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Negative Regulators of Schwann Cell Differentiation—Novel Targets for Peripheral Nerve Therapies?

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Abstract As myelinating glial cells of the peripheral nervous system, Schwann cells wrap around axons and thereby provide insulation, acceleration of electric signal propagation, and axonal protection and maintenance. Schwann cells are main effectors for regeneration in a variety of peripheral neuropathic conditions, including inherited, inflammatory, toxic, and diabetic neuropathies, as well as traumatic injuries to peripheral nerve fibers. Due to their high differentiation plasticity, these cells can respond to injury and disease by myelin sheath degradation, dedifferentiation into an immature Schwann cell-like phenotype, proliferation, and remyelination of sprouting axons. In doing so, they can support and promote axonal regrowth and target tissue innervation. Developmental differentiation as well as regenerative de- and redifferentiation are tightly controlled by a balance of positive and negative regulators of Schwann cell maturation. Since misregulated expression of such negative regulators is potentially involved in inefficient or failed regeneration, we will provide an overview about recent work revealing the complex interactions between extrinsic and intrinsic signals in the inhibition of Schwann cell differentiation.

Keywords Peripheral neuropathies · nerve injury · regeneration · myelin · repair · inhibitors · glia · maturation

Introduction

Schwann cells and oligodendrocytes are the myelinating glial cells of the peripheral (PNS) and central nervous system (CNS), respectively. These cells generate myelin sheaths that insulate axons and thereby accelerate electrical signal propagation. Schwann cell development from the neural crest is comprised of the transition from immature Schwann cells to mature myelin-forming Schwann cells that spirally wrap around large-diameter axons at a 1:1 ratio, thus generating the myelin sheath. Remarkably, and in contrast to oligodendrocytes, fully-differentiated myelinating Schwann cells maintain the ability to revert to an immature phenotype in response to nerve injury and, by doing so, can actively promote nerve repair and functional recovery [1]. As a consequence of axonal degeneration following peripheral nerve injury or in peripheral neuropathies, Schwann cells dedifferentiate and contribute to macrophage-mediated myelin removal and degradation (reviewed in [2]). Following dedifferentiation, Schwann cells re-enter the cell cycle, proliferate, and form bands of Büngner, which support and direct outgrowing axons to sites of innervation [3]. Moreover, these cells express and secrete axonal growth-promoting factors, then redifferentiate and myelinate regenerated axons, which eventually leads to substantial functional recovery. This sequence emphasizes the central function of these cells in injury and disease outcome.

Differentiation and maturation of Schwann cells—in particular, the formation of the lipid-rich multi-lamellar myelin sheath and the morphological changes which lead to and enable axonal wrapping—are tightly-regulated processes that depend on the coordinated expression of differentiation-promoting genes and repression of inhibitory determinants (previously reviewed by [4]). Varying aspects of Schwann cell differentiation, including survival, proliferation, and instruction of myelination, strictly depend on axonal signals such as type III neuregulin-1 (Nrg1-III; [5]). Importantly, signalling between Schwann cells and axons has to be maintained throughout the cell's lifetime, since Schwann cells in vivo and in vitro rapidly dedifferentiate upon axonal loss.

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Hence, axonal signals clearly direct (and maintain) cellular differentiation via promoting factors such as Krox-20 (Egr-2, [6, 7]) and Sox10 [8, 9]. These transcription factors are crucial for myelin generation and maintenance. Other factors which promote Schwann cell differentiation and interact with Krox-20 and Sox10 are Oct-6 (Pou3f1, [10]) and NFκB [11]. These transcriptional regulators are transiently expressed in premyelinating Schwann cells prior to myelin gene induction. Recent evidence reveals that loss of axonal signalling coincides with reexpression of Schwann cell differentiation-inhibitory genes, indicating that the subsequent dedifferentiation process is probably not solely induced as a consequence of reduced or absent (axonal) promoting factors [12]. Hence it is conceivable that, similar to nerve development, nerve regeneration is critically dependent on balanced positive and negative regulators, and that differentiation inhibitors significantly contribute to both avoidance of premature differentiation and promotion of dedifferentiation. This remarkable differentiation plasticity accounts for the high regeneration capacity of the PNS. However, a number of neuropathic conditions are able to prevent, decelerate, or impair repair activities. These include inherited, immunoinflammatory, toxic, or metabolic neuropathies. Given the possibility that under such conditions Schwann cell homeostasis, dedifferentiation, and redifferentiation might be influenced by inhibitory components, knowledge about their nature and mode of action is of increasing importance and might eventually aid in the design of novel therapeutic strategies that promote the endogenous regenerative potential. This review describes the currently known genes that encode negative regulators of Schwann cell differentiation and discusses evidence as to whether these cells experience multiple negative inputs or whether the currently known factors are likely to converge on a single inhibitory signalling cascade.

