwann cells located in the intramuscular nerve plexus of the intestine (Goncalves et al. 2014a).

24.3 Effect of Schwann Cells on the DRG Sensory Neuron

Each DRG sensory neuron is completely surrounded by satellite Schwann cells. The distance between the neuron and satellite cells is 20 nm on average, the requisite proximity to make a functional unit. This structure suggests that TTR secreted from satellite cells may have a paracrine effect on sensory neurons, acting as a neurotrophic factor. Cell culture experiments have indeed shown that normal TTR is incorporated into sensory neurons and promotes neurite outgrowth (Fleming et al. 2009). This suggests that, in the early stages of FAP, aggregates of variant TTR may increase around the sensory neurons and exert an inhibitory effect on neurite outgrowth, leading to axonal degeneration.

To understand how Schwann cells expressing variant TTR might impact the function of DRG sensory neurons, our laboratory established an immortalized Schwann cell line (TgS1) derived from DRG of transgenic mice expressing human variant TTR gene in a mouse TTR-null background. TgS1 cells produce and secrete variant TTR into the surrounding cell culture medium (Murakami et al. 2015). Using this system, we found that conditioned medium derived from cultured TgS1 cells inhibits neurite outgrowth from adult mouse DRG neurons (Murakami et al. 2015). In follow-up experiments, we used mouse Schwann cells (IMS32) to establish stable cell lines expressing human normal and variant TTR. Only medium derived from cells expressing variant TTR inhibited neurite outgrowth, suggesting that variant, pathogenic TTR is able to inhibit neurite outgrowth from DRG neurons (Murakami et al. 2015).

During the course of TTR amyloidogenesis, several TTR intermediates may form, including native monomers, misfolded monomers, soluble aggregates, and insoluble aggregates. It was reported amyloidogenic intermediates of TTR, but not mature fibrils, are cytotoxic in vitro (Andersson et al. 2002). It was also shown that TTR monomers and non-native oligomers ($<$100 kDa) are cytotoxic in cell culture (Reixach et al. 2004). Consistent with these findings, the TTR secreted into the medium in our experiments consists of monomers or oligomers, rather than mature amyloid fibrils.

24.4 TTR Aggregates in Schwann Cells

TTR aggregates formed within Schwann cells have not been reported in FAP patients. However, TTR amyloid fibrils and amorphous substances, termed insoluble TTR aggregates, were found to be in close contact with Schwann cells of sural nerves in FAP patients (Coimbra and Andrade 1971a; Inoue et al. 1998). Non-fibrillar TTR aggregates were also observed in sural nerves in the early stage of FAP (Sousa et al. 2001).

We observed TTR granular aggregates in Schwann cells and satellite Schwann cells in DRG of aged FAP transgenic mice (Murakami et al. 2015). TTR-immunoreactive inclusions were also found in the satellite cells. No amyloid deposits were found in the peripheral nervous system in these mice, nor were any

neurological symptoms such as paralysis in the lower limbs observed (Kohno et al. 1997; Murakami et al. 2015). These aged mice might correspond, in humans, to pre-symptomatic gene carriers or FAP patients at the early stage of the disease. These findings in mice are thus valuable, as to date pathological findings in DRG have not been reported for early-stage FAP patients.

To reproduce Schwann cell TTR aggregates in vitro, we treated TgS1 cells with the proteasome inhibitor MG132. We observed TTR aggregates as aggresomes within the cells. Aggresomes are considered both protective and toxic in the cells (Kopito 2000). When the activity of the proteasome system decreases as cells age, the aggresome-autophagy system may compensate for the protein quality control system. FAP onset typically occurs during middle age or later, and the decreasing protein quality control system concomitant with aging may, therefore, be related to the onset of the disease.

Using a *Drosophila* model for TTR amyloidosis, it was reported that fat cells and glia cells internalize variant TTR and form 20 nm spherules or amyloid fibrils (Pokrzywa et al. 2010). The authors speculated that these aggregates neutralize the toxicity of variant TTR, and the TTR aggregates within cells are overlooked owing to the late diagnosis of FAP.

