

● PERSPECTIVES

Neuregulin 1 isoforms could be an effective therapeutic candidate to promote peripheral nerve regeneration

Traumatic injuries of peripheral nerves represent common casualties and their social impact is considerably high. Although peripheral nerves retain a good regeneration potential, the clinical outcome after nerve lesion is far from being satisfactory and functional recovery is almost never complete, especially in the case of large nerve defects, that result in loss or diminished sensitivity and/or motor activity of the innervated target organs. Therefore, to improve the outcome after nerve damage, or in peripheral neuropathies, there is a need for further research in nerve repair and regeneration to identify factors that promote axonal regrowth, remyelination and target reinnervation.

Among the different factors involved in these processes (Taveggia et al., 2010; Pereira et al., 2012), stands out neuregulin 1 (NRG1), a factor which plays a role both in the myelination occurring during development (Lemke, 2006) and in the response to peripheral nerve injury (Syed and Kim, 2010; Fricker and Bennett, 2011).

NRG1 is a pleiotropic factor characterized by the existence of numerous isoforms arising from alternative splicing of exons that confer to the protein with deeply different characteristics (Falls, 2003; Mei and Xiong, 2008).

NRG1 can be produced as a secreted or as a transmembrane protein ready to interact with its receptor, or as a transmembrane pro-protein that needs a proteolytic cleavage to release a soluble fragment or to protrude its receptor binding domain in the extracellular environment (**Figure 1**). According to its structure, NRG1 signals in a paracrine, autocrine or juxtacrine manner; moreover, juxtacrine interactions can signal both in a forward and reverse manner due to the production of a fragment containing the intracellular domain (ICD, **Figure 1B**) that can translocate into the nucleus and influence gene transcription (Bao et al., 2003; Bao et al., 2004; Chen et al., 2010). NRG1 interacts directly with two of the four members of the tyrosine kinase receptor family ErbB: ErbB4, that signals as homo or heterodimer, and ErbB3, that forms a heterodimer with ErbB2.

In the peripheral nervous system, NRG1 soluble isoforms are mainly released by Schwann cells, while transmembrane isoforms are mainly expressed by the axon and both interact with the heterodimer receptor ErbB2-ErbB3, generally expressed by Schwann cells. NRG1 plays an important role both in the myelination occurring during development and in the different phases occurring after injury in the peripheral nerve: axon degeneration, axon regrowth, remyelination and target reinnervation (Taveggia et al., 2010; Fricker and Bennett, 2011; Pereira et al., 2012; Salzer, 2012; Gambarotta et al., 2013; Heermann and Schwab, 2013).

These processes respond to different cues, as can be inferred from the analysis of transgenic mice models summarized in **Figure 2**. The difference between the myelination process occurring during development and the regeneration process occurring after nerve injury is underlined by the fact that soluble NRG1 isoforms play an important role after nerve injury, while their lack seems irrelevant during development.

Membrane bound NRG1 determinates the myelination fate during development

During development, the absence of soluble NRG1 in Schwann cells does not affect myelination (Stassart et al., 2013) and, accordingly, soluble NRG1 over-expression in motoneurons and dorsal root ganglia (DRG) neurons does not influence myelination (Michailov et al., 2004).

Conversely, axonal transmembrane NRG1 expression level determines the myelination fate of axons and the thickness of the myelin sheath: animals lacking axonal transmembrane NRG1 show hypo-

myelination (Michailov et al., 2004; Taveggia et al., 2005), while its over-expression causes hypermyelination (Michailov et al., 2004) and conversion of normally non-myelinated neurons to myelinated neurons (Taveggia et al., 2005).

Soluble and membrane bound NRG1 play different roles after peripheral nerve injury

Animals lacking soluble NRG1 in Schwann cells display peripheral nerve regeneration severely impaired (Stassart et al., 2013). Accordingly, soluble NRG1 over-expression in motoneurons and dorsal root ganglion neurons improves remyelination after injury (Stassart et al., 2013).

Immediately after injury, we (unpublished results) and others (Stassart et al., 2013) observed that the soluble NRG1 transcript is strongly upregulated in the distal and proximal nerve. Because RNA extracted from the nerve belongs mainly to Schwann cells, this observation suggests that Schwann cells, following nerve injury, produce high amounts of soluble NRG1 that could stimulate, in an autocrine manner, Schwann cell survival and, likely, migration of macrophages that remove myelin debris in the early phases of Wallerian degeneration to allow remyelination (Fricker and Bennett, 2011).

The soluble NRG1 upregulation observed in Schwann cells immediately after nerve injury suggests that denervated Schwann cells require autocrine stimulation with soluble NRG1 for survival and that the peripheral nerve regeneration impairment observed in animals lacking Schwann cell derived soluble NRG1 is the indirect consequence of problems occurring during the early phases of axon degeneration and axon regrowth, not during the following phases of remyelination and target reinnervation.