Novel Inhibitory Determinants of Schwann Cell Differentiation

Despite the functional importance of negative regulation of myelination - i.e., to prevent premature or hypermyelination of peripheral nerves and to promote dedifferentiation, allowing efficient regeneration upon nerve injury or neuropathies - relatively few such regulators were described until recently. So far, an apparent role for the inhibition of Schwann cell differentiation has been described for the activation of Notch receptors [13] and the two transcription factors Sox2 [14] and c-Jun [12], the latter of which was shown to antagonize differentiation promoting expression of Krox20 (all reviewed by [4]). Rapid upregulation and activation of c-Jun upon sciatic nerve transection is critical for myelin removal, and a delayed dedifferentiation of Schwann cells was observed in c-Jun ablated cells after nerve transection. In addition, upregulation of c-Jun was also observed in human Schwann cells under pathological conditions, suggesting regeneration associated functions [15]. The central function of c-Jun was underscored by a recent report which revealed that Schwann cell specific deletion of c-Jun leads to impaired motoneuron survival and axonal regeneration that can be ameliorated by application of GDNF and Artemin [16]. Of note, ablation of c-Jun did not affect Schwann cell differentiation, emphasizing that peripheral nerve regeneration is not a recapitulation of developmental gene expression but that denervation induces a regeneration specific gene expression program which can be clearly distinguished from that of immature Schwann cells. Further negative regulators of Schwann cell differentiation are Id2, Pax3, Krox24, nitric oxide (NO), and the Ras/Raf/ERK signalling cascade ([17–25], reviewed by [4]). Additional inhibitory determinants are just emerging, revealing that several distinct pathways, rather than single signals, are involved in negative control of cellular maturation and myelination. This will be discussed below.

p57kip2

Apart from their ascribed function as cell cycle inhibitors, cyclin-dependent kinase inhibitors (CKI) such as p21cip1, p27kip1, and p57kip2 are also directly implied in regulation of differentiation. In this regard, p57kip2 appears to exert an essential function, as only deletion of this CKI leads to perinatal death as a result of severe developmental abnormalities [26, 27]. The protein domain structure of p57kip2 remarkably differs from that of other CKIs, including a unique LIM protein binding domain, and binding of p57kip2 to LIMK1 leading to nuclear translocation has been demonstrated to interfere with actin filament assembly [28]. p57kip2 may therefore exert differentiation-associated functions via interaction with LIM-proteins, regulating either their activity or subcellular localization. We demonstrated that p57kip2 interferes with Schwann cell and oligodendroglial differentiation [29, 30]. Chromatin immunoprecipitation (ChIP) analysis with primary rat Schwann cells revealed that p57kip2 activity is controlled by the histone methyltransferase EZH2 [31]. p57kip2 is downregulated during postnatal development and differentially expressed in the adult upon nerve injury [29, 32]. Of note, the postnatal decline in p57kip2 expression parallels that of Oct-6 and coincides with the onset of myelin gene induction. Since Schwann cell proliferation is still present at this stage, this suggested that p57kip2 is not involved in the control of the Schwann cell cycle. In fact, the related CKI proteins p21cip1 and p27kip1 are induced postnatally and required for Schwann cell cycle control [33, 34]. Further cell culture experiments revealed that control of both morphological maturation and differentiation associated gene expression depend on p57kip2 expression levels. Long-term suppression of p57kip2 expression led to a prominent elongation of Schwann cell processes,

resulting in a morphology which resembled that of elongated Schwann cells in dorsal root ganglion (DRG) cocultures prior to myelination. Elongated processes of p57kip2 suppressed Schwann cells contained long actin filaments, which led to the conclusion that actin filament stabilization contributed to the observed morphological alterations. Both actin filament assembly and activation of the Rho pathway are necessary for induction of myelination by Schwann cells [35, 36]. Since LIMK1 as Rho pathway effector is known to stabilize actin filaments, and p57kip2 was shown to translocate LIMK1 from cytoplasm to nuclei [28, 37], p57kip2 knockdown is likely to affect cell morphology via an enhanced cytoplasmic activity of LIMK1.

