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This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/148698 since 2019-05-03T00:21:12Z

Publisher:

Elsevier Inc. Academic Press

Published version:

DOI:10.1016/B978-0-12-410499-0.00009-5

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03 May 2024

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This is an author version of the contribution published on:

Gambarotta G, Fregnan F, Gnavi S, Perroteau I
Neuregulin 1 role in Schwann Cell regulation and potential applications to promote peripheral nerve regeneration.
Editor: Elsevier Inc. Academic Press
2013
ISBN: 9780124104990

in

Tissue Engineering of the Peripheral Nerve: Stem Cells and regeneration promoting factors
223 - 256

The definitive version is available at:
http://linkinghub.elsevier.com/retrieve/pii/B9780124104990000095

Neuregulin1 role in Schwann cell regulation and potential applications to promote peripheral nerve regeneration

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Running title: Neuregulin 1 and peripheral nerve regeneration

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Abstract

Neuregulin 1 (NRG1) is a multifunctional and versatile protein: its numerous isoforms can signal in a paracrine, autocrine or juxtacrine manner, playing a fundamental role during the development of the peripheral nervous system and during the process of nerve repair, suggesting that the treatment with NRG1 could improve functional outcome following injury. Accordingly, the use of NRG1 in vivo has already yielded encouraging results.

The aim of this review is to focus on the role played by the different NRG1 isoforms during peripheral nerve regeneration and remyelination and to identify good candidates to be used for the development of tissue engineered medical devices delivering NRG1, with the final goal to promote better nerve repair.

Keywords: myelination, re-myelination, peripheral nerve regeneration, Schwann cells, axon-glia interactions, factor delivery, tissue engineered medical devices

Introduction to Neuregulin 1

Neuregulins are a family of soluble and transmembrane growth factors encoded by four different genes. Among them, the most studied is Neuregulin 1 (NRG1) which is involved in the development of heart, mammary gland, brain and nerve through the activation of different combinations of the tyrosine kinase receptors ErbB2, ErbB3 and ErbB4 (Mei & Xiong, 2008; Yarden & Sliwkowski, 2001). NRG1 plays an important role in myelination and peripheral nerve regeneration, and - for this reason – was the main subject of recent comprehensive reviews, to which this review will refer also for the references that for space limits have not been cited here (Fricker & Bennett, 2011; Nave & Salzer, 2006; Pereira, Lebrun-Julien, & Suter, 2012; Salzer, 2012; Syed & Kim, 2010; Taveggia, Feltri, & Wrabetz, 2010).

This review aims to give an overview of what is known about the role of NRG1 in axonal growth, myelination and re-myelination, focusing the attention on its prospective clinical application to promote peripheral nerve regeneration, taking into consideration the different possible delivery systems.

Neuregulin1 gene products

Over time, different NRG1 isoforms were identified and named according to the context in which they were found (neu differentiation factor/NDF, heregulin/HRG, acetylcholine receptor-inducing activity/ARIA, glial growth factor/GGF, sensory and motor neuron-derived factor/SMDF), until all of them were recognized to belong to the same gene, and named neuregulin1/NRG1 (Falls, 2003; Marchionni et al., 1993).

In this review, the different NRG1 isoforms will be named according to the scheme proposed and shared by different authors (Falls, 2003; Mei & Xiong, 2008), based on exon composition (Figure 1). NRG1 is encoded by a gene spanning 2,6 mbp in humans and rats, 2,4 mbp in mice (Chou &

Ozaki, 2010). All NRG1 isoforms contain an epidermal growth factor (EGF)-like domain that is located in the extracellular portion of the protein and is necessary and sufficient for the receptor activation.

Six types of NRG1 were described (I-VI), which differ for N terminal exons, arising from alternative splicing and use of alternative promoters. Type I (NDF/HRG/ARIA), II (GGF) and III (SMDF) are encoded in a wide range of vertebrate genomes, type IV appears to be restricted to mammalian, type V and VI appear to be restricted to primates (Chou & Ozaki, 2010).

An immunoglobulin (Ig)-like domain – located between the N-terminal sequence and the EGF-like domain - characterizes NRG1 type I, II, IV and V (with or without a spacer region), whereas NRG1 type III and VI present an N-terminal region connected directly to the EGF-like domain. In Type III NRG1 the N- and the C-terminal regions are both located inside the cell (except one isoform - NRG1-type III-β3 - which presents inside the cell only the N-terminal). The type III N terminal sequence contains also a cystein-rich domain (CRD) with an additional transmembrane domain (TMn).

The number (greater than 30) and the nomenclature of the identified NRG1 derives also from the alternative use of different exons located downstream the EGF-like domain: the first exon can be *α* or *β* or can be missing (*γ*); the following exon can be *1,* or *4*, or *3* (followed by a stop codon) or missing (isoform *2*), the next exon is a transmembrane domain (TMc), followed by a cytoplasmic exon *c*, that can be followed by a stop codon (isoform *c*), by exon *a* or exon *b*. The activity of *β* isoforms is 100 fold higher than that of *α* isoforms.

Cleavage by the β-secretase BACE-1 (β-site of amyloid precursor protein-cleaving enzyme) or by the α-secretases TACE (tumour necrosis factor-α-converting enzyme) or ADAM (members of “a disintegrin and metalloprotease” family), generates soluble NRG1, except in the case of Type III NRG1, that remains trans-membrane. As discussed in a following paragraph, BACE-1 cleavage of

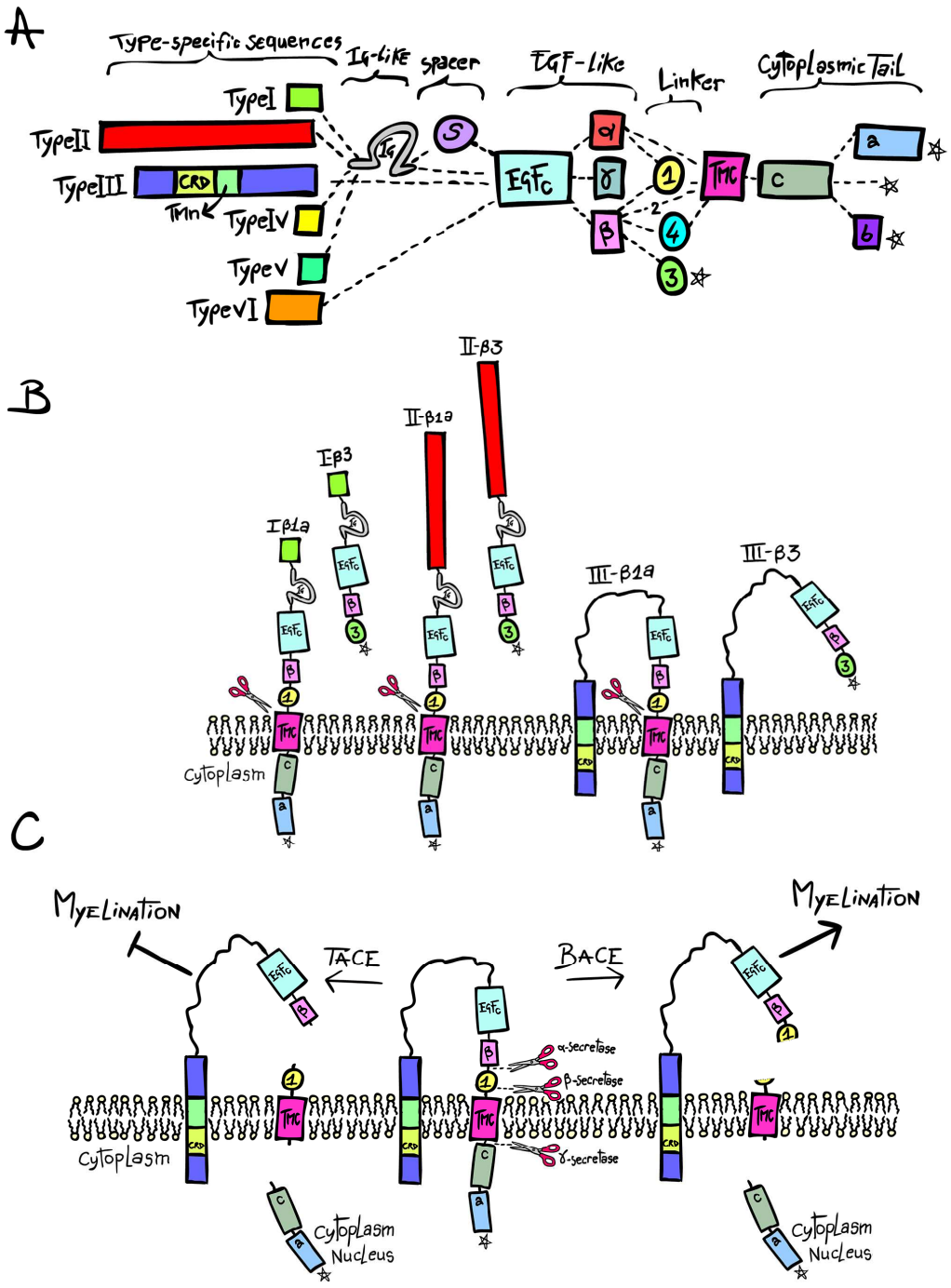
axonal NRG1-type III positively regulates myelination (Hu et al., 2006; Willem et al., 2006) and remyelination (Hu et al., 2008), while TACE cleavage has a negative effect on myelination (La Marca et al., 2011).