Animals lacking axonal transmembrane NRG1 isoforms show an impaired rate of remyelination and functional recovery at early phases after nerve injury; at later stages, the myelination thickness is not strictly dependent on axonal NRG1 and it has been hypothesized a compensation effect mediated by other factors (Fricker et al., 2013). Accordingly, axonal transmembrane NRG1 over-expression improves peripheral nerve regeneration (Stassart et al., 2013).

Strategies to promote nerve regeneration

These observations suggest that soluble NRG1 plays a role during the early phases following nerve injury corresponding to axon degeneration and regrowth, while transmembrane NRG1 plays a role during later phases corresponding to the remyelination process. Therefore, soluble NRG1, already used in human trials for heart failure treatment, could be an effective therapeutic candidate to promote nerve regeneration. Accordingly, it has been already demonstrated that nerve regeneration is successfully promoted by subcutaneous NRG1 injection (Chen et al., 1998; Yildiz et al., 2011), by NRG1 released by biomaterials (Mohanna et al., 2003; Cai et al., 2004; Mohanna et al., 2005) or by adenovirus encoded NRG1 (Joung et al., 2010). Moreover, it has been suggested that NRG1 is released by the degenerating muscle successfully used to fill a non-nervous conduit graft consisting of a vein to bridge the proximal and the distal stumps after substance loss (Nicolino et al., 2003).

However, we think that treatment with recombinant soluble NRG1 should be carried out in a well-defined time window, during early phases following nerve injury, to improve survival, migration and redifferentiation of Schwann cells, in synergy with endogenous NRG1 released by Schwann cells immediately after injury, that in cases of severe damage may not be sufficient.

Furthermore, NRG1 treatment should be finely regulated, because it has been demonstrated *in vitro* that different NRG1 isoforms have different pro-myelinating activities and a too high concentration can inhibit myelination (Syed et al., 2010).

A second strategy to promote myelination could be the over-expression of recombinant transmembrane NRG1 in axons during later phases following nerve injury. However, to express transmembrane isoforms, the use of viral vectors would be necessary; to bypass this critical step, manipulation of the processing of endogenously expressed NRG1 could increase its pro-myelinating activity. Actually,

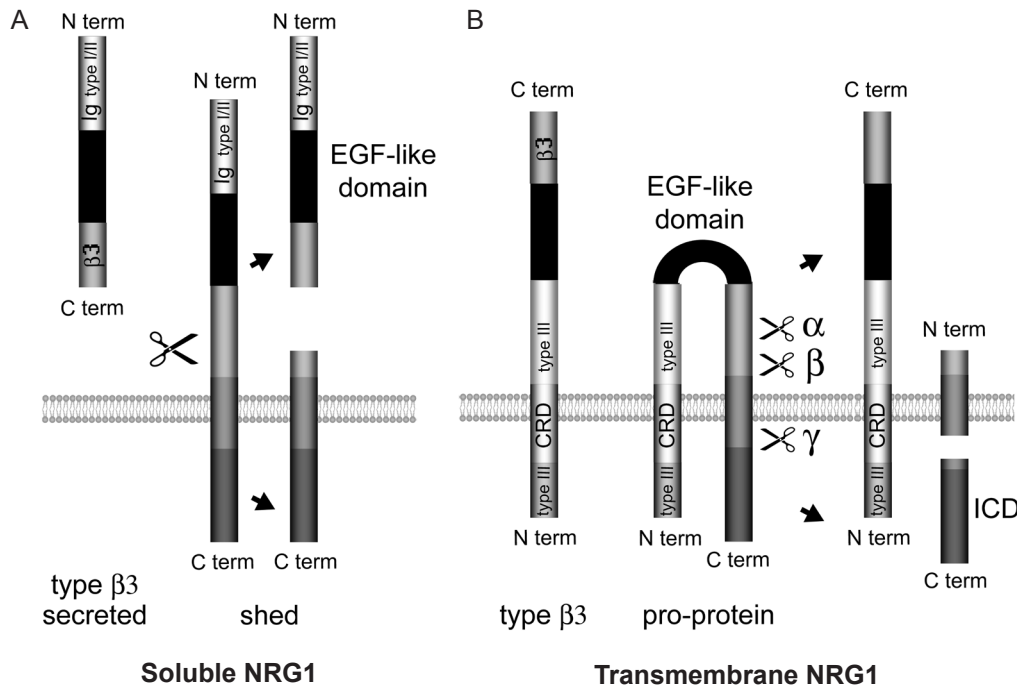


Figure 1 Structure of soluble or transmembrane neuregulin 1 (NRG1) isoforms.