Apart from its described function in regulating Schwann cell morphology, GeneChip analysis and quantitative RT-PCR showed that suppression of p57kip2 led to an overall gene expression pattern which closely resembled the pattern that can be observed during peripheral nerve development, in that p57kip2 suppression induced myelin gene and protein expression and reduced levels of Oct-6, Krox24, and Sox2 [29]. Notably, c-Jun expression was also lowered, although to a lesser extent, independently from Krox20 expression, which remained unchanged (AH and PK, unpublished data). In addition, expression of Hes5, which is known to interfere with oligodendroglial differentiation [38] was reduced upon p57kip2 knockdown. Hes5 directly binds to myelin gene promoters and inhibits their expression [39], suggesting a similar inhibitory function in Schwann cells. Indeed, knockdown of Hes5 in Schwann cells induced myelin gene and protein expression, revealing Hes5 as potent inhibitory determinant the expression of which is regulated upon p57kip2 suppression [31].

Importantly, reduction of p57kip2 expression led to an acceleration of in vitro myelination, strengthening the conclusion that p57kip2 negatively interferes with Schwann cell differentiation. Given that cultured Schwann cells have so far been known as reluctant to differentiate spontaneously, it was concluded that the suppression of p57kip2 is necessary to induce Schwann cell maturation. This notion is additionally supported by the observation that EZH2 suppressed Schwann cells have a reduced ability to form myelin in vitro, presumably due to p57kip2 and Hes5 induction. Thus, p57kip2 encodes a central negative regulator of several aspects of Schwann cell differentiation. Whether the observed gene and protein regulatory changes are secondary to morphological alterations and whether p57kip2 ablation leads to aberrant morphology or myelination in vivo has yet to be shown.

Dlg1/PTEN

Signals that direct the extent of myelination are constitutively expressed by axons, revealing the necessity of control mechanisms that negatively interfere with myelin gene expression to prevent hypermyelination once the myelin sheath is generated. The membrane-associated disks large homolog 1 (Dlg1) and its interaction with the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) fulfill this important function and hence regulate myelin thickness. Silencing of Dlg1 in Schwann cells led to migration defects, resulting in an inability to myelinate axons in vitro. However, those axons that were myelinated displayed hypermyelinating phenotypes, indicating a function in the control of myelin thickness [40]. Lentivirus-mediated silencing of either Dlg1 or PTEN in sciatic nerve Schwann cells in vivo resulted in severe axonal hypermyelination. Accordingly, when Dlg1 was overexpressed in sciatic nerves, most of the Schwann cells failed to myelinate. Mechanistically, Cotter and colleagues found that Dlg1 suppression in cultured Schwann cells stimulated the PI3K pathway, leading to enhanced Akt phosphorylation. This is normally activated by axonal type III neuregulin 1 (Nrg1-III), thus revealing Dlg1 as antagonizing Nrg1-III signalling. In vivo, PTEN ablation also led to hypermyelination and thus to generation of myelin outfoldings which resemble those found in Charcot-Marie-Tooth (CMT) type 4B1 and hereditary neuropathy with liability to pressure palsies (HNPP). As expected, this phenotype was caused by hyperactivation of the PI3K pathway [41]. Enhanced Akt phosphorylation in Dlg1-suppressed Schwann cells was shown to depend on the ability of Dlg1 to interact with and to stabilize PTEN [40]. Hence, lowered Dlg1 levels led to a reduction of Dlg1/PTEN complexes and enhanced Akt phosphorylation. In addition, stimulation of Schwann cells with Nrg1-III led to increased Dlg1 and PTEN protein expression levels and an inhibition of their ubiquitination. These findings suggest a dual function for Nrg1-III: 1) stimulation of the PI3K pathway, hence promoting Schwann cell myelination, and 2) stabilization of Dlg1/PTEN complexes to prevent hypermyelination.