Types IV- VI NRG1 are less characterized, but it is likely that they are processed like Types I- II (Mei & Xiong, 2008).

The expression of the different isoforms is spatially and temporally regulated, suggesting that different isoforms display specific and unique characteristics.

Different types of NRG1 I, II and III are expressed in the PNS; they activate – in *cis* and in *trans* - the heterodimeric receptor ErbB2/ErbB3 expressed by Schwann cells (SC). After ligand binding to ErbB3, ErbB3 and ErbB2 heterodimerize, ErbB2 tyrosine kinase switches on, and phosphorylates intracellular tyrosine residues which became docking sites for adaptor proteins involved in different signal transduction pathways, which eventually regulate the transcription of genes implicated in survival, migration, differentiation, proliferation and myelination (Fricker & Bennett, 2011; Yarden & Sliwkowski, 2001) (and references therein).

NRG1 is involved in many steps of the peripheral nerve development, playing a fundamental role in the development of SC precursors and in the interactions between axons (expressing mainly transmembrane NRG1-type III) and Schwann cells (expressing ErbB2-ErbB3 and soluble type I/II NRG1). The absence of NRG1-type III (Wolpowitz et al., 2000) or of the co-receptors ErbB3 (Riethmacher et al., 1997) or ErbB2 (Morris et al., 1999; Woldeyesus et al., 1999) give rise to animals without or with severely reduced SC precursors; the absence of NRG1-type I/II give rise to normal SC (Meyer et al., 1997), suggesting that NRG1-type III is the most important isoform for SC development. Nevertheless, it has been shown - in the embryonic chick - that soluble NRG1 is also released by axons during the critical period of SC survival (Ma, Wang, Song, & Loeb, 2011).


A
Type-specific sequences
Ig-like
spacer
EGF-like
Linker
Cytoplasmic Tail
TypeI
TypeII
TypeIII
CRD
TMIn
TypeIV
TypeV
TypeVI
Ig
S
EGFc
α
γ
β
1
2
4
3
TMc
C
a
b
B
II-β3
II-β1a
Iβ3
Iβ1a
III-β1a
III-β3
Cytoplasm
C
MYELINATION
TACE
BACE
α-secretase
β-secretase
γ-secretase
Cytoplasm
Nucleus

- Figure 1 –

Legend to Figure 1 - NRG1 isoforms. Panel A - Six types of NRG1 are described (I-VI), which differ for N terminal exons. NRG1-type I, II, IV and V (with or without a spacer region) are characterized by an immunoglobulin (Ig)-like domain, located between the N-terminal sequence and the EGF-like domain; NRG1-type III and VI present an N-terminal region connected directly to the EGF-like domain. The type III N- terminal sequence contains a cystein-rich domain (CRD) with an additional transmembrane domain (TMn). Variants derive also by splicing in the linker and in the cytoplasmic tail; the C-terminal transmembrane domain (TMc) is located between these two regions.
The great number of NRG1 isoforms derives also from the alternative use of different exons located downstream the EGF-like domain: the first exon can be *α* or *β* or can be missing (isoform *γ*); the second exon can be *1,* or *4*, or *3* (followed by a stop codon) or can be missing (isoform *2*); the third exon can be a transmembrane domain (TMc), followed by a cytoplasmic exon *c*, that can be followed by a stop codon (isoform *c*), by exon *a* or exon *b*.

Panel B - In this scheme is reported the structure of those isoforms (I, II and III, β1a and β3) cited in this review which is focused on peripheral nerve regeneration (see also Table I). Many soluble and mature NRG1 proteins are produced in the form of transmembrane precursors and are generated by the cleavage by different secretases, except in the case of NRG1- β3 isoforms, which are already released as soluble mature proteins for autocrine/paracrine interactions (type I/II) or as transmembrane mature proteins for juxtacruine interactions (type III).
Panel C - NRG1-type III can be cleaved by α-secretases belonging to TACE (tumour necrosis factor-α-converting enzyme) or ADAM (a disintegrin and metalloprotease) family (eg TACE/ADAM17) or by β-secretases (BACE-1/β-site of amyloid precursor protein-cleaving enzyme). BACE-1 cleavage stimulates myelination (Hu et al., 2006; Willem et al., 2006) and remyelination (Hu et al., 2008), TACE cleavage inhibits myelination (La Marca et al., 2011) . A second cleavage by a γ-secretase

dependent protease, in those NRG1 isoforms containing the TMc, generates a cytoplasmic fragment that can translocate into the nucleus and influence gene transcription (Bao et al., 2004). For the contents of this figure, the authors got inspiration from different reviews and papers (Bao et al., 2003; Falls, 2003; La Marca et al., 2011; Mei & Xiong, 2008; Velanac et al., 2011).

NRG1 isoforms and their role in Schwann cell myelination

The existence of a signal instructing SC to deposit layers of myelin - the thickness of which is proportional to the diameter of the axon - had long been hypothesized. Transgenic and knock out mice (Table 1) allowed to demonstrate that neuronal NRG1-type III plays an instructive role on myelination, determining the ensheathment fate of axons: reduced NRG1 expression causes hypo-myelination (Michailov et al., 2004; Taveggia et al., 2005), which can be rescued by lentiviral-mediated expression of NRG1-type III-β1a (Taveggia et al., 2005). Moreover, neuronal NRG1-type III has been shown to be required for remyelination and regeneration after nerve injury: single axons in which NRG1 has been ablated are hypomyelinated and regenerate more slowly (Fricker et al., 2011).

Neuronal transmembrane NRG1-type III-β1a overexpression induces hyper-myelination (Michailov et al., 2004), converts unmyelinated axons of sympathetic neurons to myelination (Taveggia et al., 2005), improves remyelination after peripheral nerve injury (Stassart et al., 2013).

Neuronal NRG1-type III-β3 overexpression has no effect on myelin thickness (Gomez-Sanchez et al., 2009), but stimulates SC proliferation and enlarges peripheral nerve and ganglia.

Neuronal soluble NRG1-type I-β1a overexpression does not alter myelination (Michailov et al., 2004), but improves remyelination after peripheral nerve injury (Stassart et al., 2013). These authors demonstrate that NRG1 produced by SC is not necessary for myelination, but it is strongly involved in remyelination, which is strongly impaired after nerve crush in mice lacking SC NRG1,

resulting in severe hypo-myelination. In these mice, the ectopic neuronal expression of NRG1-type I-β1a does not completely rescue for the absence of SC NRG1, suggesting that NRG1 released by SC is relevant for remyelination. This role is confirmed by the observation that NRG1-type I (both α and β isoforms) is strongly up-regulated following injury (Carroll, Miller, Frohnert, Kim, & Corbett, 1997; Ronchi et al., 2013; Stassart et al., 2013).

