Hedgehog Signaling in CNS Remyelination

Subjects: Developmental Biology

Contributor: Minxi Fang , Tao Tang , Mengsheng Qiu , Xiaofeng Xu

Remyelination is a fundamental repair process in the central nervous system (CNS) that is triggered by demyelinating events. In demyelinating diseases, oligodendrocytes (OLs) are targeted, leading to myelin loss, axonal damage, and severe functional impairment. While spontaneous remyelination often fails in the progression of demyelinating diseases, increased understanding of the mechanisms and identification of targets that regulate myelin regeneration becomes crucial. Several signaling pathways have been implicated in the remyelination process, including the Hedgehog (Hh) signaling pathway.

hedgehog signaling pathway oligodendrocytes remyelination

1. The Promotion of Remyelination by the Hedgehog(Hh) Signaling Pathway

1.1. The Contribution of the Hh Signaling Pathway in Myelination and Remyelination

There exist sequential waves of oligodendrocyte progenitor cells (OPCs) generation in vertebrates, firstly localized in the ventral progenitor domains and later in dorsal regions [1]. Although most generated OPCs differentiate into OLs and contribute to myelination, a small fraction of OPCs remain in a low-proliferative or quiescent state in the adult [2]. Cell-tracing experiments revealed that most adult OPCs are dorsally-derived in the forebrain [3]. After a demyelinating insult, these OPCs undergo activation, proliferation, migration, and differentiation until the formation of new myelin sheaths [4]. As mentioned before, NPCs from in the ventricular-subventricular zone (V-SVZ) also can generate OLs after demyelination [5][6][7][8].

The contribution of the Shh signaling pathway in the context of CNS demyelination and remyelination has been addressed by several groups. In addition to its early role in the induction of embryonic OPCs [9], Shh signaling is also implicated in the generation of postnatal OPC populations [10][11]. Exogenous Shh is able to increase the OPC population and premyelinating OLs in the adult forebrain [12]. Genetic cell-fate labeling experiments revealed that neural stem cells (NSCs) in the dorsal V-SVZ respond to Shh and generate OPCs that come to reside in the corpus callosum. These cells persist into adulthood and contribute to remyelination after Cuprizone-induced demyelination [13]. In a model of focal demyelination induced by lysophosphatidyl choline (LPC), the major components of the Hh signaling pathway (including Shh and Smo) were upregulated in the oligodendroglial cells in the area of a lesion. Further gain- and loss-of-function experiments demonstrated that Shh promotes the proliferation and differentiation

of OPCs and decreases astrogliosis and macrophage infiltration altogether, leading to the attenuation of the lesion extent during myelin repair [14].

The stimulation of Smo activity is compatible with the positive influence that Shh exerts on remyelination. For instance, the microinjection of SAG, a Smo agonist, into the corpus callosum of LPC-induced demyelinated mouse significantly increased OPC proliferation and enhanced remyelination [15], in accordance with the recent results in Cuprizone-induced demyelinating models [16]. Conversely, GDC-0449 (also referred to as Vismodegib), a specific Smo antagonist, has been reported to repress Gli-mediated transcription in different types of cells [17], significantly aggravating disease severity and increasing the extent of demyelination in the EAE model of demyelination [18].

Recently, another crucial component of the Hh signaling pathway, the type I transmembrane receptor Boc, was identified as a new regulator of myelin formation and repair [19]. During development, Boc forms a Shh receptor with Ptch1 and is necessary for the Shh-mediated proliferation of cerebellar progenitor cells [20]. The Boc-null mutant mice displayed delayed myelination, associated with a reduction in callosal axon diameter. In the context of demyelination induced by LPC injection, Boc was significantly up-regulated in the lesion. During myelin repair, Boc mutants exhibit aberrant OPC differentiation, reminiscent of the phenotypes observed after blockade of the Hh signaling pathway.

1.2. Identification of Clobetasol as a Smo agonist for Promoting Remyelination

Although the Hh/Smo signaling pathway possesses an important role in promoting remyelination, the development of related therapeutic strategies has been impeded by the lack of U.S. Food and Drug Administration (FDA)-approved Smo agonists. By using high-throughput screening for cells that express the Smo receptor, four FDA-approved drugs, clobetasol, halcinonide, fluticasone, and flucinonide, were identified as agonists of Smo [21]. These drugs have the capacity to bind Smo, promote the internalization of Smo, cause the activation of Gli factors, and increase the proliferation of neuronal progenitor cells. Meanwhile, several bioactive drugs have also been selected in phenotypic screens for their ability to promote MBP expression in different cell-based assays, including primary OPC cultures [22][23][24], mouse OPC cell lines such as Oli-neuM [25], and epiblast-derived OPCs [26]. These independent drug screens, performed with different libraries and OPC models, support Clobetasol as one of the top-ranking drugs in promoting OPC differentiation and myelin development.