TACE (ADAM17)

Given that it constitutes an important myelination-instructive signal, ablation of Nrg1-III expression resulted in aberrant myelination of peripheral nerves [5]. Therefore, inactivation of Nrg1-III can be expected to interfere with Schwann cell differentiation. Nrg1-III is cleaved and thus activated by the β-site amyloid precursor protein cleaving enzyme (BACE1), the knockout of which resulted in hypomyelination of peripheral nerves [42, 43]. The tumor necrosis factor-α–converting enzyme (TACE; ADAM17), a proteolytic enzyme involved in the shedding of several membrane-bound proteins, has recently been demonstrated to inhibit Schwann cell myelination via alternative cleavage of Nrg1-III [44]. TACE is expressed in Schwann cells and DRG neurons, and lentiviral-mediated knockdown of TACE expression in DRG cocultures substantially enhanced myelination and myelin protein zero (MPZ) expression. Importantly, this effect was seen only when TACE

was suppressed in neurons, whereas knockdown in Schwann cells did not enhance myelination of DRG cocultures. Conditional ablation of TACE in motor neurons in vivo led to hypermyelination of nerves, which was accompanied by higher concentrations of MBP and enhanced activation of the PI3K pathway, indicative of an increased Nrg1-III activity. On the other hand, conditional ablation of TACE in Schwann cells in vivo did not alter myelination of peripheral nerves, confirming that the function of TACE is neuron autonomous. Additional experimental evidence revealed that recombinant TACE cleaves recombinant Nrg1-III, which is then no longer able to activate the PI3K pathway in cultured Schwann cells. Owing to these reported results, TACE can be considered an extrinsic inhibitory determinant of Schwann cell differentiation that controls the activity of axonally-expressed Nrg1-III by proteolytic cleavage, thus counteracting and regulating the Nrg1-III processing activity of BACE. Hence Nrg1-III amounts are under positive and negative control by proteolytic cleavage as a result of BACE and TACE activity, thus regulating myelin thickness.

Dock7

Another protein the expression of which demonstrates a dual role of Nrg1-III signalling is dedicator of cytokinesis protein 7 (Dock7). Expression of Dock7 is highest in migrating Schwann cells, and stimulation of ErbB2 receptors with Nrg1-III directly activates Dock7 [45]. As a guanine-nucleotide exchange factor (GEF), Dock7 catalyzes the exchange of GDP to GTP and thus activates G proteins. The activation of Rac/Cdc42 by Dock7, which antagonizes Rho signals and leads to a downstream activation of c-Jun, regulates Schwann cell migration prior to myelination. A recently published report revealed that Dock7 actively inhibits Schwann cell myelination by promoting Rac/Cdc42 signalling, thus inhibiting Rho activation [46]. Dock7 expression is downregulated during postnatal peripheral nerve development, with a transient peak between postnatal days 1 and 4. Treatment of Schwann cells with siRNA directed against Dock7 led to induction of MPZ and MBP protein expression, and shRNA mediated knockdown of Dock7 in vivo led to sciatic nerve hypermyelination in 7-day-old transgenic mice. Interestingly, the hypermyelination effect decreased during later development, suggesting a role for Dock7 at myelination onset, consistent with its early expression peak during postnatal development. This was further supported by the notion that transgenic mice exhibited lowered Oct-6 and slightly increased Krox20 expression levels. Additional analyses exposed that myelin gene induction in both Dock7-suppressed Schwann cells and Dock7 knockdown sciatic nerves was accompanied by reduction of Rac and Cdc42 levels and subsequent c-Jun reduction, while activation of RhoA was accelerated and prolonged. Of note, the described results do not exclude the possibility that further targets of Rac/Cdc42 contribute to the induction of myelin gene expression.

LXR and Oxysterols

Oxysterols derive either from autooxidation of cholesterol or enzymatically through cholesterol hydroxylases. Makoukji and colleagues have shown that Schwann cells contain significant amounts of oxysterols and express liver X receptors (LXR). These nuclear receptors can be activated upon oxysterol stimulation and directly bind to DNA via LXR-responsive elements, thus regulating gene expression. The inhibitory nature of this signalling pathway was demonstrated by treatment of the Schwann cell line MSC80 with oxysterol 25-hydroxycholesterol or the synthetic LXR ligand TO901317, both of which resulted in significantly reduced levels of MPZ and PMP22 expression [47]. LXR recruitment to MPZ and PMP22 promoters was raised by 25-hydroxycholesterol treatment, and promoter activities were subsequently reduced. On the other hand, knockdown of LXR led to increased MPZ and PMP22 promoter activities and reversed the inhibitory effect of 25-hydroxycholesterol treatment. In addition to LXR's negative effect on myelin gene transcription, the authors provide evidence that the Wnt/β-catenin signalling cascade, which is an important activator of myelin gene expression [48], is also disrupted by LXR. Myelin promoter occupancy by β-catenin was reduced upon 25-hydroxycholesterol treatment. Consequently, β-catenin activity was induced in LXR knockout mice. Unexpectedly, although LXR knockout resulted in higher levels of myelin gene expression, presumably due to increased β-catenin activity, myelin protein expression was reduced and mutant nerves were therefore hypomyelinated. The authors conclude that this hypomyelination may reflect an altered cholesterol homeostasis in LXR knockout mice. Since elevated cholesterol levels were shown to be implied in MPZ trafficking from the endoplasmic reticulum [49], diminished MPZ protein levels may thus result from inefficient trafficking - an assumption that remains to be proven in future studies.