Intriguingly, Syed and colleagues (Syed et al., 2010) asked whether NRG1-type III - provided in a paracrine manner - would still promote myelination and they demonstrated that this is the case: a recombinant "soluble" (but containing all domains, including the CRD and the TMn domain) NRG1-type III-β3 is able to promote in vitro myelination of dissociated dorsal root ganglia (DRG) neurons and of normally non myelinated superior cervical ganglion (SCG) neurons, and is able to rescue the myelination defects on Nrg1-typeIII $^{+/-}$ neurons (but not on Nrg1-typeIII $^{-/-}$ neurons). These data suggest that NRG1-typeIII must be expressed – even at low levels - as a transmembrane protein in order to allow early events of axonal segregation and ensheathment but, to further promote myelination, it can be delivered as a soluble protein.

Then, they asked whether also the NRG1 concentration played a role on myelinating activity and they, amazingly, demonstrated that soluble Nrg1-typeII-β3 - when used at very low concentration - promotes myelination, while at higher concentration inhibit it, as previously shown by others (Zanazzi et al., 2001). Similarly, they demonstrated that "soluble" NRG1-type III – when used at high concentration - inhibits myelination (while at low concentration stimulates it). These data show that the concentration of soluble NRG1 (regardless of the isoform) plays an important role in determining the myelination fate of axons. Syed and colleagues demonstrate that there is a concentration threshold beyond which the AKT pathway is activated, playing a pro-myelinating effect, and a higher concentration threshold beyond which the ERK pathway is activated, leading

to myelination inhibition (and the thresholds are lower for NRG1-typeII, higher for “soluble” NRG1-typeIII).

Following nerve injury, SC respond to axonal damage up-regulating NRG1-type I/II (Carroll et al., 1997; Ronchi et al., 2013; Stassart et al., 2013) and activating ERK signalling pathway (Harrisingh et al., 2004). Accordingly, it has been shown that activation of the ERK-signalling pathway in myelinating SC drives them back to a dedifferentiated state, that is reversible: as soon as ERK signal diminishes, SC respond to axonal signals and redifferentiate (Napoli et al., 2012). Indeed, when *Mycobacterium leprae*, the leprosy pathogen, binds to and activates ErbB2, it activates ERK pathway and demyelination (Tapinos, Ohnishi, & Rambukkana, 2006). On the contrary, it has been recently shown that sustained activation of ERK in SC and oligodendrocytes stimulates myelin growth and increases myelin thickness (Ishii, Furusho, & Bansal, 2013). To explain these different results, it has been suggested that ERK activation can be promyelinating or demyelinating, depending on the context, on the strength and on the duration of activation; it has been proposed that, during development, ERK activation promotes myelination, while its activation in differentiated SC promotes demyelination.

NRG1 ISOFORM	GENOTYPE	AXONAL EXPR.	GLIAL EXPR.	*in vitro* ASSAYS	*in vivo* ASSAYS	PERIPHERAL NERVE PHENOTYPE	REFERENCES
II-β3 (soluble)	WT			+		soluble NRG1-II-β3 (GGF2) isoform blocks axon ensheathment and myelination, and leads to extensive demyelination when added to mature cocultures.	Zanazzi *et al.*, *Journal of Cell Biology* 2001 "Glial growth factor/Neuregulin inhibits Schwann Cell myelination and induces demyelination"
all	Nrg1 $^{+/-}$ x ErbB2 $^{+/-}$ (KO)				+	reduced NRG1 expression causes hypomyelination (fig.2a)	Michailov *et al.*, *Science* 2004 "Axonal Neuregulin-1 regulates myelin sheath thickness"
III-β-1a (transmembrane)	Thy1.2-NRG1-III-β-1a (transgenic mice expressing NRG1 in postnatal motoneurons and DRG neurons)	+			+	Nrg1-III-β1a overexpression causes hypermyelination (fig.4-5)	
I-β-1a (soluble)	Thy1.2-NRG1-I-β-1a (transgenic mice expressing NRG1 in postnatal motoneurons and DRG neurons)	+			+	Nrg1-I-β1a overexpression does not alter myelination (fig.4c)	
III (transmembrane)	NRG1-type III $^{-/-}$ (KO)	-		+	+	NRG1-type III +/- mice are hypomyelinated and aberrantly ensheathed (fig.7); Nrg1-III-β1a forced expression rescues myelination defects (fig.3)	Taveggia *et al.*, *Neuron* 2005 "Neuregulin-1 type III determines the ensheathment fate of axons"
III-β-1a (transmembrane)	WT	+		+		Nrg1-III-β1a forced expression converts unmyelinated axons of sympatetic neurons to myelination (fig.4)	
III-β-3 (transmembrane)	NSE-hSMDF (transgenic mice expressing NRG1-III-β-3 under the promoter of neuron specific enolase)	+			+	Nrg1-III-β3: no major effects on myelin thickness; peripheral nerves are enlarged; Remak bundles altered: small caliber axons not separated, but closely packed and ensheathed as a single unit	Gomez-Sanches *et al.*, *Journal of Neuroscience* 2009 "Sustained axon-glial signaling induces Schwann cell hyperproliferation, Remak bundle myelination and tumorigenesis"
III-β-3 ("soluble", but containing all domains, including CRD and TMn; produced as a soluble factor by R&D)	WT			+		Soluble Nrg1-III-β3 promotes Schwann cell myelination (fig. 1, cocultures of SC and dissociated DRG neurons)	Syed *et al.*, *Journal of Neuroscience* 2010 "Soluble Neuregulin-1 has bifunctional, concentration-dependent effects on Schwann Cell myelination
	WT			+		Soluble Nrg1-III-β3 induces myelination on normally nonmyelinated superior cervical ganglion (SCG) neurons (fig.3)	
	WT			+		Soluble Nrg1-III-β3 at high concentration inhibits Schwann cell myelination (fig.6)	
	NRG1 type III $^{-/-}$ (KO)			+		Soluble Nrg1-III-β3 rescues the myelination defect on Nrg1 type III $^{+/-}$ neurons, but not on Nrg1 type III $^{-/-}$ neurons (fig.2)	
II-β3 (soluble, produced by Acorda Therapeutics)	WT			+		Soluble Nrg1-II-β3 (GGF2) at very low concentration promotes Schwann cell myelination (fig.7), at medium-high concentration inhibits it.	
III (transmembrane)	WT + Tace shRNA			+		TACE downregulation induces precocious myelination and hypermyelination in vitro (fig.1) that is neuron-autonomous (fig.2)	La Marca *et al.*, *Nature Neuroscience* 2011 "TACE (ADAM17) inhibits Schwann cell myelination"
	Tace fl/fl x HB9-cre (transgenic line that drives motor neuron specific recombination using the promoter of Mnx1 gene)				+	TACE inactivation in motor neurons leads to precocious myelination (fig.3); nerves are hypermyelinated during development and in the adult, and Remak fibers are aberrantly ensheathed (fig.4-5)	
	Tace fl/fl x Mpz-cre (transgenic line that drives Schwann cell specific recombination using the promoter of Mpz gene)				+	TACE inactivation in SC does not alter myelination (fig.5); myelinated fibers have more periaxonal space and an accumulation of organelles in the inner cytoplasmic collar (Fig.S5), suggesting that glial TACE process molecules implicated in myelin compaction and/or adhesion.	
	NRG1 type III $^{-/-}$ (KO) + Tace shRNA			+		Ablation of Tace in type III $^{-/-}$ neurons does not rescue myelination: Tace inhibits myelination by modulating NRG1 type III	
III (transmembrane)	SLICK-A Cre; NRG$^{fl/fl}$	+			+	Axonal NRG1 ablation (single neuron labelling with inducible Cre-mediated knock-out) causes severe deficits in remyelination.	Fricker *et al.*, *Journal of Neuroscience* 2011 "Axonally derived neuregulin-1 is required for remyelination and regeneration after nerve injury in adulthood.
III-β-1a (transmembrane)	Thy1.2-HA-NRG1-III-β1a^{FL} & Thy1.2-HA-NRG1-III-β1a^{GIEF} (transgenic mice expressing NRG1 in postnatal motoneurons and DRG neurons)	+			+	Neuronal overexpression of NRG1-III-β1a^{GIEF} - designed to mimic BACE cleavage - induces hypermyelination in vivo	Velanac *et al.*, *Glia* 2012 "BACE1 processing of NRG1 Type III produces a myelin-inducing signal but is not essential for the stimulation of myelination"
	NRG1-type III $^{-/-}$ (KO)			+		neuronal overexpression of NRG1-III-β1a^{GIEF} is sufficient to restore myelination in NRG1-III$^{-/-}$ sensory neurons, suggesting that the C terminal domain and the cytoplasmic tail are not required for myelination	
	BACE1 $^{-/-}$				+	overexpression of full-length NRG1-III-β1a promotes hypermyelination in BACE1$^{-/-}$ mice. NRG1 processing is impaired but not abolished in BACE1$^{-/-}$. BACE1 is not essential for the activation of NRG1 type III to promote myelination.	
III-β-1a (transmembrane)	Thy1.2-NRG1-III-β1a (transgenic mice expressing NRG1 in postnatal motoneurons and DRG neurons)	+			+	overexpression of the axonal Nrg1-III-β1a isoform improves remyelination after peripheral nerve injury (fig.1S)	Stassart *et al.*, *Nature Neuroscience* , 2013 "A role for Schwann cell-derived neuregulin-1 in remyelination"
I-β-1a (soluble)	Thy1.2-NRG1-I-β1a (transgenic mice expressing NRG1 in postnatal motoneurons and DRG neurons)	+			+	overexpression of the soluble Nrg1-I-β1a isoform (which has not effect on normal myelination) improves remyelination after peripheral nerve injury (fig.1)	
all	Dhh-Cre x Nrg1$^{loxP/loxP}$ (Cre-lox mice missing NRG1 exons α and β in Schwann Cells)		-		+	SC NRG1$^{+/-}$: no defects in myelination; remyelination is strongly impaired after nerve crush, resulting in severe hypomyelination (fig.4)	
I-β-1a (soluble)	Thy1.2-NRG1-I-β-1a x Dhh-Cre X Nrg1$^{loxP/loxP}$ (transgenic mice expressing NRG1 in postnatal motoneurons and DRG neurons, and missing NRG1 in Schwann cells)	+	-		+	remyelination after crush is inefficient: neuronal Nrg1-I-β1a does not compeletely compensate for the absence of NRG1 expression in SC (fig.5e)	
I (soluble)	WT		+		+	type I NRG1 expression is induced after nerve injury (fig.2c)	
I/II (α/β) (soluble)	WT and BALB-neuT (ErbB2 overexpression)		+		+	type I/II NRG1 (α and β isoforms) is upregulated after nerve injury (fig.12)	Ronchi *et al.*, *PlosONE* 2013 "ErbB2 receptor over-expression improves post-traumatic peripheral nerve regeneration in adult mice"

