Molecular control of Schwann cell migration along peripheral axons

Keep moving!

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The development of the peripheral nervous system (PNS) is a highly dynamic process, during which motor and sensory axons innervate distal targets, such as skeletal muscles and skin. Axonal function depends critically on support from Schwann cells, the main glial cell type in the PNS. Schwann cells originate from the neural crest, migrate along outgrowing axons and associate with axons along their entire length prior to ensheathment or myelination. How axonal growth and the migration of Schwann cells is coordinated at the level of reciprocal axon-glial signaling is the fascinating subject of ongoing research. Neuregulin-1 (NRG1) type III, an axonal membrane-bound ligand for receptor tyrosine kinases of the ErbB family, acts as a “master regulator” of peripheral myelination. In addition, NRG1-ErbB signaling directs the development of the Schwann cell lineage and regulates the proliferation and survival of Schwann cells. Studies in zebrafish have identified a direct role of NRG1 type III in Schwann cell migration, but to what extend NRG1 serves a similar function in the mammalian PNS is not clear. We have employed a mouse superior cervical ganglion explant culture system, in which the migration of endogenous Schwann cells along outgrowing axons can be visualized by time-lapse imaging. Using this approach, we found that NRG1 type III-ErbB signaling regulates the colonization of distal axonal segments by Schwann cells. However, our data suggest an indirect effect of NRG1 type III-ErbB signaling via the support of Schwann cell survival in proximal axonal regions rather than a direct effect on Schwann cell motility.

Development of the Schwann Cell Lineage Involves Extensive Migration

Schwann cells are the main glial cell type of the peripheral nervous system (PNS). They derive from pluripotent neural crest cells, which emerge at the dorsal neural tube, from where they migrate ventrally to sites of gliogenesis in dorsal root ganglia (DRG) and nerve trunks. Following glial specification, Schwann cell precursors (SCP) proliferate and populate nascent nerves by migration in close contact with outgrowing axons. Early postnatally, Schwann cells associated with small-diameter axons mature into “non-myelinating” Schwann cells that ensheath groups of small-caliber axons, whereas Schwann cells in contact with large-diameter axons differentiate into myelinating Schwann cells. Thus, to myelinate (or ensheath) axons along their entire longitudinal axis, matching numbers of Schwann cells must extensively migrate from nerve roots over considerable distances to the periphery.1,2 Besides nerve development, migratory activity of Schwann cells is also an important aspect of remyelination after nerve injury,3,4 and tumor formation,5 yet the cellular and molecular processes that control Schwann cell migration during development, repair and neoplasia are not fully understood.
**Molecular Signals that Control Schwann Cell Migration**

Directed cell migration requires exposure to gradients of chemical cues (chemotaxis) and/or vectored substrates (e.g., axons) that provide migration “corridors.” Using in vitro assays such as the “scratch assay,” “Boyden assay” or “Varani migration assay,” several signaling molecules have been identified that promote the motility and migration of Schwann cells. These include neurotrophins, integrins, erythropoietin and GDNF (glial cell line derived neurotrophic factor). Of particular importance for many aspects of Schwann cell development is a family of EGF-like domain containing signaling molecules encoded by the Nrg1 gene. Due to differential promoter usage and alternative splicing multiple transmembrane and soluble NRG1 variants exist, all of which act as ligands for glial transmembrane receptor tyrosine kinases of the ErbB family. A soluble NRG1 variant (glial growth factor, GGF) increases the motility and directed migration of primary Schwann cells, independent of their mitotic activity. Already the soluble EGF domain of NRG1 (“NRG1β”) is sufficient to promote Schwann cell motility in vitro (in a “scratch assay”), a function that requires activation of the MAP kinase pathway. In addition, “NRG1β” enhances the migration of peripheral nerve sheath tumor cells (which derive from the Schwann cell lineage) suggesting a role for NRG1 signaling in tumor cell invasion. The principal NRG1 receptor in Schwann cells is an ErbB2-ErbB3 heterodimer, which is essential for normal Schwann cell development. ErbB2 promotes the migration of rat primary Schwann cells in vitro (in a “Boyden assay”) through activation of the GTPases Rac1 and Cdc42. Moreover, ErbB2 and ErbB3 receptors are crucial for Schwann cell migration along the posterior lateral line (PLL) nerve in zebrafish. Furthermore, in ErbB2 mouse mutants, SCP are present in DRG, but their ability to migrate is decreased. The axonal membrane-bound type III variant of NRG1 serves as a master regulator of peripheral nerve myelination. Recently, NRG1 type III was also identified as a critical regulator of Schwann cell migration in peripheral nerves of zebrafish and the rodent sympathetic nervous system. Together these findings identify an important role for NRG1-ErbB signaling during Schwann cell migration in vivo.

**A PNS Tissue Explant Assay for the Study of Schwann Cell Development**

The zebrafish (Danio rerio) is a suitable vertebrate model organism with which to monitor dynamic developmental processes, such as Schwann cell migration, in vivo. The main advantages of zebrafish embryos are that their development is extra-uterine and they appear transparent during early developmental stages. In contrast, mouse embryos are not accessible for in vivo time-lapse imaging and must be explanted and cultured ex utero for time-lapse imaging of developmental processes. In addition, the intransparent embryo hinders imaging of cellular dynamics at deeper locations in the embryo. To circumvent some of these restrictions, we recently adapted a tissue explant assay. To this end we used superior cervical ganglia (SCG) explants from mouse embryos cultured on a collagen gel matrix. Axon outgrowth from sympathetic neurons within the ganglia can be induced by treatment with nerve growth factor (NGF). Endogenous Schwann cell progenitors then migrate from the explant along these growing axons toward the periphery. Thus, this migration assay provides outgrowing axons, the physiological “substrate” for migratory Schwann cells, which can be monitored “on route” for long time periods by time-lapse imaging. A similar assay using DRG explants was established by Gumy and colleagues and may likewise be adapted for time-lapse imaging.

