**Self-delivery siRNA as a versatile tool to study molecular effectors of myelin maintenance in vivo**

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**Abstract**

The myelination in Schwann cells that occurs during the fœtal life is governed by several transcription factors, including Egr2 gene. Unfortunately, mutations affecting this gene result in various human hereditary peripheral neuropathies, such as the Charcot-Marie-Tooth disease. There are few informations on myelin maintenance that is a dynamic process which is under the control of various genes.We have developed a technique to identify genes in peripheral myelin maintenance, using small interfering ribonucleic acids(siRNAs) to induce extinction of target genes *in vivo.* Modified self-delivery siRNAswere usedto induce extinction of candidate genes without using transfection reagents. These siRNAs were injected into the sciatic nerves of adult rats. We showed that control non-targeting siRNAs did not induce significant demyelination after direct injection into sciatic nerves. The injection of anti-Egr2 siRNAs into the sciatic nerves of adult rats induced a significant and rapid demyelination, as shown by the loss of Myelin Protein Zero expression in the injected area on immunohistochemistry experiments (optic microscopy) and by direct evidence of demyelination on epon-embedded transversal sections of sciatic nerves (optic and electron microscopy). These results confirm previous research findings involving Egr2 in active myelin maintenance. Injections of anti-Dicer siRNAs similarly induced sciatic nerve demyelination, establishing that Dicer expression in adult peripheral nerves is necessary for proper myelin maintenance. This paper also reports on an original method for the use of self-delivery siRNAs to study the molecular mechanisms of myelin maintenance *in vivo.*

**Key words**: siRNA, sciatic nerves, myelin.

**Les siRNA auto-délivrés : des outils polyvalents pour l’étude *in vivo* des effecteurs moléculaires de la maintenance myélinique**

**Résumé**

La myélinisation dans les cellules de Schwann se produit au cours de la vie fœtale et est régie par plusieurs facteurs de transcription, y compris le gène EGR2. Malheureusement, les mutations affectant ce gène entraînent diverses neuropathies périphériques héréditaires humaines, comme la maladie de Charcot-Marie-Tooth. Il y a peu d'informations sur la maintenance de la myéline qui est un processus dynamique contrôlé par différents gènes. Nous avons développé une technique pour identifier les gènes impliqués dans le maintien de la myéline périphérique, en utilisant de petits acides ribonucléiques interférents (siRNA), dans le but d’induire l'extinction de gènes cibles *in vivo*. Des siRNA modifiés pour permettre leur auto-délivrance ont été utilisés pour induire l'extinction de gènes candidats sans utiliser de réactifs de transfection. Ces siRNA ont été injectés dans les nerfs sciatiques de rats adultes. Nous avons tout d’abord montré que les siRNA non ciblés témoins n'induisaient pas de démyélinisation significative après injection directe dans les nerfs sciatiques. L'injection de siRNA anti-Egr2 dans les nerfs sciatiques de rats adultes a induit une démyélinisation significative et rapide, comme le montre la perte d'expression de Myelin Protein Zero dans la zone injectée sur des expériences d'immunohistochimie (microscopie optique) et par la preuve directe de la démyélinisation sur coupes transversales incluses en Epon des nerfs sciatiques (microscopie optique et électronique). Ces résultats confirment les résultats de recherches antérieures impliquant Egr2 dans la maintenance active de la myéline. Des injections de siRNA anti-Dicer induisent de la même manière une démyélinisation du nerf sciatique, établissant que l'expression de Dicer dans les nerfs périphériques adultes est nécessaire pour une bonne maintenance de la myéline. Cet article présente également une méthode originale d'utilisation des siRNA auto-administrés pour étudier les mécanismes moléculaires de la maintenance de la myéline *in vivo*.