24.5 Mechanisms of Peripheral Nervous System Involvement

Several lines of evidence suggest that DRG may be the initial lesion sites in FAP: (1) sensory dysfunction is usually observed at the onset of FAP; (2) in DRG, blood vessels exist abundantly while blood–nerve barriers are absent; (3) amyloid is deposited markedly in the DRG of FAP patients; (4) TTR is expressed in the Schwann cells of DRG; (5) variant TTR inhibits neurite outgrowth from DRG sensory neurons in vitro; and (6) TTR aggregates form within Schwann cells of DRG in aged FAP transgenic mice. Variant TTRs might aggregate within aging Schwann cells of DRG and contribute to cellular dysfunction. Subsequently, extracellular variant TTRs and TTR aggregates may increase, damaging DRG sensory neurons and leading to progressive ascending neuropathy (dying-back axonal degeneration) (Fig. 24.1a). Pain, numbness, and impaired thermal sensitivity are the first symptoms in typical FAP cases, and small-diameter sensory neurons, which convey pain and temperature senses, may be more vulnerable to variant TTR.

Motor impairment has been explained by the mechanical compression of peripheral nerves by amyloid fibrils. It was initially thought that amyloid deposits occur randomly along the peripheral nerve at multiple sites, with the accumulative effects of these deposits eventually causing distal axonal degeneration. However, postmortem studies of FAP patients have shown that amyloid deposits occur preferentially in the proximal region of peripheral nerves. Segmental demyelination is observed at proximal sites, while axonal degeneration is observed at the more distal sites. In support of these postmortem studies, amyloid fibrils were found by electron microscopy directly to contact demyelinating axons (Toyooka et al. 1995). These lesions may cause axonal degeneration in the more distal sites. Magnetic resonance

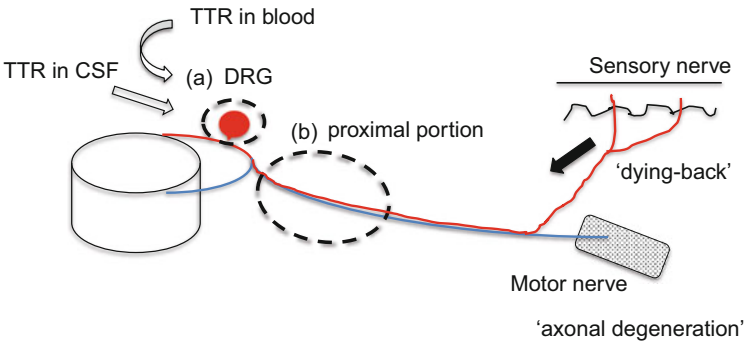

Fig. 24.1 Hypothesis of neurodegeneration in FAP. The TTR gene is expressed in glial cells of DRG. In addition, TTR is transported via blood, as there is no blood–brain barrier in DRG. TTR is also transported from cerebrospinal fluid (CSF) to DRG. DRG may represent the initial site of lesion formation in FAP, leading to dying-back axonal degeneration of the sensory nerve (**a**). Subsequently, segmental degeneration and multiple TTR deposits preferentially occur in the proximal portion of the peripheral nerve, resulting in axonal degeneration of motor nerve (**b**)

neurography recently revealed that nerve lesions and nerve caliber increases are found at the proximal sciatic nerve of FAP patients and asymptomatic carriers, compared with healthy individuals (Kollmer et al. 2015). Furthermore, no amyloid of the sural nerve has been reported in late-onset FAP cases (Koike et al. 2016; Murakami et al. 2017). Similar to pathological findings of DRG in aged FAP transgenic mice, TTR aggregates and Schwann cell dysfunction may initially occur in the proximal peripheral nerve (Fig. 24.1b). This may result in disrupted Schwann cell–axon interactions, amyloid fibril formation, and distal axonal degeneration.

24.6 Conclusion

Schwann cell abnormality was reported early on in studies of TTR amyloid neuropathy. In 1971, Coimbra and Andrade studied the sural nerves of five FAP patients using electron microscopy (Coimbra and Andrade 1971a, b). They found that Schwann cell and myelin changes occur prior to axon damage in myelinated fibers. Moreover, they observed no relationship between amyloid deposits and nerve fiber loss. They described that "it is then possible that the genetic anomaly first caused the myelin and other nerve fiber lesions, and that some noxious agent which was released, diffused to the endoneurium to produce amyloid deposition." They further investigated the sural nerves of asymptomatic children in FAP families and found abnormal changes in Schwann cells, and in the myelin of myelinated fibers (Carvalho et al. 1976). They concluded that "the findings in the 4 cases. . .support the hypothesis that the pathological process in this hereditary disease attacks the Schwann cells directly." In the years following these studies, mutations in TTR were implicated in FAP (Mita et al. 1984; Murakami et al. 1995). Studies in our laboratory