NRG1 cleavage and consequences on myelination activity

All NRG1 are synthesized with the EGF like domain exposed to the extracellular environment (Figure 1). With the exception of the β3 isoforms - lacking the transmembrane domain (TMc) - which are produced as soluble or transmembrane proteins (Falls, 2003), ready to interact with their receptors, most NRG1 isoforms are synthesized as transmembrane precursor proteins and need a proteolytic cleavage to release a soluble ligand for paracrine/autocrine signals (all type isoforms, except type III) or to expose the EGF like domain toward the extracellular environment for juxtacrine interactions (isoform type III).

As discussed above, axonal NRG1-type III regulates myelin sheath thickness (Michailov et al., 2004), determines the ensheathment fate of axons (Taveggia et al., 2005), is required for remyelination (Fricker et al., 2011). NRG1-type III can be cleaved by α-secretases belonging to the ADAM family (eg TACE/ADAM17) or by the β-secretase BACE-1 (Figure 1, panel C). BACE-1 cleavage stimulates myelination (Hu et al., 2006; Willem et al., 2006) and remyelination (Hu et al., 2008), ADAM cleavage inhibits myelination (La Marca et al., 2011).

Moreover, it has been shown that in NRG1 isoforms containing the TMc and the cytoplasmic tail (all but the β3 isoforms) a second cleavage by a γ-secretase dependent protease can occur following ligand-receptor interactions, to activate “reverse signalling” (Bao, Wolpowitz, Role, & Talmage, 2003). A cytoplasmic fragment is released, that can translocate into the nucleus and influence transcription (Bao et al., 2004).

TACE/ADAM17 cleaves NRG1 in the exon “1” a few aminoacids (3-6) upstream the cleavage site of BACE-1 (La Marca et al., 2011) and its down-regulation leads to precocious- and hyper-myelination, that is neuron-autonomous (indeed, TACE inactivation in SC does not alter myelination). These results suggest that TACE inhibits myelination by limiting the amount of functional axonal NRG1-typeIII.

It has been also demonstrated that ADAM10 cleaves NRG1 in the same region, although its down-regulation in a co-culture system was unable to affect normal myelination (Luo et al., 2011). Conversely, BACE-1 inhibition impaired normal myelination (Luo et al., 2011) and neuronal overexpression of NRG1-typeIII-β1a^{GIEF}, a recombinant protein designed to mimic BACE-1 cleavage, induces hyper-myelination in vivo and is sufficient to restore myelination in NRG1-typeIII$^{-/-}$ sensory neurons (Velanac et al., 2011), suggesting that the C terminal domain - that is missing in NRG1-typeIII-β1a^{GIEF} recombinant protein - is not required for myelination. Nevertheless, NRG1-typeIII-β1a overexpression promotes myelination also in BACE-1$^{-/-}$ mice, suggesting that BACE-1 promotes myelination, but is not essential for NRG1 processing and pro-myelination activity (Velanac et al., 2011).

It will be intriguing to understand how a few aminoacids in the NRG1-type III C terminus are able to guide the fate of axon myelination: NRG1-typeI-β1a cleaved by TACE or BACE-1 differ for a few Aminoacids; moreover, the C terminal aminoacids that characterize β3 are just 11 and, as previously mentioned, the neuronal overexpression of NRG1-typeIII-β3 does not induce hyper-myelination (Gomez-Sanchez et al., 2009). As already discussed by Velanac et al., these 11 aminoacids can serve as an acylation-like modification site, that tightly associate this isoform to the membrane (Cabedo, Luna, Fernandez, Gallar, & Ferrer-Montiel, 2002), likely activating different signalling pathway. On the other hand, β3 exon aminoacids are present in the isoform NRG1-typeII-β3 (GGF2) which is currently used for clinical trials and, provided at very low concentration, promotes myelination, at higher concentration inhibit it (Syed et al., 2010; Zanazzi et al., 2001). Understanding the role played by the “GIEF” aminoacids which remain following BACE-1 cleavage (Velanac et al., 2011), and by the β3 exon aminoacids, will contribute to understand the myelination process.