SSeCKS

The rodent orthologue of human gravin, Src-suppressed protein kinase C substrate (SSeCKS), was recently found to negatively regulate Schwann cell differentiation [50]. SSeCKS expression was transiently induced and then rapidly downregulated upon peripheral nerve injury, indicating a role in the promotion of dedifferentiation. Lentivirus-mediated knockdown of SSeCKS led to process elongation in cultured Schwann cells and was accompanied by a transient induction of myelin gene expression that was explained by elevated Akt phosphorylation rates. SSeCKS knockdown also promoted in vitro myelination in DRG cocultures. The authors suggest that since SSeCKS acts on protein kinase A (PKA) activity [51], the observed changes in morphology and myelin gene expression might account for PKA signalling, the expression of which was previously shown to promote Schwann cell myelination [52]. However, whether

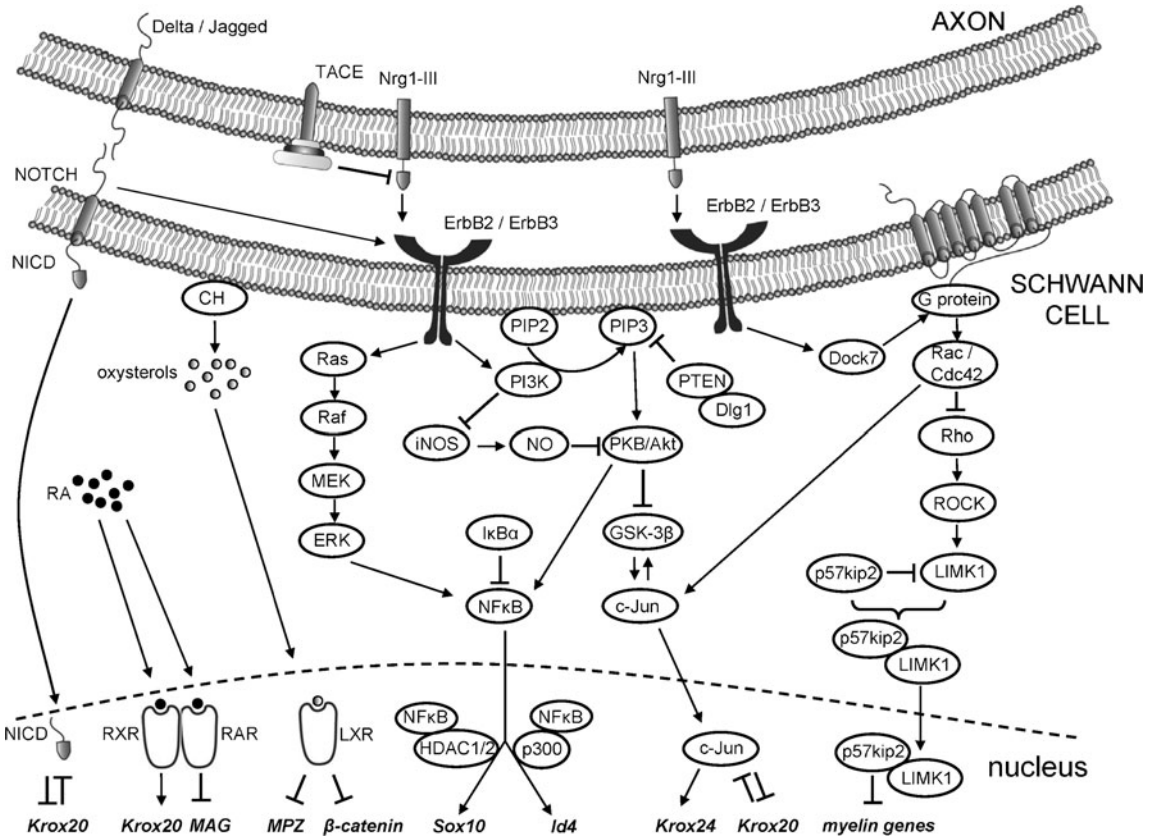

Fig. 1 Molecular pathways implied in the negative control of Schwann cell differentiation. Schwann cell differentiation is controlled by axonal (extrinsic) inhibitors that regulate the temporal control of myelination onset by induction of intrinsic inhibitory signalling cascades. Extrinsic inhibitors comprise Nrg1-III, TACE, and Delta/Jagged activation of Notch in Schwann cells. Although primarily considered to positively affect Schwann cell proliferation and differentiation by activation of PI3K and, hence, Akt phosphorylation, axonally expressed Nrg1-III is also able to induce the Ras/Raf/ERK signalling cascade, leading to ERK phosphorylation, a signal which inhibits differentiation and leads to dedifferentiation of myelinated DRG cocultures. Neuron-specific conditional ablation of TACE (ADAM17), a protein shown to cleave and inactivate Nrg1-III, leads to hypermyelination of nerves, accompanied by PI3K activation. Axonal Delta and Jagged regulate varying aspects of Schwann cell differentiation via Notch activation. Notch signalling, via its intracellular domain (NICD), promotes proliferation of Schwann cell precursor cells, presumably due to its ability to activate ErbB2 expression; however, it also inhibits myelinating Schwann cell differentiation by interfering with Krox20 activity, the expression of which activates differentiation-associated genes, while repressing immature Schwann cell markers. Further transcription factors inhibiting Schwann cell differentiation by either directly or indirectly repressing myelin gene expression involve the nuclear receptors