NRG1 isoforms are tissue-specifically expressed and are classified into types I–VI according to their N-terminus domain, and into types α/β , 1–4, a–c, according to their C-terminus domains, as previously shown (Falls, 2003; Mei and Xiong, 2008). To simplify, in this figure only soluble (type I–II) and transmembrane (type III) NRG1 isoform structures are shown, because type IV–VI role in the peripheral nerve is still not known. All NRG1 isoforms contain an epidermal growth factor (EGF)-like domain (here in black). Panel A: Soluble NRG1 contain a type I or type II N-terminal domain, an immunoglobulin (Ig)-like domain and can be expressed as secreted proteins released in the extracellular environment (type β) or as transmembrane pro-proteins susceptible to proteolytic shedding and consequent release of the soluble ligand in the extracellular environment. Panel B: Transmembrane NRG1 are characterized by the presence of a type III N-terminal domain, containing a cysteine rich domain (CRD) and can be expressed with the EGF-like domain ready to interact with receptors (type β) or as transmembrane pro-protein that requires a proteolytic cleavage to expose the EGF-like domain towards receptors. These isoforms can be cleaved by either the α secretase TACE or the β secretase BACE1, and by a γ secretase that releases an NRG1 intracellular domain (ICD).





	NRG1	Developmental myelination	Peripheral nerve regeneration
Soluble	Knockout 	+	-
	Overexpression 	+	++
Transmembrane	Knockout 	-	-
	Overexpression 	++	++

Figure 2 The role played by soluble and transmembrane neuregulin 1 (NRG1) isoforms in the myelination occurring during development and in the different phases occurring after nerve injury (axon degeneration, axon regeneration, remyelination and target reinnervation) as inferred from transgenic and conditional knockout mice.

The absence of soluble NRG1 in Schwann cells does not affect myelination during development (+), but nerve regeneration is severely impaired (-) (Stassart et al., 2013). The over-expression of soluble NRG1 in motoneurons and dorsal root ganglia neurons does not affect myelination (+) during development (Michailov et al., 2004), but improves nerve regeneration after injury (++) (Stassart et al., 2013). Animals lacking axonal transmembrane NRG1 show hypomyelination (-) (Michailov et al., 2004; Taveggia et al., 2005) and an impaired rate of peripheral nerve regeneration (-) (Fricker et al., 2013). Animals over-expressing axonal transmembrane NRG1 show hypermyelination (++) (Michailov et al., 2004; Taveggia et al., 2005) and an improvement of peripheral nerve regeneration (++) after nerve injury (Stassart et al., 2013).

transmembrane NRG1 can be cleaved by different metalloproteases, including the α secretase TACE (also known as ADAM17) and the β secretase BACE1, and other not yet identified proteases, that cleave the transmembrane NRG1 in the same stalk region, leaving the EGF-

like domain exposed and C terminal domains that differ by a few amino acids (Figure 1).

The effect on myelination of these proteases seems to be opposite: the β secretase BACE1 cleavage activates the pro-myelinating activity

of NRG1, as shown in BACE1 knockout mice characterized by an hypo-myelination phenotype (Willem et al., 2006; Hu et al., 2008) and in transgenic mice over-expressing a recombinant NRG1 mimicking the BACE1 cleavage, characterized by an hyper-myelinated phenotype (Velanac et al., 2012). It would be interesting to analyze the remyelination efficiency in these mice, to understand if BACE1 plays a role only during developmental myelination or also during remyelination occurring after peripheral nerve injury and repair. However, a pro-myelinating strategy including the treatment with BACE1 stimulators, if any, would not be recommended, because BACE1 is a major drug target for Alzheimer's disease: BACE1-mediated cleavage of amyloid precursor protein (APP) is the first step in the generation of the pathogenic amyloid- β peptides and recent studies demonstrate a wide range of BACE1 physiological substrates and functions (Vassar et al., 2014).

Conversely, the α secretase TACE cleavage inhibits the pro-myelinating activity of NRG1 and its inactivation in motor neurons—obtained through conditional knockout mice—correlates with a hyper-myelination phenotype during development and in the adult (La Marca et al., 2011). No data concerning remyelination efficiency following peripheral nerve injury in mice in which TACE is inactivated or inhibited by pharmacological treatments are available and it would be really useful to test if TACE inactivation promotes remyelination during peripheral nerve regeneration.

Different TACE inhibitors are already available and used in preclinical trials anti rheumatoid arthritis and anti breast cancer (DasGupta et al., 2009; Rego et al., 2014) and could be useful tools to promote remyelination.

Because regeneration is spontaneous, but often incomplete, the development of new strategies to promote peripheral nerve regeneration is a significant goal to achieve, and the pleiotropic NRG1 isoforms appear to be good candidates for therapeutic treatments.

Giovanna Gambarotta^{1,2}, Giulia Ronchi^{1,3}, Stefano Geuna^{1,2,3}, Isabelle Perroteau^{1,2}

1 Department of Clinical and Biological Sciences, University of Turin, Italy

2 Neuroscience Institute of Turin (NIT), University of Turin, Italy

3 Neuroscience Institute of the "Cavaliere Ottolenghi" Foundation (NICO), University of Turin, Italy

Corresponding author: Giovanna Gambarotta, Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Ospedale San Luigi, Regione Gonzole 10, 10043 - Orbassano (TO), Italy, giovanna.gambarotta@unito.it.

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