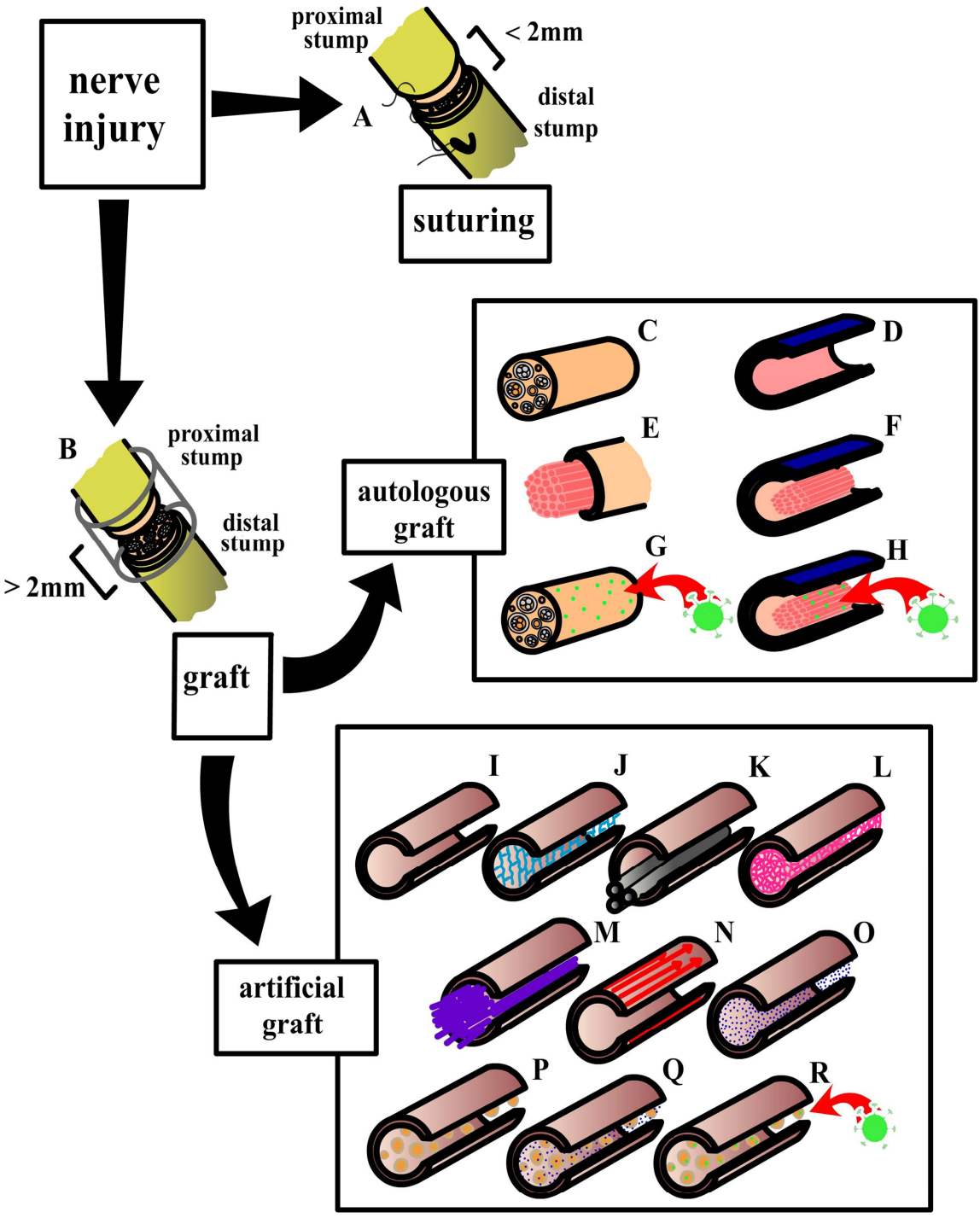
NRG1 to promote nerve repair

Peripheral nerve injury and repair

Axons in the adult peripheral nervous system (PNS) undergo spontaneous regeneration after injury. Following a peripheral nerve injury, the type of nerve repair depends on the length of the nerve gap between the proximal and the distal stump (Siemionow & Brzezicki, 2009), as shown in Figure 2. Short gaps (few mm), can be repaired by direct suture of the proximal and the distal stumps with end-to-end coaptation and epineural suturing. The most common treatment for longer nerve gaps is surgical repair using autologous nerve grafts (autografts) (Deumens et al., 2010; Kandenwein, Kretschmer, Engelhardt, Richter, & Antoniadis, 2005). Autografts have shown high efficacy in inducing nerve regeneration because they provide to the regenerating axons a natural guidance channel with SC surrounded by their basal lamina, allowing appropriate alignment of outgrowing axons (Deumens et al., 2010). However, there are some drawbacks in using autografts: donor site morbidity and sensory loss, scarring, neuroma formation and limited length of available graft material encouraging the search of alternatives for nerve gap reconstruction. On the other hand, current techniques and artificial nerve conduit devices available to support regeneration across large lesion gaps have limited success (Deumens et al., 2010; Steed, Mukhatyar, Valmikinathan, & Bellamkonda, 2011). Allografts have been used, but immunosuppression is required and this technique results in poor success rates (Mackinnon & Dellon, 1990). Autologous and autogenous blood vessels (Chiu et al., 1988) and muscle fibres (Glasby, Gschmeissner, Hitchcock, & Huang, 1986) have also been used as conduits for nerve regeneration with different success rates; however, they present the same disadvantages as auto and allografts (Doolabh, Hertl, & Mackinnon, 1996). In 1993, Brunelli et al. described a new biological conduit represented by a vein segment filled with fresh skeletal muscle (Brunelli, Battiston, Vigasio, Brunelli, & Marocolo, 1993). Both the vein and the skeletal muscle of this

autologous graft are withdrawn in the site of surgery and are sutured to bridge the two stumps of a severed nerve. The clinical use of this autologous device led to good results in terms of motor and sensory recovery in 85% of patients in which it was applied (Battiston, Tos, Cushway, & Geuna, 2000).

Instead of autografts, artificial Nerve Guidance Channels (NGC) may be used to enhance regeneration avoiding availability and immune rejection problems (Kehoe, Zhang, & Boyd, 2012; Steed et al., 2011). The basic design are hollow tubes in which the proximal and the distal stumps of the injured nerve are inserted. More recently, research has been focused mainly on improving the single lumen nerve tube to bridge larger nerve gaps (de Ruiter, Malessy, Yaszemski, Windebank, & Spinner, 2009; de Ruiter, Spinner, Yaszemski, Windebank, & Malessy, 2009). The artificial conduit may be implanted empty, or it may be filled with collagen and laminin-containing gels (Labrador, Buti, & Navarro, 1998; Madison, Da Silva, & Dikkes, 1988; Verdu et al., 2002), internal frameworks (de Ruiter, Spinner et al., 2009; Francel, Francel, Mackinnon, & Hertl, 1997; Lundborg & Kanje, 1996; Meek et al., 2001; Nakamura et al., 2004; Yoshii & Oka, 2001; Yoshii, Oka, Shima, Taniguchi, & Akagi, 2003), supportive cells (Ansselin, Fink, & Davey, 1997; Evans et al., 2002; Guenard, Kleitman, Morrissey, Bunge, & Aebischer, 1992; Kim et al., 1994; Rodriguez, Verdu, Ceballos, & Navarro, 2000; Sinis et al., 2005), growth factors (Derby et al., 1993; Fine, Decosterd, Papaloizos, Zurn, & Aebischer, 2002; Hollowell, Villadiego, & Rich, 1990; Lee et al., 2003; Midha, Munro, Dalton, Tator, & Shoichet, 2003; Sterne, Brown, Green, & Terenghi, 1997), and conductive polymers, but combinations have also already been used (Figure 2).


nerve injury
proximal stump
< 2mm
distal stump
A
suturing
B
proximal stump
distal stump
> 2mm
graft
autologous graft
C
D
E
F
G
H
artificial graft
I
J
K
L
M
N
O
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- Figure 2 -

Legend to figure 2 - Short gap versus long gap peripheral nerve injury. (A) Short gaps (< 2mm) can be directly repaired by suturing the proximal and the distal stumps one to each other without tension. (B) Longer gaps (> 2 mm) can be repaired by using autologous or artificial nerve grafts, also called Nerve Guidance Channels (NGC). Autologous grafts are: (C) nerve autograft , (D) blood vessel, (E) muscle fibre, (F) vein filled with muscle (muscle-vein combined conduit). Modifications of autologous grafts are: (G) autograft infected with viruses, (H) vein filled with muscle infected with viruses.

Artificial grafts can be: (I) empty artificial nerve graft, that can be used to repair gaps (2-10 mm); modifications to the lumen of empty artificial NGC may be helpful to repair longer gaps (> 20 mm): (J) internal framework-filled artificial NGC, (K) multichannel-filled artificial NGC, (L) sponge-filled artificial NGC, (M) filament-filled artificial NGC, (N) conductive artificial NGC, (O) artificial NGC incorporated with growth factors, (P) artificial NGC incorporated with supportive cells, (Q) artificial NGC incorporated with supportive cells and (R) growth factors and artificial NGC incorporated with virus infected supportive cells.