of retinoic acid (RAR and RXR), oxysterol-activated nuclear LXR receptors, c-Jun, and NFκB acetylated by p300. RA was shown to possess bivalent functions with respect to Schwann cell differentiation. RAR activation upon RA treatment leads to inhibition of the MAG expression, whereas activation of RXR induces Krox20 and, subsequently, myelin gene expression. Another nuclear receptor implicated in the inhibition of myelin gene expression is LXR. LXR activation by oxysterols leads to inhibition of myelin gene expression, most likely by interfering with β-catenin promoter binding activity. NFκB is another transcriptional regulator with bivalent functions. NFκB can be activated by Akt or ERK and, depending on the context, either inhibits Schwann cell differentiation when acetylated by p300 via Id4 induction or, when deacetylated upon interaction with HDAC1/2, induces Sox10. NFκB expression can be inhibited by IκBα, therefore representing yet another negative regulator of Schwann cell differentiation. An important function for the inhibition of Schwann cell differentiation and induction of dedifferentiation is achieved by c-Jun. c-Jun activation is inhibited by the PI3K/Akt pathway, and its expression counteracts Krox20 expression. Hence, Krox20 antagonizes expression of both Notch and c-Jun, while it promotes the expression of myelin genes. A further group of differentiation inhibitors comprising NO and Dlg1/PTEN interferes with the PI3K pathway, leading to reduced Akt phosphorylation. The inhibition of the PI3K pathway by Dlg1/PTEN was shown to exert an important function in the control of the myelin thickness, thus preventing excessive myelination of axons. An additional group of differentiation inhibitors, including p57kip2 and Dock7, blocks morphological maturation by interfering with Rho pathway components, subsequently blocking further differentiation. Dock7 is activated by Nrg1-III signalling, leading to activation of Rac/Cdc42. This inhibits Rho signalling and promotes activity of c-Jun. p57kip2 interacts with LIMK1 and controls LIMK1 activity by translocation from cytoplasm to Schwann cell nuclei. Abbreviations: MEK, mitogen-activated protein kinase kinase; GSK-3β, glycogen synthase kinase 3 beta; iNOS, inducible nitric oxide synthase; CH, cholesterol; all other abbreviations are defined in the text

SSeCKS expression also interferes with Schwann cell differentiation in vivo, and whether the observed reexpression of SSeCKS promotes dedifferentiation upon injury, remains to be elucidated.