now show that TTR is expressed in Schwann cells and that TTR aggregates form in Schwann cells in aged FAP mice, collectively implicating Schwann cells in FAP pathogenesis. We thus appreciate these early insights into the neuropathogenesis of FAP. Further studies will reveal the mechanisms of neurodegeneration in TTR amyloidosis, and Schwann cell protection may represent an important future therapeutic avenue for this intractable disease.

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References

Andersson K, Olofsson A, Nielsen EH, Svehag SE, Lundgren E (2002) Only amyloidogenic intermediates of transthyretin induce apoptosis. Biochem Biophys Res Commun 294:309–314

Andrade C (1952) A peculiar form of peripheral neuropathy; familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. Brain 75:408–427

Carvalho J, Coimbra A, Andrade C (1976) Peripheral nerve fibre changes in asymptomatic children of patients with familial amyloid polyneuropathy. Brain 99:1–10

Coimbra A, Andrade C (1971a) Familial amyloid polyneuropathy: an electron microscope study of the peripheral nerve in five cases. I. Interstitial changes. Brain 94:199–206

Coimbra A, Andrade C (1971b) Familial amyloid polyneuropathy: an electron microscope study of the peripheral nerve in five cases. II. Nerve fibre changes. Brain 94:207–212

Colon W, Kelly JW (1992) Partial denaturation of transthyretin is sufficient for amyloid fibril formation in vitro. Biochemistry 31:8654–8660

Connors LH, Lim A, Prokaeva T, Roskens VA, Costello CE (2003) Tabulation of human transthyretin (TTR) variants, 2003. Amyloid 10:160–184

Costa PP, Figueira AS, Bravo FR (1978) Amyloid fibril protein related to prealbumin in familial amyloidotic polyneuropathy. Proc Natl Acad Sci U S A 75:4499–4503

Dyck PJ, Lambert EH (1969) Dissociated sensation in amyloidosis. Compound action potential, quantitative histologic and teased-fiber, and electron microscopic studies of sural nerve biopsies. Arch Neurol 20:490–507

Fleming CE, Mar FM, Franquinho F, Saraiva MJ, Sousa MM (2009) Transthyretin internalization by sensory neurons is megalin mediated and necessary for its neuritogenic activity. J Neurosci 29:3220–3232

Goncalves NP, Costelha S, Saraiva MJ (2014a) Glia cells in familial amyloidotic polyneuropathy. Acta Neuropathol Commun 2:177. https://doi.org/10.1186/s40478-014-0177-8

Goncalves NP, Teixeira-Coelho M, Saraiva MJ (2014b) The inflammatory response to sciatic nerve injury in a familial amyloidotic polyneuropathy mouse model. Exp Neurol 257:76–87

Hanyu N, Ikeda S, Nakadai A, Yanagisawa N, Powell HC (1989) Peripheral nerve pathological findings in familial amyloid polyneuropathy: a correlative study of proximal sciatic nerve and sural nerve lesions. Ann Neurol 25:340–350

Holmgren G, Steen L, Ekstedt J, Groth CG, Ericzon BG, Eriksson S, Andersen O, Karlberg I, Norden G, Nakazato M et al (1991) Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met30). Clin Genet 40:242–246

Inoue S, Kuroiwa M, Saraiva MJ, Guimarães A, Kisilevsky R (1998) Ultrastructure of familial amyloid polyneuropathy amyloid fibrils: examination with high-resolution electron microscopy. J Struct Biol 124(1):1–12

Kohno K, Palha JA, Miyakawa K, Saraiva MJ, Ito S, Mabuchi T, Blaner WS, Iijima H, Tsukahara S, Episkopou V, Gottesman ME, Shimada K, Takahashi K, Yamamura K,