An artificial graft can meet many of the needs of regenerating fibres by concentrating neurotrophic factors, reducing cellular invasion and providing directional neuritis outgrowth to prevent neuroma formation.

NRG1 isoform expression during nerve regeneration

SC dedifferentiation and proliferation are a precondition for axonal regeneration in the lesioned peripheral nervous system (Carroll et al., 1997). The different NRG1 isoforms play an important role in this process, thanks to their ability to stimulate dedifferentiation, proliferation and differentiation of SC in vitro (Stassart et al., 2013).

Many studies have examined the expression of the different NRG1 isoforms at various times following peripheral nerve injury. It has been reported that expression of NRG1-type I and -type II isoforms is induced after sciatic nerve transection (3-30 days post injury) and is strictly associated with SC, strengthening the idea that NRG1 acts not only by juxtacrine interactions, but also by autocrine/paracrine communication (Carroll et al., 1997; Ronchi et al., 2013; Stassart et al., 2013). Interestingly, these authors reported that NRG1-typeII-β3 expression in SC coincides with the DNA synthesis phase and this expression lasts in spite of markedly diminished SC mitogenesis, rising the question whether SC could regulate mitosis in the presence of persistent NRG1.

A rapid but transient activation of the NRG1 receptor ErbB2 in myelinating SC after sciatic nerve axotomy was detected (Guertin, Zhang, Mak, Alberta, & Kim, 2005). The authors defined the role of this activation using an ErbB2 antagonist so preventing, in vivo, SC response to axotomy and, in vitro, SC demyelination in neuron-SC co-cultures. Two days post-nerve injury, NRG1 receptors are strongly down-regulated (Ronchi et al., 2013); 4-5 days post-nerve injury, when Wallerian degeneration and demyelination occur, ErbB2 is expressed and activated (Carroll et al., 1997;

Kwon et al., 1997). An earlier increase, at 3 days post injury, is observed for Erbin, an ErbB2 interacting protein whose expression is required for remyelination (Liang et al., 2012).
Indeed, the heterodimer receptor ErbB2-ErbB3 is co-ordinately induced in axotomized nerve SC, suggesting that the density of functional NRG1-receptors may modulate NRG1 activity during the process of peripheral nerve Wallerian degeneration.
These results are in accordance with a previous study (Li, Terenghi, & Hall, 1997) in which chronically denervated SC were missing ErbB2. It was proposed that the lack of ErbB2 renders SC chronically insensitive to axonal derived NRG1, so contributing to the failure of axonal regeneration through chronically denervated distal nerve stumps.
Neuronal NRG1-type III, as previously discussed, plays an important role in myelination and nerve regeneration. This isoform is anchored to the axonal surface and directly communicates with adjacent glial cells. Recent studies have confirmed the actual importance of the axon-derived NRG1 in nerve regeneration (Fricker et al., 2011). Through single-neuron labelling - by means of inducible Cre-mediated knock-out mice (Young et al., 2008) - it was demonstrated that juxtacrine NRG1 signalling is not essential for maintenance of the myelin sheath and neuromuscular junctions, but it is necessary for the reparative response after nerve injury, including remyelination, axon regeneration, and reinnervation of the neuromuscular junctions.
The NRG1 importance in the nerve regenerative process and in remyelination is more relevant when the functional outcome of non-nervous nerve grafts is analyzed. The analysis of a successful graft represented by a fresh muscle-vein-combined conduit (Geuna et al., 2007; Nicolino et al., 2003; Tos et al., 2007) revealed that SC, in supporting axonal regeneration, very early and massively colonize the tubular graft migrating from both the proximal and the distal nerve stumps (Fornaro, Tos, Geuna, Giacobini-Robecchi, & Battiston, 2001; Raimondo et al., 2005). SC graft colonization is not only supported by migration from the nerve stumps, but also by active SC

proliferation accompanied by NRG1 up-regulation (Geuna et al., 2003). mRNA expression analysis of the early events occurring in this non-nervous tube placed to bridge a peripheral nerve gap has highlighted NRG1 α2 isoform - released by the muscle used to fill the tube - as suitable candidate to promote SC survival and activity in early post-operative phases, when regenerating axons are still not present. It has been proposed that this molecule may promote SC survival and differentiation rather than proliferation (Raabe, Clive, Neuberger, Wen, & DeVries, 1996). These results suggest that NRG1 α2 isoform plays a role in supporting early SC survival and activity in the absence of axons and provides a possible explanation for the observed effectiveness of the fresh muscle-vein-combined technique for nerve repair .

Biomaterials and delivery system

As discussed previously, several studies demonstrated that endogenous NRG1 is required for nerve repair, suggesting that the treatment with recombinant NRG1 could improve peripheral nerve regeneration and functional outcome following injury. Different strategies have been used to deliver NRG1: subcutaneous injection, protein release by biomaterials, transplantation of NRG1 expressing cells and injection of adenoviruses coding for NRG1 (Table 2).

NRG1 subcutaneus injection

Treatment with 1 mg/kg NRG1-II-β3 (recombinant human glial growth factor 2) via subcutaneous injection promotes nerve regeneration and accelerates functional recovery after rat sciatic nerve injury (Chen et al., 1998). Histological assessment shows less severe degeneration and earlier robust axon remyelination, with improvement in axon diameter and myelin thickness, in the treated group. Epinerium injection with 500ng “GGF” (heuregulin α, Sigma Aldrich) after rabbit facial nerve anastomosis results in high SC and glial cell proliferation and in better nerve regeneration (Yildiz et al., 2011).

NRG1 release by biomaterials

The most promising approach for growth factor delivery to the site of nerve injury is to load growth factors directly into the conduit (Simon, Terenghi, Green, & Coulton, 2000; Sterne et al., 1997; Whitworth, Dore, Green, & Terenghi, 1995). Alginate hydrogel provides a sustained and controlled release of neurotrophich factors in vitro and in vivo (Austin, Bower, Kurek, & Muldoon, 1997; Ko, Dixit, Shaw, & Gitnick, 1995) and freeze-dryed alginate supports axonal regeneration across 50-mm gap in the cat sciatic nerve (Suzuki et al., 1999). Moreover, coating of alginate hydrogel with fibronectin, supports SC viability and neuronal regeneration (Mosahebi, Wiberg, & Terenghi, 2003). An ultrapure endotoxin-free low-viscosity alginate with high manuronic content was used for recombinant NRG-1-type II-β3 (human GGF2, CeNes Pharmaceutical USA) delivery in a poly 3-hydroxybutyrate (PHB) polymer conduit to repair 20mm and 40mm gaps in rabbit common peroneal nerve (Mohanna, Young, Wiberg, & Terenghi, 2003). The same authors demonstrated that NRG-1-type II-β3 addition significantly increased SC quantity, sustained axonal regeneration over short and long gaps in comparison with empty and alginate conduits and improved target muscle reinnervation (Mohanna, Terenghi, & Wiberg, 2005).

Nerve conduits can be produced to have characteristics as similar as possible to the native nerve, by combining growth factors, aligned extracellular matrix and biomaterial filaments, improving the nerve conduit performance and ensuring better functional recovery after *in vivo* transplantation. In vitro oriented collagen and a combination of differentiation factors (NGF, NRG-1, TGF-β) induce SC alignment (Ribeiro-Resende, Koenig, Nichterwitz, Oberhoffner, & Schlosshauer, 2009). TGF-β stimulates α1αβ1 and α6β1 integrin expression and affects cell polarity (Rogister et al., 1993; Stewart, Turner, Jessen, & Mirsky, 1997); NRG1 increases N-cadherin expression (Gess et al., 2008); at low concentration NRG1 accelerates SC migration (Meintanis, Thomaidou, Jessen, Mirsky, & Matsas, 2001), at high concentration increases proliferation via activation and

phosphorylation of the ErbB2/ErbB3 receptor (Porter, Clark, Glaser, & Bunge, 1986; Rosenbaum et al., 1997). Poly-ε-caprolacton (PCL) filaments induce pronounced SC alignment with a polarized expression of the cell adhesion molecule L1 similar to that seen in vivo in bands of Büngner after sciatic nerve crush in adult rats (Ribeiro-Resende et al., 2009). Integration of bioengineered bands of Büngner - which would guide axonal regrowth - and growth factors for the development of innovative nerve guide implants, may be a promising strategy to facilitate and accelerate axonal regeneration.