**Mots clés :** siRNA, nerfs sciatiques, myéline.

**Introduction**

The higher vertebrate nervous system is characterized by the coexistence of neurons and glial cells. Schwann cells are responsible for producing myelin that is wrapped around axons in the peripheral nerves. The myelin serves as an insulator to facilitate signal propagation along axons. The transcription factor Egr2/Krox20 is a prime regulator of Schwann cells myelination, nerves from Egr2- knockout mice being hypomyelinated and populated with Schwann cells that fail to exit the cell cycle (Topilko et al., 1994). In patients with congenital hypomyelinating neuropathy, Charcot-Marie-Tooth disease or Dejerine-Sottas syndrome; mutations in EGR2 are found (Warneret al., 1998; Timmerman et al., 1999). Many molecular effectors of peripheral nerve myelination have been identified during the last two decades (Woodhoo et al., 2009). Egr2 is permanently expressed in myelinating schwann cells and downregulated after axonal injury, which results in the elimination of myelin sheath (Ghislain et Charnay, 2006). This raised the possibility that Egr2 expression might be required for myelin maintenance. Specific inactivation of Egr2 in adult mouse sciatic nerve by inducible knockout experiments resulted in severe demyelination, involving rapid Schwann cells dedifferentiation and increased proliferation, followed by an attempt to remyelinate and a block at the promyelinating stage. These data established that Egr2 is not only required for the onset of myelination but that it is also crucial for the maintenance of the myelinating state (Decker et al., 2006).

Apart from this study using conditional knockout mice, very little knowledge is available on genes and mechanisms responsible for maintenance of peripheral myelin (Canu et al., 2009, Bremer et al., 2011).

We have developed a new technique to identify genes involved in peripheral myelin maintenance, using small interfering RNAs (siRNAs) which have the ability to silence target genes *in vivo*. For this purpose, we used “self-delivery” siRNAs (Accell siRNA, Dharmacon) to silence candidate genes without using transfection reagents. These modified siRNAs were previously shown to be able to down-regulate target genes *in vivo* after direct injection into the skin (Gonzalez-Gonzalez et al., 2010, Lara et al., 2012).

Another siRNA targeting KLF6-SV1 was shown to inhibit ovarian cancer development after intraperitoneal injection (DiFeo et al., 2009). In our study, anti-Egr2 and anti-Dicer siRNAs were used in sciatic nerves of adult rats (direct injection of siRNAs, *in vivo* model). This allowed us to confirm the involvement of EGR2 in peripheral myelinmaintenance *in vivo*, and to show that the miRNA-processing protein Dicer (which wasrecently shown to be involved in peripheral nerve myelination (Pereira et al., 2010) is also involved inthemyelin maintenance in adult rat sciatic nerves.

1. **Materials and methods**
   1. **Animals**

For in vivo study of myelin maintenance and injection of siRNAs, we used adult male Sprague-Dawley rats between 10 and 12 weeks of age. All rats were obtained from DEPRE breeding center, Saint-Doulchard, France.

There were two animals housed per cage, in a temperature and humidity-controlled room with 12:12 h light/dark cycle. The animals were fed with standard chow and water *ad libitum*.

Adequate measures were taken to minimize pain and discomfort. The animals were housed for a minimum duration of one week before being subjected to the experiments. All procedures were performed with the approval of the animal ethics committee of the University of Limoges (authorization number 15-2013-15).