In order to study NRG1 functions during Schwann cell migration in our SCG explant assay, we blocked ErbB signaling with a chemical inhibitor. When ErbB signaling was inhibited at day in vitro 3 (DIV3), a time when large numbers of Schwann cells are actively migrating along sympathetic axons, the distance from the explant to the most distal Schwann cell was significantly reduced (to 2/3 of control levels). Thus, blocking ErbB receptor signaling slows the colonization of distal axonal regions by Schwann cells. In addition, ErbB inhibition also strongly reduces Schwann cell proliferation. We thus investigated whether direct inhibition of Schwann cell proliferation similarly affects the colonization of distal axonal segments. However, when we blocked Schwann cell proliferation with a DNA polymerase inhibitor (aphidicolin), we observed only a modest reduction in the distance Schwann cells traveled (by 15%). Thus, we propose that Schwann cells can migrate along sympathetic axons even when proliferation is inhibited, similar to findings in the zebrafish PNS. In addition to reduced proliferation, we also observed increased Schwann cell apoptosis when ErbB signaling was inhibited. This is in line with earlier findings in the sciatic nerve. Unexpectedly, when apoptosis was blocked with a caspase inhibitor in the presence of ErbB inhibitor, Schwann cell migration was restored. Thus, we conclude that ErbB signaling indirectly affects Schwann cell migration by controlling the survival of Schwann cells in proximal axonal regions.

Consistent with this idea, we observed many stalling Schwann cells after ErbB inhibition. We hypothesize that due to Schwann cell apoptosis in proximal regions, distally located Schwann cells lose crucial cell-cell contacts to neighboring Schwann cells and therefore stall or even move “backwards” to contact more proximal Schwann cells (Fig. 1, middle part) leaving distal axonal regions uncovered (red dotted line in Fig. 1, middle part). Stalling and “backwards” movement of Schwann cells were also observed along the lateral line in zebrafish when ErbB signaling was blocked, however, this occurred in the absence of increased apoptosis (Fig. 1, lower part).

In mutant zebrafish lacking NRG1 type III, the number of Schwann cells associated with the posterior lateral line nerve is severely reduced, whereas pan-neuronal overexpression in CNS neurons was sufficient to direct ectopic Schwann cell...
migration into the spinal cord. In an attempt to identify the responsible axonal ligand in mice, we identified the NRG1 type III variant as the most prominently expressed NRG1 isoform in SCG explants. When we examined SCG explants from NRG1 type III deficient mice, axonal outgrowth was unaltered, but the number of migrating Schwann cells was strongly reduced. As a consequence, distal regions of NRG1 type III-deficient axons almost completely lacked Schwann cells. Importantly, since Schwann cells were present in the vicinity of NRG1 type III-deficient SCG explants, NRG1 type III appears not to be essential for the initial production of SCPs per se. We conclude that NRG1 type III is the responsible axonal ErbB ligand in mammals, which regulates the colonization of sympathetic axons by Schwann cells.

**Perspectives**

Recent studies in zebrafish and the mouse sympathetic nervous system strongly suggest that axonal NRG1 type III signaling through glial ErbB2/3 receptors promotes Schwann cell migration along peripheral axons. However, several aspects of Schwann cell migration require further investigations. In general, a more detailed (time-lapse) analysis of Schwann cell migration (including stalling and backward movements) is required to provide further insights into cellular dynamics during migration. In addition to axon-glial interactions, it will be of particular interest to determine the role of contacts between migrating Schwann cells.

In addition, it is unclear whether (axonal) NRG1 serves as a true chemoattractant during directed murine Schwann cell migration in vivo, as observed for soluble NRG1 in vitro. A chemoattractive function of NRG1 has recently been described also for oligodendrocyte precursors and interneurons in the mouse brain. To this end the regional presentation and the exact structure of “active” axonal NRG1 type III during Schwann cell migration is an important research topic. A hypothetical scenario is that a gradient of “active” NRG1 type III is linked to axonal elongation, such that it precedes migrating Schwann cells (Fig. 1,

![Figure 1](image-url)
Importantly, the function of axonal NRG1 during migration might be further modulated by interactions (in cis or trans) with other signaling molecules, such as neurotrophins \(^{30,31}\) (Fig. 2).

NRG1-ErbB signaling is involved in all aspects of Schwann cell development, (i.e., proliferation, survival, migration, myelination and remyelination). Most likely, multiple signaling cascades are employed in Schwann cells during these processes (Fig. 2), and it is of great interest to reveal how the respective NRG1 signal is transferred to distinct signaling effectors downstream of glial ErbB2/3 receptors and to what extent the dosage of “active” NRG1 determines the “choice” of a particular signaling cascade (Fig. 2). These and related questions can be addressed in the future with the SCG explant migration assay using in vivo reporters, e.g., for proliferation or cell death \(^{32-34}\) in combination with time-lapse imaging.

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