Silicone implants containing matrigel, poly-L-lactic acid (PLLA) microfilaments and NRG1-type I-β1 (human heuregulin-β1, R&D systems) have been used to bridge a 14 mm gap in adult rats (Cai, Peng, Nelson, Eberhart, & Smith, 2004). Microfilaments provide organized guidance channels that direct SC migration, enhance cable formation and axonal regeneration longitudinally across nerve stumps (Lundborg & Kanje, 1996; Ngo et al., 2003; Zhao, Lundborg, Danielsen, Bjursten, & Dahlin, 1997). NRG1 treatment caused an increase in SC number by inducing proliferation by an autocrine mechanism (Carroll et al., 1997). Application of a low dose of NRG1 promotes SC migration and trophic factor release that support neuronal survival and regeneration (Mahanthappa, Anton, & Matthew, 1996). Implants containing NRG1 and microfilaments act synergistically inducing a significant improvement in the number and longitudinal organization of both SC and axons, leading to axonal regeneration and nerve repair (Cai et al., 2004).

Transplantation of NRG1 expressing cells

The transplantation of cultured cells, transfected to express growth factors, into bio-engineered conduits, may be used to improve nerve regeneration. Due to their role in peripheral nerve regeneration SC may be used as a tool to deliver growth factors. Adult stem cells from adipose tissue or bone marrow can be differentiated into a SC-like phenotype and used as SC replacements (Faroni et al., 2011). Transplantation of cultured SC (Magnaghi, Procacci, & Tata, 2009; Mosahebi,

Woodward, Wiberg, Martin, & Terenghi, 2001), bone marrow mesenchymal stem cells (MSC) (Tohill, Mantovani, Wiberg, & Terenghi, 2004) and adipose-derived regenerative cells (ADRC) (Suganuma et al., 2013) has been used to promote peripheral nerve regeneration.

In vitro, MSC exposed to NRG-1-type II-β3 (human GGF2, CeNes Pharmaceutical USA) express s100 and glial fibrillary acidic protein (Tohill et al., 2004). Following transplantation into 10 mm nerve conduits in the rat sciatic nerve, MSC maintained s100 expression and promote nerve regeneration (Tohill et al., 2004).

ADRC in combination with type I collagen gel have been used to bridge 10 mm sciatic nerve gaps in Wistar rats (Suganuma et al., 2013). ADRC promote peripheral nerve regeneration not by differentiating into SC, but – probably – by secreting NRG1 and VEGFA factors that promote proliferation and migration of SC (Suganuma et al., 2013).

These data suggest that NRG1 stimulates the proliferation of glial cells, inhibits apoptosis and facilitates repair and regeneration of injured nerves in the peripheral nervous system.

NRG1 has been successfully used in combination with different kinds of supportive cells, also to repair spinal cord injury. SC transfected to express high levels of NRG1 (of a non specified isoform) have been implanted into rats with hemisection spinal cord injury (J. Zhang, Zhao, Wu, Li, & Jin, 2010). Transfected SC secreted a large amount of NRG1 both in vitro and in vivo, which results - in vivo - in ErbB2-ErbB4 up-regulation in neurons and neuroglia cells. After transplantation, cells survived and migrated into the spinal cord injuried areas. Significant recovery of hemisection spinal cord injury was observed in the group of rats implanted with transfected SC expressing NRG1. These results suggest that SC expressing NRG1 can significantly improve the repair of spinal cord injury by up-regulating ErbB receptor expression in the target cells, increasing proliferation of glial cells, and protecting neurons from apoptosis (J. Zhang et al., 2010).

Co-transplantation of BMSC with transfected SC expressing NRG1 (of a non specified isoform) into a rat model of spinal cord hemisection injuries, reduced the size of cystic cavities, promoted axonal regeneration and hind limb functional recovery in comparison with SC or BMSC transplantation alone or together (J. F. Zhang et al., 2011). This treatment could provide important insights into potential therapies of spinal cord hemisection injuries to improve functional recovery. Delivery of NRG1 with transfected supportive cells may be a promising strategy for the repair of both spinal cord and peripheral nerve.

Injection of NRG1 coding viruses

Gene therapy, using adeno-associated viral (AAV) vectors (Kaplitt et al., 1994) and lentiviral (LV) vectors (Naldini et al., 1996) may be a promising strategy to promote peripheral nerve regeneration (Hoyng, Tannemaat, De Winter, Verhaagen, & Malessy, 2011).

Recombinant adenoviruses have been used to express NRG1 to improve axonal regeneration in the injured peripheral nervous system (Joung et al., 2010). Rats, injected into both proximal and distal stump of the sciatic nerve with a recombinant adenovirus expressing the EGFβ- domain of NRG1-type I, display an augmented expression of neurofilaments, GAP43 and S100 in the distal stump of the injury site and increased the length of regenerating axons leading to sensory and motor functions improvement (Joung et al., 2010). These results suggest a therapeutic potential for β-EGF-like domain of NRG1 in the treatment of peripheral nerve injury.

NRG1 subcutaneus injection

NRG1 ISOFORM	DOSE	TREATMENT / NERVE TUBE	ANIMAL	NERVE	INJURY	GAP SIZE (mm)	METHODS	CONTROLS	FOLLOW UP	OUTCOME	REFERENCES
II-β3 soluble (GGF2, recombinant human glial growth factor 2)	1mg/kg	subcutaneous injection one day before surgery and daily for the following 4 days	rat	sciatic nerve	crush injury	5	motor functional test, muscle contractility test and histological examination	injection equivalent volume of saline	49 days	Nerve function recovery at 11 days post-surgery, stronger isometric tetanic contractile force 11-21 days post-surgery. Less severe degeneration and earlier remyelination.	Chen et al., 1998
α (Heregulin-α,by Sigma Aldrich)	500 ng	epineurin injection at time of surgery, 24 and 48 hours post-surgery	female New Zeland rabbit	facial nerve	anastomosis	1	electron microscopy	no medication with NRG1	2 months	Increased regeneration, new axons and myelin formation, higher SC proliferation, lower number of myelin debris.	Yildiz et al., 2011

NRG1 release by biomaterials

NRG1 ISOFORM	DOSE	TREATMENT / NERVE TUBE	ANIMAL	NERVE	INJURY	GAP SIZE (mm)	METHODS	CONTROLS	FOLLOW UP	OUTCOME	REFERENCES
II-β3 soluble (recombinant human GGF2, by CeNes Pharmaceutical)	1.250 mg/ml	poly 3-hydroxybutyrate (PHB) conduits filled with GGF suspended in alginate hydrogel	female New Zeland rabbit	common peroneal nerve	nerve transection	20 and 40	immunohistochemisty	PHB conduits filled with alginate hydrogel and empty PHB conduits	63 days	SC number and axonal regeneration increased. 20 and 40 mm gaps bridged by axons after 63 days.	Mohanna et al., 2003
I-β1 soluble (human heuregulin β1, by R&D system)	800 ng	silicone conduits filled with matrigel containing poly-L-lactic acid (PLLA) microfilaments containing heuregulin-b1	female Sprague Dawley rats	sciatic nerve	nerve transection	14	immunohistochemistry	silicone conduits filled with matrigel containing poly-L-lactic acid (PLLA) microfilaments	10 weeks	Extensive regeneration with improvement of the number and longitudinal organization of SC and axons.	Cai et al., 2003
II-β3 soluble (recombinant human GGF2, by CeNes Pharmaceutical)	1.250 mg/ml	poly 3-hydroxybutyrate (PHB) conduits filled with GGF suspended in alginate hydrogel	female New Zeland rabbit	common peroneal nerve	nerve transection	20 and 40	immunohistochemisty, histological examination, electron microscopy and motor reinneravtion evaluation	PHB conduits filled with alginate hydrogel and empty PHB conduits	120 days	SC number and axonal regeneration increased. Reduction of muscle mass percentage loss.	Mohanna et al., 2005