* 1. ***In vivo* injection of siRNA in the rat sciatic nerve**

Rats were deeply anesthetized by inhalation of isoflurane mixed to O2. All surgical techniques were done under aseptic conditions and performed using a standard dissecting microscope. After anesthesia, the hind legs were attached; the sciatic nerve was exposed unilaterally after skin and muscle incision. The injection point was located above the bifurcation of the nerve in the mono fascicular part of the sciatic nerve. Injections were performed under a dissection microscope, using a manually controlled micro-syringe (see supplementary figure 1). The needle was inserted across the perineurium, then the selected volume of siRNA diluted in isotonic NaCl was slowly injected into the nerve (in the endoneurium). After injection, a mark was made with a non-resorbable suture fixed at the level of the injection point, on the lateral muscle. The muscle and skin were closed with some staples. The rats were returned to their cages and regained mobility. At various times after injection, the rats were again anesthetized in order to remove the sciatic nerve, then sacrificed by intra-cardiac injection of ketamine (0.5 ml at 10 mg/ml; PANPHARMA). A rather large fragment of the sciatic nerve (approximately 1 cm-long) was removed in order to have oth parts of the nerve above and below the injection point. For each time and each siRNA pool (non-targeting siRNAs and anti-Egr2 siRNAs), we obtained three formalin-fixed paraffin-embedded sciatic nerves and three glutaraldehyde-fixed epon-embedded nerves.



Supplementary figure 1: Injection of siRNAs in the

endoneurium of an adult rat sciatic nerve using a

micro-syringe.

**Tables:**

**Table 1. Oligonucleotide primers used for RT-qPCR**

**Gapdh**

Forward primer TGCACCACCAACTGCTTAG

Reverse primer GGATGCAGGGATGATGTT

**Hprt**

Forward primer CTCATGGACTGATTATGGACAGGAC

Reverse primer GCAGGTCAGCAAAGAACTTATAGCC

**Egr2**

Forward primer CAGTACCCTGGTGCCAGCTG

Reverse primer TGTGGATCTCTCTGGCACGG

**Dicer**

Forward primer GAGTCTCTTGCTGGTGCCAT

Reverse primer CGCCTGTGAGTCTGGAAGC

**Table2. Self-delivery siRNA nucleotide sequences**

**Dicer**

Sequence 1 5'CCCCUAUCGUGAAAUUAUG 3'(13546.4 g/mol)

Sequence 2 5'CCAGGAUCCACAAAACGAA 3' (13514.0 g/mol)

Sequence 3 5'UGAUCAGCCUCAUCGAUUU 3' (13572.2 g/mol)

Sequence 4 5'GUCUGAAGCUCGUAUAGGC 3' (13566.6 g/mol)

**Egr2**

Sequence 1 5'CCAGAAGGCAUCAUCAAUA 3' (13510.8 g/mol)

Sequence 2 5'CUGACUUGUUUGGUUAUUA 3' (13546.3 g/mol)

Sequence 3 5' GGUUUAAGUAUGGCUGUAU 3' (13535.2 g/mol)

Sequence 4 5' CCAGUAACUCUCAGUGGUU 3' (13561.4 g/mol)

* 1. **Fixation and inclusion of sciatic nerves**

For the immunohistochemical study of the nerve, nerve fragments were fixed in formalin and

included in paraffin. Five micrometer-thick sections were performed using a Leica microtome. Dewaxing was done through two baths in toluene and two others in absolute alcohol. The rehydration was performed using a water bath for 10 min.

For the semi-thin and ultrastructural analysis of sciatic nerves, samples were fixed in 2.5% glutaraldéhyde diluted in cacodylate buffer, postfixed in 1% osmic acid, dehydrated in successive acetone baths and included in epoxy resin. Dehydratation was obtained by increasing concentrations of acetone diluted in H2O and inclusion was carried out in epoxy resin. Semi-thin transverse sections were performed using an ultramicrotome, mounted on glass slides and stained with toluidine blue. An ultrastructural study was performed on ultrathin sections (60-100 nm), after staining with acetate uranyl and lead citrate, using an electron microscope (JEOL 1011).

* 1. **Immunohistochemistry of sciatic nerves**

Unmasking of antigenic sites on rehydrated sections was performed at 98°C for 25 min in retrieval solution (Dako). The slides were then rinsed with water. 100 μl of peroxydase blocking solution was placed on the slide for 5 min and then rinsed with water and PBS. 100 μl of goat serum was placed on each slide for 30 min and then 100 μl of primary antibodies.

