PERSPECTIVE

# Soluble neuregulin-1 (NRG1): a factor promoting peripheral nerve regeneration by affecting Schwann cell activity immediately after injury

Neuregulin-1 (NRG1) is a well known growth factor playing contradictory roles in myelination depending on the existing isoform. Transmembrane NRG1 acts as a promyelinating factor, while the soluble isoform inhibits myelination. In this perspective, we would like to emphasize this conflicting role played by NRG1 isoforms regarding their roles in myelination, remyelination and the entire process of peripheral nerve regeneration.

NRGs belong to a family of growth factors encoded by four different genes (NRG1–4); among them, NRG1 and its generated isoforms are the most well studied owing to their involvement in the developmental stages of several different systems including nervous, cardiac and muscular systems. NRG1 is a complex gene comprised of multiple promoters and several exons, consequently up to 30 different NRG1 isoforms can be produced by alternative splicing (Mei and Xiong, 2008). NRG1 mainly exists in two forms based on the N-terminal motif, either a transmembrane or a soluble form. A core epidermal growth factor like domain that is responsible for NRG1 binding to and activation of ErbB receptors is common to all isoforms. Most of the soluble NRG1 isoforms contain an additional immunoglobulin like (Ig) domain in their N-terminus, thus are called "Ig-NRG1". These isoforms can be synthesized either as a soluble ligand released in the extracellular environment or as a single pass transmembrane anchored precursor which needs to be further processed by metalloproteases to release a soluble ligand acting in a paracrine or autocrine manner.

Transmembrane NRG1 isoforms lack the Ig domain, but possess a cysteine rich domain (CRD) in their N-terminus, hence are called "CRD-NRG1". Because CRD contains an additional transmembrane domain, precursors of these isoforms pass the cell membrane twice, thus needing a proteolytic cleavage to signal in a juxtracrine manner. NRG1 acts by binding to ErbB3 and ErbB4 receptors, which belong to the ErbB family of tyrosine kinase receptors. They are involved in many cellular processes such as proliferation, growth, migration, adhesion, differentiation and survival (Mei and Xiong, 2008).

As mentioned above, in the peripheral nervous system different NRG1 isoforms play different roles (Fricker and Bennett, 2011): transmembrane NRG1 isoforms are expressed by myelinating axons, while soluble NRG1 isoforms are expressed by Schwann cells immediately after injury. These NRG1 isoforms, signaling respectively in a juxtacrine or autocrine manner, play opposite roles, giving to Schwann cells opposite messages: while axonal transmembrane NRG1 during development and after injury promotes myelination and remyelination, soluble NRG1 expressed by Schwann cells immediately after injury, inhibits myelination genes and promotes Schwann cell survival and dedifferentiation. Furthermore, it has been shown that axonal transmembrane NRG1 negatively affects the expression of soluble NRG1 in Schwann cells (Stassart et al., 2013): when, following nerve injury, axon-Schwann cell interaction is interrupted, the interaction between axonal transmembrane NRG1 and glial ErbB receptors is lost and soluble NRG1 transcription is switched on.

Myelination fate of Schwann cells is determined by the amount of the expressed transmembrane NRG1 on the axonal surface: an axon diameter > 1 μm expresses the adequate NRG1 threshold for stimulating myelination. It has been found that the transmembrane NRG1 knock-out mice show poor nerve ensheathment and myelination (Michailov et al., 2004; Taveggia et al., 2005), while the overexpression of transmembrane NRG1 in transgenic mice renders the typically un-myelinated sympathetic neurons myelinated (Taveggia et al., 2005). The pro-myelinating activity of transmembrane NRG1 is not exclusively dependent on its molecular level of expression, but also on the proteolytic cleavage that NRG1 precursor is subjected to, which can be mediated either by β-secretase 1 (BACE, promoting myelination) or tumor necrosis factor-α-convertase (TACE, inhibiting myelination) (Taveggia, 2016). Thus, the modulation of different protease activities could be an encouraging therapeutic strategy to promote axon remyelination following a nerve injury, as direct transmembrane NRG1 administration is not applicable, since its expression has to be axonal and would require virus transduction of sensitive neurons localized in the dorsal root ganglia and motor neurons localized in the ventral root of the spinal nerve.

In the healthy nerve, myelinating Schwann cells are highly differentiated, but in response to injury they exhibit a great capacity of plasticity, and transdifferentiate into a repair phenotype (Jessen and Mirsky, 2016). Repair Schwann cells support nerve regeneration by proliferating and organizing themselves in a tubular structure called "Büngner bands", which acts as a scaffold upon which the regenerating axon can grow and direct its path to reinnervate the target organs. Later, when the axon regeneration is successfully accomplished, Schwann cells redifferentiate into a myelinating phenotype and remyelinate regenerated peripheral axons (**Figure 1**).

Changes in gene expression in response to nerve injury were addressed by Stassart et al. (2013) and Ronchi et al. (2016). The expression analysis of different soluble NRG1 isoforms demonstrates that it strongly increased immediately post-nerve injury, followed by an increase of NRG1 co-receptors ErbB2 and ErbB3.