Transplantation of NRG1 expressing cells

NRG1 ISOFORM	DOSE	TREATMENT / NERVE TUBE	ANIMAL	NERVE	INJURY	GAP SIZE (mm)	METHODS	CONTROLS	FOLLOW UP	OUTCOME	REFERENCES
II-β3 soluble (recombinant human GGF2, by CeNes Pharmaceutical)	$15x10^7$ cells/animal	poly 3-hydroxybutyrate (PHB) conduits filled withPHB fibres and differentiated marrow stromal cell (MSC)	Sprague Dawley rats	sciatic nerve	nerve transection	10	immunohistochemistry	poly 3-hydroxybutyrate (PHB) conduits filled withPHB fibres and SC	15 days	Folllowing transplantation, MSC mantain S100 expression and enhance nerve regeneration.	Tohill et al., 2004
?	$5x10^5$ cells/animal	injection of differentiated SC trasfected with pcDNA3.1-NRG1	male and female Wistar rats	spinal cord (CNS)	hemisection spinal cord injury model	20	immunohistochemistry and Basso, Beattie Bresnahan locomotor rating score (BBB)	DMEM and DMEM containing SC injection	8 weeks	Increased spinal cord injury repair. Increased proliferation of glial cells and protection of neurons from apoptosis.	Zhang et al., 2010
?	$3x10^5$ cells/animal	injection of differentiated SC trasfected with pcDNA3.1-NRG1	male and female Wistar rats	spinal cord (CNS)	hemisection spinal cord injury model	/	immunohistochemistry and Basso, Beattie Bresnahan locomotor rating score (BBB)	SC injection	4 weeks	Reduced size of cystic cavities; increased axonal regeneration and hind limb functional recovery.	Zhang et al., 2011
?	$1x10^6$ cells/tube	silicon tube filled with a mixture of type I collagen gel and adipose-derived regenerative cells (ADRC)	Wistar rats	sciatic nerve	nerve transection	10	immunohistochemistry	silicon tube filled with saline solution or type I collagen gel	2 weeks	Increased SC migration and proliferation, and axons regrowth. ADCR express high NRG1 (Neu-1) level	Suganuma et al., 2013

Injection of NRG1 coding adenoviruses

NRG1 ISOFORM	DOSE	TREATMENT / NERVE TUBE	ANIMAL	NERVE	INJURY	GAP SIZE (mm)	METHODS	CONTROLS	FOLLOW UP	OUTCOME	REFERENCES
EGF-like domain of NRG1 β soluble	2 µl $1x10^{11}$ PFU/ml	injection of recombinant adenovirus coding for EGF-like domain of NRG1-β	Male Sprague Dawley rats	sciatic nerve	nerve transection	/	immunohistochemistry and motor function test	injection of recombinant adenovirus LacZ or saline injection	5 weeks	Augmented expression of neurofilaments, GAP43 and S100 in the distal stump of the injury site; increased length of regenerated axons, recovery of sensory and motor function.	Joung et al., 2010

Conclusions

When a peripheral nerve is severely injured, the use of an artificial conduit becomes necessary if the nerve gap is too long to be directly sutured, as an alternative to the autograft, that gives good results, but has some clinical drawbacks. It has been shown that SC of the nerve autograft and the muscle of the muscle-vein conduit release soluble NRG1 which contributes to the effectiveness of this approaches (Nicolino et al., 2003). The aim of this review is to get a clear view of the role played by NRG1 during peripheral nerve regeneration, to identify the right isoform to be delivered to the injured nerve by grafted artificial nerve guidance channels.

NRG1-type III could be the candidate with the therapeutic potential to improve nerve repair: literature data demonstrate that axonal transmembrane NRG1 is required for remyelination and regeneration after nerve injury, while it is dispensable for myelin maintenance (Fricker & Bennett, 2011; Fricker et al., 2011). However, exogenous axonal transmembrane expression implies the use of viruses, that present some problems and risks (toxicity, immune and inflammatory responses, gene control and targeting issues).

An alternative strategy to increase the amount of NRG1-type III suitable to stimulate myelination, is the inhibition of those α-secretases (eg TACE/ADAM17, ADAM10), whose activity negatively interferes with NRG1, impairing myelination. Several TACE (tumour necrosis factor alpha converting enzyme) inhibitors have been developed for the treatment of rheumatoid arthritis and other inflammatory disorders (Bahia & Silakari, 2010; DasGupta, Murumkar, Giridhar, & Yadav, 2009), because tumour necrosis factor alpha is one of the most common pro-inflammatory cytokines involved in rheumatoid arthritis and in other autoimmune diseases. However, most of them have broad spectrum inhibitory activity for other matrix metalloproteases involved in other processes and are not suitable candidates for clinical trials.

Several authors demonstrated that different soluble isoforms of NRG1 (including a type III isoform provided as “soluble” recombinant protein) can improve remyelination after peripheral nerve injury (Tables 1 and 2), with an activity that is concentration and isoform dependent (Syed et al., 2010). They demonstrated that soluble type III isoforms have pro-myelinating activity in a broad concentration range, while type II isoforms have pro-myelinating activity when supplied at very low concentration, below the threshold that stimulates ERK activation. Therefore, soluble NRG1 could be the candidate with the therapeutic potential to improve nerve repair (Syed & Kim, 2010). However, because the pro-myelinating activity of NRG1 seems to depend also on the exons which are downstream the EGF-like domain (α, β, γ, 1, 3, 4, a, b, c…), it is important to understand which isoforms have been used by the different authors. Nevertheless, in many papers it is really difficult to find this information, because the authors call it “NRG1” or use the old nomenclature.

One of these soluble isoforms, type II-β3 (often called “GGF2”), has recently completed the Phase 1 of a clinical trial in patients with heart failure: Acorda Therapeutics is conducting a clinical program for GGF2 in heart failure, and preclinical development to treat peripheral nerve injury and stroke. Other groups performed a Phase II clinical trial to study the efficacy and the safety of recombinant human NRG1 in patients with chronic heart failure (Gao et al., 2010).

It has been shown that after damage to peripheral nerve, the injured nerve reacts with a peak of production of soluble NRG1-type I/II (Carroll et al., 1997; Ronchi et al., 2013; Stassart et al., 2013), ERK and AKT pathway activation (Harrisingh et al., 2004; Napoli et al., 2012; Sheu, Kulhanek, & Eckenstein, 2000) - that in vitro can be activated either by soluble NRG1 (Syed et al., 2010) either by other factors - SC dedifferentiation and proliferation. Subsequently, NRG1 type I/II level decreases, ERK pathway is switched off, and SC remyelinate axons expressing transmembrane NRG1-type III.

These data suggest that soluble NRG1-type I/II could be the candidate to improve nerve repair when there is a large lesion and endogenous SC are not enough to produce an adequate amount of NRG1. Therefore, when designing tissue engineered medical devices to bridge large gap injuries and deliver NRG1, it is essential to get an early release of highly concentrated soluble NRG1 to stimulate ERK pathways and SC dedifferentiation, followed by a late release of NRG1 at low concentration to stimulate AKT activation and remyelination.

In addition to NRG1, other molecules are certainly involved in the regeneration process (Pereira et al., 2012; Taveggia et al., 2010); it is expected that the regenerative research will go towards the concomitant use of different factors, to obtain synergistic effects and better outcome.

Acknowledgments

We apologize for any omissions in citing other relevant publications, because of space limitations. We sincerely thank Silvia Grifoni, for drawing figures 1 and 2. The research leading to this paper has received funding from the European Community's Seventh Framework Programme (FP7-HEALTH-2011) under grant agreement no. 278612 (BIOHYBRID), from MIUR and from Compagnia di San Paolo (MOVAG).

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