Schwann Cell Determinants with Bivalent Roles: Context-Dependent Promotion or Inhibition of Cellular Differentiation

Apart from simply interfering with maturation and myelination of Schwann cells, several proteins with bivalent functions - both promoting and inhibiting Schwann cell differentiation, depending on the developmental stage and a context-dependent expression of putative binding partners - were described in recent publications.

Nrg1-III

As outlined above, promotion of myelination via Nrg1-III is achieved by activation of the PI3K pathway, leading to phosphorylation of Akt. However, although being a central instructive signal for myelination, application of high Nrg1-III doses inhibits myelination and leads to dedifferentiation of myelinated DRG cocultures. This negative regulatory effect is mediated by activation of the Ras/Raf/MEK/ERK signalling pathway [20], since inhibition of ERK signalling prevented demyelination, while inhibition of the PI3K pathway did not. Of note, ErbB2 receptor inactivation in adult nerves using conditional knockout animals did not induce dedifferentiation, revealing that maintenance of the myelin sheath is supported by additional signals independent of Nrg1-III [53]. Whether, on the other hand, elevated Nrg1-III levels are instructive for dedifferentiation upon peripheral nerve transection via ERK activation remains to be shown in suitable in vivo studies. However, expression profiles of both ErbB2 and Nrg1-III would point to a late function in the dedifferentiation process, rather than to one instructing it.

RA and RAR/RXR

The vitamin A metabolite retinoic acid (RA) is known to play pivotal roles during neuronal differentiation, and a maturation-promoting function has recently been revealed for oligodendroglial cells [54]. RA binds to the nuclear receptors RAR and RXR, enabling their association with retinoic acid response elements (RARE) and thereby regulating gene expression. In the peripheral nervous system, retinoic acid signalling pathway components, including retinaldehyde dehydrogenase (RALDH), retinoic acid receptors (RAR) and retinoic X receptors (RXR) are mainly expressed by Schwann cells of the uninjured nerve [55], and the RA receptor expression profile coincides with myelin gene expression [56]. Since RA receptor (RAR and RXR) expression was induced upon peripheral nerve injury [57] this suggests a function in the regeneration process and raises the question of whether it is also implied in peripheral nerve development. Interestingly, application of RA to DRG cocultures strongly inhibited myelin formation and led to a reduction of the expression of myelin-associated glycoprotein (MAG), while MPZ and myelin-basic protein (MBP) expression levels were increased [56]. RA treatment of DRG cocultures and Schwann cell cultures strongly induced Krox20 expression dependent on RXR, while inhibition of MAG was mediated by RAR. Although this implies that RA may exert conflicting effects, it could act in vivo on both receptors but at different stages, thus either promoting myelination via RXR activation or inhibiting it as a consequence of binding to RAR. Such a dual role suggests a balancing function and could be used to control myelin sheath thickness. Of note, the utility of RA as potential treatment has been assessed in preclinical models of diabetic and chemotherapy-induced polyneuropathy (CIPN) [58, 59]. In mice with streptozotocin-induced diabetes, the subcutaneous injection of all-trans RA restored reduced NGF serum and nerve levels and improved nerve regeneration [58]. Likewise, rats exposed to cisplatin or paclitaxel, two drugs frequently associated with CIPN, displayed a less severe sensory neuropathy upon treatment with all-trans RA [59]. These promising results were corroborated in a small, randomized, controlled trial in which all-trans RA improved electrophysiological measures in lung cancer patients during chemotherapy treatment [59].

IκBα and NFκB/p300

Nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) was demonstrated to be involved in peripheral nerve myelination [11]. NFκB is formed by homo- or heterodimers of structurally similar proteins, with p65/p50 being the most abundant heterodimer. NFκB expression peaks in premyelinating Schwann cells and declines thereafter, and its DNA-binding activity is stimulated by Nrg1-III, both via PI3K and ERK activation [60]. DRG cocultures prepared from p65 knockout mice displayed reduced in vitro myelination, suggesting an important function for p65 in myelin formation. This promoting effect was confirmed by SN50 peptide-mediated inhibition of NFκB and by overexpression of IκBα mutant (IκBm), which inhibits nuclear localization of NFκB p65/p50. In both cases, significant reduction of myelin formation in DRG cocultures was observed. IκBm-expressing Schwann cells were adjacent to but did not ensheath axons, suggesting that IκBα activity may stall Schwann cells at the premyelinating stage [11]. Therefore, it can be assumed that IκBα acts as an inhibitor of NFκB activity in Schwann cells, blocking differentiation and myelination. Of note, forced expression of IκBα and thus inhibition of NFκB transcriptional activation in Schwann cells in vivo did not impair Schwann cell developmental differentiation, but led to a delayed regeneration following crush injury of peripheral nerves [61], indicating a function rather in the regeneration process.