Clobetasol is a member of the glucocorticoid family and is commonly used to treat a number of skin disorders [27]. It is a potential remyelinating agent that has been demonstrated to promote the differentiation of OPCs in vitro, as well as remyelination in vivo [28]. Najm et al. reported that Clobetasol, as a modulator of the glucocorticoid receptor, specifically promotes rapid myelination in organotypic cerebellar slice cultures, as well as in the CNS of postnatal mouse pups [26]. Systematic administration of Clobetasol resulted in a significant increase in newly differentiated OLs and enhanced myelin regeneration in the LPC-induced mouse models of focal demyelination. In an EAE mouse model of chronic progressive MS, an impressive reversal in disease severity was observed when Clobetasol was administrated at the peak of the disease. Furthermore, an assessment of the immune response demonstrated that Clobetasol was able to serve as a robust immunosuppressant in addition to inducing

remyelination [26]. In addition, Clobetasol enhanced OL production from human OPCs in vitro [26]. Neuromyelitis optica (NMO) is a CNS disorder that involves inflammation and demyelination of the spinal cord and optic nerve [29]. In a mouse model of NMO produced by an injection of an anti-AQ4 antibody, an intraperitoneal administration of Clobetasol significantly reduced the myelin loss and increased the number of myelinating OLs within the lesions [30]. Recent studies further demonstrated that Clobetasol significantly improved NSC survival and prompted the differentiation of NSCs into neurons and OLs while inhibiting astrocyte differentiation, providing a potentially novel mechanism underlying the therapeutic effect of Clobetasol in CNS-related disease [31].

Altogether, the identified Smo agonist Clobetasol might function in multiple cell types and act via a range of targets to promote myelin repair. Importantly, Clobetasol is able to pass through the blood–brain barrier, raising the exciting possibility that Clobetasol could advance to clinical trials for the currently unavailable chronic progressive phase of MS.

2. Negative Regulation of Myelination and Remyelination by the Hh Signaling Pathway

2.1. Inhibitory Effect on Myelination by the Hedgehog Signaling

In the transgenic mice that ectopically expressed Shh in the dorsal neural tube, spinal precursor cells were arrested in an undifferentiated state and exhibited elevated levels of proliferation [32]. Recently, The team discovered a stage-specific activity of Hh signaling in OL development and showed that persistent activation of Smo in OPCs inhibited their differentiation [33]. Thus, Smo-mediated Hh signaling appeared to robustly promote NPC or OPC proliferation and resulted in the inhibition of OPC differentiation and subsequent myelination during early developmental stages. This observation is in agreement with the blockade of myelin development by the Smo agonist, SAG [15]. Moreover, the fact that appropriate myelination during development requires down-regulation of Hh is consistent with the thin corpus callosum observed in patients with Gorlin syndrome [34]. This syndrome is associated with a mutation in the Hh receptor, Ptch1, that blocks the repression of Smo activity, allowing for the increased activation of Hh signaling. In summary, Smo-mediated Hh signaling has an apparent inhibitory effect on OPC differentiation and developmental myelination.

2.2. Down-Regulation of Gli1 during Myelination and Remyelination

In fact, Gli1, originally considered to be a reliable readout of Hh signaling activity, has proven to be detrimental during myelination and remyelination. During development, the genetic ablation of Gli1 in NPCs appeared to lead to precocious myelination [35]. Specifically, the inhibition of Gli1 through specific-inhibitor GANT61 in human iPSCs-derived neural stem cells (NSCs) resulted in the increased generation of OPCs. These GANT61-induced OPCs are more migratory, in agreement with the single-cell RNA sequencing that show up-regulated cytoskeletal reorganization pathways. The differentiated OLs were proven to be functional and able to generate compact myelin both in vitro and in vivo [36]. Thus, the inhibition of Gli1 in NSCs facilitates OPC generation and OL maturation during development.