To further investigate the role of soluble NRG1 in Schwann cells, we analyzed the regulated genes at 6 hours following soluble NRG1 stimulation in an *in vitro* adult Schwann cell model (El Soury et al., 2018). Our data showed that, at 6 hours post stimulation, NRG1 down-regulates several genes involved in myelination, Schwann cell differentiation and apoptotic processes. Since these are the changes required *in vivo* for Schwann cell transdifferentiation, it can be suggested that NRG1 plays a significant role in this process in the injured nerve environment (**Figure 1**). Moreover, the comparison of our *in vitro* data with *in vivo* transcriptomic data on injured nerves obtained by other groups had shown that several genes are overlapping. The immediate up-regulation of soluble NRG1 could play a pivotal role in the early stage following peripheral nerve injury. Using conditional knock-out it has been shown that nerve remyelination is strongly impaired when soluble NRG1 expressed by Schwann cells is lacking (Stassart et al., 2013). We hypothesize that soluble NRG1 affects the process of remyelination only indirectly: soluble NRG1 itself is not directly implied in the remyelination process, but it is directly involved in Schwann cell transdifferentiation, a limiting step for Wallerian degeneration and subsequent nerve regeneration.

The expression of soluble NRG1 in the distal nerve stump is strongly increased immediately after mild injury, such as crush or transection followed by end-to-end repair (Ronchi et al., 2016). When the nerve lesion is severe and is accompanied by substance loss, autografts are considered the gold standards in bridging the two nerve stumps. Unfortunately, there are numerous drawbacks related to this technique as secondary inju-

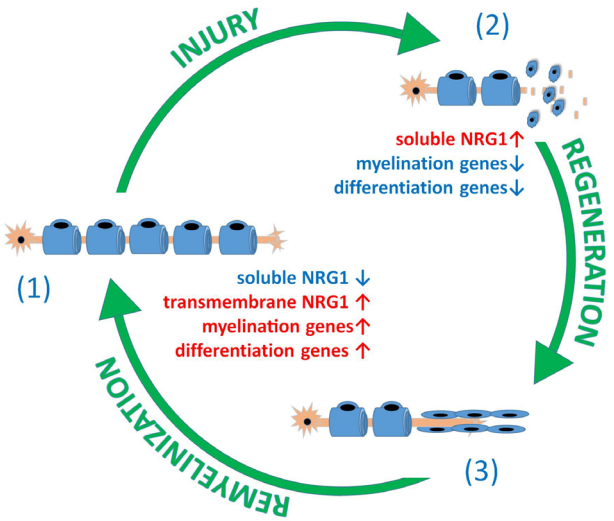


**Figure 1 Schematic representation of the hypothetical role of soluble neuregulin1 (NRG1) in Schwann cell transdifferentiation.**
*In vitro* experiment demonstrates that Schwann cell stimulation with soluble NRG1 down-regulates genes involved in myelination and differentiation. We hypothesize that *in vivo*, in response to nerve injury, the immediate increase of soluble NRG1 might contribute to the process of Schwann cell transdifferentiation from a myelinating Schwann cell to a repairing Schwann cell, through the down-regulation of genes involved in myelination and differentiation in the distal nerve portion. During the axonal regeneration, the interaction between Schwann cells and axonal transmembrane NRG1, and the concomitant down-regulation of soluble NRG1, might stimulate the expression of myelination and differentiation genes, thus promoting the remyelination process. In the figure, the healthy nerve (1), the injured nerve undergoing Wallerian degeneration (2), the regenerating nerve with Schwann cells organized in Büngner bands guiding axon regrowth (3) and the regenerated nerve (1) are shown. Soluble NRG1 is released by Schwann cells, transmembrane NRG1 is expressed by neurons in the axons. Up-regulated genes in the distal nerve portion are represented in red, down-regulated genes in blue.

ries, donor site morbidity and loss of sensitivity. Tubulization technique is considered a promising alternative to autografts. Nevertheless, while soluble NRG1 level increases immediately when using autografts to repair nerve injury, produced by the autograft Schwann cells, it takes up to 2 weeks in a hollow tube to reach the same NRG1 levels (Ronchi et al., 2018) which is likely the time required for Schwann cells to colonize the hollow tube. Several attempts have been made to improve the tubulization technique and its outcomes. One of them is enriching the hollow tube with soluble NRG1 or other growth factors.

Data obtained by different research groups and us suggest that the delivery of recombinant soluble NRG1 should be finely regulated and restricted to an early time window, to promote Schwann cell dedifferentiation and survival, while in later stages of regeneration and remyelination soluble NRG1 levels should be reduced, to avoid remyelination inhibition.

The observation that soluble NRG1 promotes demyelination *in vitro* (Zanazzi et al., 2001), and that it is dramatically over-expressed *in vivo* in peripheral nerves of chronic demyelinating neuropathy rats (Fornasari et al., 2018), further suggests that soluble NRG1 could be deleterious if supplied in the inappropriate time-window.

To conclude, NRG1 isoforms could be a promising therapeutic target for peripheral nerve regeneration: soluble NRG1 should be supplied immediately and transiently after injury, to promote Schwann cell survival and dedifferentiation, while transmembrane NRG1 could be activated later, acting on BACE or TACE as discussed above, to further promote remyelination. As soluble NRG1 and activators of transmembrane NRG1 might have negative side effects when supplied in the incorrect time window, further studies will be necessary to better define doses and timing of administration.

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