Interestingly, a recent report revealed that p65 activity is modulated by means of acetylation and deacetylation [62]. At

the onset of postnatal nerve development, p65 is associated with the histone acetyltransferase p300, leading to high levels of acetylated p65. During postnatal development, the p65/p300 interaction is interrupted and p65 interacts with deacetylases HDAC1 and HDAC2. This switch in the acetylation status of p65 is vital for peripheral nerve myelination, since HDAC1/2 double-knockout mice exhibited severe myelination deficits. These mutants contained Schwann cells that failed to form a 1:1 relationship with axons and displayed reduced expression of Oct6, Krox20 and myelin genes, as well as induced levels of differentiation inhibitors such as Sox2, c-Jun, and Id4. Whereas in wildtype sciatic nerves NFκB interacting with HDAC1/2 is recruited to the Sox10 promoter, recruitment of NFκB and p300 to the Id4 gene occurred in HDAC1/2 double-knockout mutants. This indicated that context dependent transactivation of different promoters by p65 either results in activation of myelination-promoting genes and repression of differentiation inhibitors or in repression of myelination-promoting genes, while inhibitors are activated. Hence p300-dependent acetylation of NFκB p65 inhibits Schwann cell maturation. In animals with streptozotocin-induced diabetes, which is a widely used model for diabetic neuropathy, NFκB activation is decreased in subsets of DRG neurons. These changes go along with a different intracellular localization of p65 and p50, which indicates that these subunits might be involved in the pathogenesis of diabetes-associated neuropathy [63].

Conclusions

Signals that promote and inhibit Schwann cell differentiation must act in concert in order to achieve and maintain proper myelination of axons. A misbalance, caused by abnormal activation of differentiation inhibitors, either causes myelination deficits or, once the myelin sheath is established, may lead to dedifferentiation of Schwann cells accompanied by loss of the myelin sheath. On the other hand, such inhibitory components might become increasingly important, considering inherited demyelinating diseases or Schwann cell plasticity in injured or diseased nerves. So, for example, hypermyelination as seen in CMT4B patients may result from a reduced activity of the Dlg1/PTEN brake in the control of myelin thickness [40]. Likewise, impaired regeneration of peripheral nerves can be expected when (re) expression of dedifferentiation signals such as c-Jun are interrupted or when differentiation-inhibitory factors cannot be inactivated or downregulated correctly [12]. Apparently it is not a single signalling cascade that inhibits premature axon myelination by Schwann cells or regulates glial dedifferentiation processes. Different extracellular stimuli, including Nrg1-III or activation of Notch receptors by axonally expressed ligands, regulate at least four different subtypes of intrinsic inhibitory determinants (Fig. 1). The first group consists of factors such as c-Jun, the intracellular domain of Notch receptor (NICD), Sox2, Krox24, NFκB/p300, LXR, and RAR; these either interact (directly/indirectly) with and repress myelin gene promoters or interfere with Schwann cell differentiation. The second group includes regulators that negatively interfere with the PI3K signalling pathway, such as Dlg1/PTEN and NO. A third group, including Ras, Raf, and ERK, can be defined along the ERK signalling cascade. Finally, a fourth group consists of inhibitory regulators that were shown to interfere primarily with morphological maturation, such as p57kip2, Dock7, and SSeCKS. Remarkably, the knowledge about the role of these inhibitory regulators in human neuropathic conditions is largely incomplete. Further studies in appropriate animal models and in humans clearly are warranted to determine to what extent these signalling cascades contribute to the pathogenesis of hereditary and acquired peripheral nerve disorders and whether misregulation of single members or of complete functional groups interferes with successful remyelination and regeneration. Given the fact that a significant percentage of the human population will be confronted at least once in their lifetime with a peripheral neuropathic condition, knowledge of such differentiation-inhibitory components and their pathophysiological relevance might provide a basis to develop novel therapeutic approaches in order to promote the endogenous PNS repair capacity.

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