The slides were incubated overnight at 4°C, then rinsed in PBS. The revelation was made using 100 μl of biotinylated secondary antibodies (vectastain diluted biotinylated antibody, VECTOR) for 30 min. The slides were again rinsed with PBS. Vectastain elite ABC kit was added to each slide for 30 min, then 100 μl of 3, 3’-diaminobenzidine was added. H2O2 was added, the slides were rinsed with water and placed for 15 s in hematoxylin, rinsed again in water, then in ammonia water and finally in water. Two baths of absolute alcohol and two others of toluene were realized, and the cuts were mounted between slide and coverslip in a non-aqueous mounting medium.

1. **Results**
   1. **Effect of siRNAs targeting Egr2 on myelin maintenance *in vivo***

Sciatic nerves were injected with anti-Egr2 and non-targeting siRNA using 30μl at 1μM of each pool. We studied the temporal pattern of demyelination by examining serial longitudinal sections of sciatic nerves removed at various times (1 day, 3 days, 5 days, one week) after injection. In the immunohistochemistry experiments, acute demyelination manifested as a loss

of Myelin Protein Zero (P0) staining, associated with invasion by macrophages (CD68- positives cells). There was no visible loss of P0 staining and no invasion by macrophages in nerves treated by non-targeting siRNAs at any time point. In the sciatic nerve that had received anti-Egr2 siRNAs, a focal demyelination (corresponding to the injected area) was observed on samples taken 3 days, 5 days and one week after injection (fig 1). No demyelination was observed in nerves taken 1 day after injection. P0-negative areas were smaller in nerves taken 3 days after injection and larger in the nerves taken at 5 days and one week (Fig. 1B-D). In addition, anti-CD68 immunostaining showed an absence of CD68 staining at 1 day and presence of an increasing number of macrophages at 3 days, 5 days and one week (Fig. 1E-G). On semi-thin (light microscopy) and ultra-thin (electron microscopy) transverse sections, marked acute demyelination was visible for the samples taken 5 days and one week after injection of anti-Egr2 siRNA. The semi-thin sections showed demyelination in nerves treated with siRNAs targeting Egr2 but no or very slight demyelination in nerves treated with nontargeting siRNAs (Fig 2A-C). Ultrastructural analysis showed a marked acute and focal demyelination after treatment with anti-Egr2 siRNAs, which was absent from the nerves treated with control siRNAs (Figure 3A-B).



**Figure 1**. Immunohistochemistry of Myelin Protein Zero (P0) and CD68 in rat sciatic

nerves injected with anti-Egr2 and control non-targeting siRNAs.

**A.** P0 staining of a sciatic nerve injected with control siRNA, showing a homogeneous staining of myelin sheaths with no areas of demyelination (5 days after injection).

**B.** 3 days after injection of anti-Egr2 siRNAs, presence of a demyelinated area of limited size (lack of brown P0 staining, arrows).

**C.** Five days after injection of anti-Egr2 siRNAs, presence of a larger area of demyelination (arrows).

**D.** One day after injection of anti-Egr2 siRNAs, absence of CD68 staining, indicating an absence of macrophages in the injected nerve.

**E.** 3days after injection of anti-Egr2 siRNAs (same nerve as in panel B), presence of CD68- positive macrophages in the demyelinated area. F. 5 days after injection of anti-Egr2 siRNAs (same nerve as in panel C), presence of a higher number of CD68-positive macrophages in the large area of demyelination.



**Figure 2:** Semi-thin sections of adult rat sciatic nerves five days after injection of siRNAs.

Injection of 30 μL of non-targeting siRNAs resulted in either no effect (A, absence of demyelination) or in a slight localized demyelination (B, arrows). Injection of 30 μL of anti Egr2 siRNAs results in a marked area of demyelination (C, arrows).