Maeda S (1997) Analysis of amyloid deposition in a transgenic mouse model of homozygous familial amyloidotic polyneuropathy. Am J Pathol 150:1497–1508
Koike H, Ikeda S, Takahashi M, Kawagashira Y, Iijima M, Misumi Y, Ando Y, Ikeda SI, Katsuno M, Sobue G (2016) Schwann cell and endothelial cell damage in transthyretin familial amyloid polyneuropathy. Neurology 87:2220–2229
Kollmer J, Hund E, Hornung B, Hegenbart U, Schonland SO, Kimmich C, Kristen AV, Purrucker J, Rocken C, Heiland S, Bendszus M, Pham M (2015) In vivo detection of nerve injury in familial amyloid polyneuropathy by magnetic resonance neurography. Brain 138:549–562
Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 10: 524–530
Luís ML (1978) Electroneurophysiological studies in familial amyloid polyneuropathy – Portuguese type. J Neurol Neurosurg Psychiatry 41:847–850
Mita S, Maeda S, Shimada K, Araki S (1984) Cloning and sequence analysis of cDNA for human prealbumin. Biochem Biophys Res Commun 124:558–564
Murakami T, Sunada Y (2014) Expression of the transthyretin gene in Schwann cells and familial amyloidotic polyneuropathy-mediated neurodegeneration. In: Sango K, Yamauchi J (eds) Schwann cell development and pathology. Springer, Tokyo, pp 103–115
Murakami T, Uchino M, Ando M (1995) Genetic abnormalities and pathogenesis of familial amyloidotic polyneuropathy. Pathol Int 45:1–9
Murakami T, Ohsawa Y, Sunada Y (2008) The transthyretin gene is expressed in human and rodent dorsal root ganglia. Neurosci Lett 436:335–339
Murakami T, Ohsawa Y, Li Z, Yamamura K, Sunada Y (2010) The transthyretin gene is expressed in Schwann cells of peripheral nerves. Brain Res 1348:222–225
Murakami T, Sango K, Watabe K, Niimi N, Takaku S, Li Z, Yamamura K, Sunada Y (2015) Schwann cells contribute to neurodegeneration in transthyretin amyloidosis. J Neurochem 134:66–74
Murakami T, Nishimura H, Nagai T, Hemm S, Kutoku Y, Ohsawa Y, Sunada Y (2017) Clinical and pathological findings in familial amyloid polyneuropathy caused by a transthyretin E61K mutation. J Neurol Sci 381:55–58
Planté-Bordeneuve V, Said G (2011) Familial amyloid polyneuropathy. Lancet Neurol 10: 1086–1097
Pokrzywa M, Dacklin I, Vestling M, Hultmark D, Lundgren E, Cantera R (2010) Uptake of aggregating transthyretin by fat body in a Drosophila model for TTR-associated amyloidosis. PLoS One 5(12):e14343
Reixach N, Deechongkit S, Jiang X, Kelly JW, Buxbaum JN (2004) Tissue damage in the amyloidoses: transthyretin monomers and nonnative oligomers are the major cytotoxic species in tissue culture. Proc Natl Acad Sci U S A 101:2817–2822
Said G, Ropert A, Faux N (1984) Length-dependent degeneration of fibers in Portuguese amyloid polyneuropathy: a clinicopathologic study. Neurology 34:1025–1032
Sekijima Y, Wiseman RL, Matteson J, Hammarstrom P, Miller SR, Sawkar AR, Balch WE, Kelly JW (2005) The biological and chemical basis for tissue-selective amyloid disease. Cell 121:73–85
Sobue G, Nakao N, Murakami K, Yasuda T, Sahashi K, Mitsuma T, Sasaki H, Sakaki Y, Takahashi A (1990) Type I familial amyloid polyneuropathy. A pathological study of the peripheral nervous system. Brain 113:903–919
Sousa MM, Saraiva MJ (2003) Neurodegeneration in familial amyloid polyneuropathy: from pathology to molecular signaling. Prog Neurobiol 71:385–400
Sousa MM, Cardoso I, Fernandes R, Guimarães A, Saraiva MJ (2001) Deposition of transthyretin in early stages of familial amyloidotic polyneuropathy: evidence for toxicity of nonfibrillar aggregates. Am J Pathol 159:1993–2000
Toyooka K, Fujimura H, Ueno S, Yoshikawa H, Kaido M, Nishimura T, Yorifuji S, Yanagihara T (1995) Familial amyloid polyneuropathy associated with transthyretin Gly42 mutation: a quantitative light and electron microscopic study of the peripheral nervous system. Acta Neuropathol 90:516–525