In addition, the negative regulation of myelin regeneration by Gli1 was also reported recently. During the demyelination and remyelination processes, the expression of Gli1 appeared to be variable depending on the animal models that were used. When demyelination was induced in the corpus callosum by an injection of LPC, a relatively moderate transcription of Gli1 was seen in OPCs within the lesions [14]. In the EAE model, Gli1 transcription was up-regulated in OPCs and neurons immediately before EAE onset but down-regulated while the demyelination stage [37]. Concerning the Cuprizone model, it was noted that little to no up-regulation of Gli1 was observed in the demyelinated corpus callosum, primarily in the reactive astrocytes [16]. Importantly, fate-mapping experiments following Cuprizone-induced demyelination showed that a subset of SVZ-derived Gli1-expressing NPCs down-regulated Gli1 expression upon arrival to the lesion site [35]. Moreover, the inhibition of Gli1 expression in the Cuprizone model was found to amplify the recruitment of NPCs, promote the migration of OPCs to the demyelinated axons, and enhance remyelination [36][38]. Concomitantly, the pharmacological inhibition of Gli1 activity directly or indirectly improved the functional outcomes in the EAE model by promoting remyelination and neuroprotection in the spinal cord [35].

3. The Complex Involvement of Canonical and Non-Canonical Hedgehog Signaling Pathways in Remyelination

3.1. The Promotion of Remyelination via the Non-Canonical Pathway

As mentioned above, Smo is able to transduce Hh signaling via both canonical and non-canonical pathways [39][40][41][42][43][44]. In agreement with the findings that Gli1 inhibition by GANT61 improves remyelination [35], the non-canonical Smo agonist GSA-10 has been recently reported to promote remyelination [43]. GSA-10 was first identified through a Smo pharmacophore-based screen [45][46], and it belongs to a new family of Smo agonists that activate the non-canonical pathways associated with Gli1 inhibition [47]. In the Oli-neuM cell line, GSA-10 was a potent activator of OPC differentiation. Upon demyelination induced by LPC, it prompted the OPC recruitment toward the lesion area without enhancing their proliferation. Notably, GSA-10 displayed the ability to promote OL maturation up to the stage of engaging artificial axons [43]. In conclusion, non-canonical Hh signaling is able to promote remyelination until axon engagement, representing a novel potential therapeutic target. Thus, together with the remyelinating effects described for the other small molecules binding Smo, the conspicuous remyelinating effects of GSA-10 support the idea that different Smo agonists can activate distinct signaling pathways presumably by activating Smo at different sites [42][45][46]. Interestingly, Smo activation by GSA-10 led to Gli2 upregulation, which is consistent with the recent report that ablation of Gli1 increased the expression of Gli2 in NPCs following Cuprizone-induced demyelination [38]. In the same line, Sox17 has also been found to induce OL regeneration in demyelinated areas through an increase in Shh/Smo/Gli2 activity [48], further supporting the importance of Gli2 upregulation for the differentiation program under Gli1 downregulation.

3.2. Hh Signaling Modulation Controls Local Inflammatory Cells

Under repairing conditions, inflammatory cells in the affected regions, including astrocytes and microglia, are endowed with beneficial or deleterious properties, promoting or impairing the endogenous capacity of OPCs to

induce spontaneous remyelination after myelin loss [49][50]. Therefore, astrocytes and microglia are becoming additional targets for assessing remyelinating properties afforded by small molecules. The observation that SAG was able to promote OPC differentiation in the context of demyelinated lesions was unexpected given its ability to also promote OPC proliferation [15][16]. During spontaneous remyelination occurring after LPC-induced demyelination, the Smo receptor is up-regulated in OLs and microglia but at a reduced level in astrocytes. Upon demyelination, SAG might promote the differentiation of OPCs indirectly by influencing microglia, inducing the expression of anti-inflammatory markers. This potential mechanism is supported by the previous report that microglia were shifted to an anti-inflammatory phenotype that could direct OL differentiation during remyelination [51]. Consistently, the conditional removal of Smo from microglia resulted in a dramatic decline of differentiated OLs, suggesting that Smo is cell-autonomously required for the response of microglia to a demyelinating event and that the pro-differentiating activity of SAG is related to its influence on microglia. Although GFAP expression did not appear to be regulated by SAG, the selective up-regulation of Smo in astrocytes in the LPC models also raises the possibility that its pro-differentiating activity might be mediated by specific subsets of astrocytes.

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