**Figure 3 :** Ultrastructural study of nerves injected with anti-Egr2 siRNAs.

**A.** After five days of treatment with anti-Egr2 siRNAs, presence of various types of demyelinating features within the lesion: myelinated fiber with a near-normal appearance (white arrowhead), actively demyelinating fiber (white arrow) and macrophages, loaded with myelin debris (black arrows).

**B.** five days after injection of anti-Egr2 siRNAs, several large axons appear as demyelinated (absence of myelin sheaths, black arrows), whereas a few other still remain myelinated (white arrows).

**C.** (detail of panel B): presence of a macrophage loaded with myelin debris immediately adjacent to a demyelinated axon.

* 1. **Effect of siRNAs targeting Dicer on gene expression and myelin maintenance**

Injection of anti-Dicer siRNAs in rat sciatic nerves resulted in active demyelination, as shown by semi-thin (Fig. 4B) and ultra-thin (Fig. 4C-D) sections, 5 days after treatment. Patterns of demyelination were not significantly different from those observed with anti-Egr2 siRNAs. These last results demonstrate that expression of Dicer is necessary for peripheral myelin maintenance in adult rat sciatic nerves.



**Figure 4 :** Effect of siRNAs targeting Dicer on gene expression and myelin maintenance.

**A.** Treatment of DRG neuron-Schwann cell co-cultures with anti-Dicer siRNAs results in a 72% decrease of Dicer mRNA.

**B.** On the semi-thin transverse section of a sciatic nerve 5 days after injection of anti-Dicer siRNAs, presence of a large area of demyelination.

**C-D:** Ultra-thin sections after injection of anti-Dicer siRNAs. Five days after injection of anti-Dicer siRNAs, presence of many large demyelinated (nude) axons (black arrows), together with macrophages loaded with myelin debris (white arrows).

1. **Discussion**

In the peripheral nerve, Egr2 is expressed in myelinating Schwann cells but remains expressed at high levels throughout life, which led to the hypothesis that Egr2 could also be involved in peripheral myelin maintenance. In experiments using inducible knockout of the Egr2 gene in mouse sciatic nerves (Cre-Lox excision of the Egr2 gene using estrogen receptor-induced Cre expression), Decker et al., showed that focal excision of the Egr2 gene after injection of hydroxytamoxiphen resulted in active demyelination in the injected area (Decker et al., 2006). This suggested that Egr2 constant expression in the peripheral nervous system is required for peripheral myelin maintenance. In the absence of Egr2 gene expression, a specific program of dedifferenciation would be engaged, leading to active myelin destruction. One possible mechanism for this active demyelination would be the reactivation of Egr2-silenced genes involved in maintaining the immature stage of Schwann cells (Decker et al., 2006).

We took advantage of self-delivery siRNAs to perform an *in situ* silencing of Egr2 mRNA in rat sciatic nerve without affecting the Egr2 gene itself. This approach combines several advantages: it avoids the generation of a transgenic rodent model, which is expensive and time-consuming; it also allows to deliver siRNAs without any transfection reagent, which could be toxic for the nerve cells, induce reactive demyelination by itself, and relatively inefficient for the transfection of primary quiescent cells. The self-delivery siRNAs have been shown to be efficient for *in vivo* inactivation of target genes.

However, this approach had been used in a limited number of *in vivo* experiments, having been tested in skin injections to down-regulate various target genes and in intra-peritoneal injections to treat an animal model of ovarian cancer ([DiFeo et al., 2009 ; Gonzalez-Gonzalez et al., 2010]). This type of approach had never been used to silence gene expression in peripheral nerves before. By this mean, we were able to confirm that Egr2 silencing results in active demyelination in the absence of non-specific effect of the siRNAs (no significant demyelination after treatment with non-targeting siRNAs). Our results confirm by a completely different technique the findings of Decker et al. (2006) suggesting that peripheral myelin maintenance is a dynamic phenomenon requiring constant expression of Egr2 in the peripheral nervous system.

These results also constitute a proof of concept for the use of self-delivery siRNAs to silence various genes in peripheral nerve Schwann cells *in vivo*, without the need for transgenic animals. Another mean to down-regulate specific mRNAs in peripheral nerves could have been to use small hairpin RNA (shRNAs) plasmids delivered by lentiviral particles into the peripheral nerves. We did not test this approach because it was previously shown that in adult nerves, lentiviral vectors were not efficient, as very few cells were infected (Özcelik et al., 2010). The siRNA induced demyelination we performed could also constitute a clever model of peripheral nerve demyelination, because chemically-induced PNS demyelination also results in significant axonal injury because of non-specific toxic effect of the chemical agents (as is the case with lyso-phosphatidyl-choline).

After showing that anti-Dicer siRNAs are able to down-regulate the target mRNA in cell cultures, we injected the siRNAs in rat sciatic nerves, which resulted here again in active demyelination. By contrast with Egr2, which is only expressed by Schwann cells in the peripheral nervous system, we cannot rule out that anti-Dicer siRNAs could also target Dicer in neurons, which could indirectly contribute to the observed demyelination through perturbed axon-Schwann cell interactions. Whatever the case, our results show that constant expression of Dicer in the peripheral nerves is necessary for peripheral myelin maintenance.

This suggests that this process is (at least in part) controlled by specific miRNAs, which could down-regulate genes associated with the immature stage of Schwann cells.

To conclude, constant expression of Egr2 is essential for peripheral myelin maintenance and Dicer expression is necessary for adult rat sciatic nerves myelin maintenance. Experimental studies are necessary to determine which miRNAs are involved in the regulation of this dynamic phenomenon.

**Bibliographic References**

Bremer J, Baumann F, Tiberi C, Wessig C, Fischer H, Schwarz P, Steele AD, Toyka KV, Nave KA, Weis J, Aguzzi A (2010) Axonal prion protein is required for peripheral myelin maintenance. Nat Neurosci 13: 310–318.

Bremer J, O’Connor T, Tiberi C, Rehrauer H, Weis J, Aguzzi A (2010) Ablation of Dicer from Murine Schwann Cells Increases Their Proliferation while Blocking Myelination. PLoS One 5: e12450.

Bremer M, Fröb F, Kichko T, Reeh P, Tamm ER, Suter U, Wegner M (2011) Sox10 is required for Schwann-cell homeostasis and myelin maintenance in the adult peripheral nerve. Glia 59: 1022–1032.

Canu MH, Carnaud M, Picquet F, Goutebroze L (2009) Activity-dependent regulation of myelin maintenance in the adult rat. Brain Res 1252: 45–51.

Decker L, Desmarquet-Trin-Dinh C, Taillebourg E, Ghislain J, Vallat JM, Charnay P (2006). Peripheral Myelin Maintenance Is a Dynamic process Requiring Constant Krox20 Expression. J Neurosci 26: 9771–9779.

DiFeo A, Huang F, Sangodkar J, Terzo EA, Leake D, Narla G and Martignetti JA. (2009) KLF6-SV1 Is a Novel Antiapoptotic Protein That Targets the BH3- Only Protein NOXA for Degradation and Whose Inhibition Extends Survival in an Ovarian Cancer Model Cancer Res 69: 4733-4741.

Ghislain J, Charnay P (2006) Control of myelination in Schwann cells: a Krox20 cis regulatory element integrates Oct6, Brn2 and Sox10 activities. EMBO Rep 7: 52–58.

Gonzalez-Gonzalez E, Speaker TJ, Hickerson RP, Spitler R, Flores MA, Leake D, Contag CH and Kaspar RL (2010) Silencing of Reporter Gene Expression in Skin Using siRNAs and Expression of Plasmid DNA Delivered by a Soluble Protrusion Array Device (PAD) Mol Ther 18: 1667–1674.

Hickerson RP, Flores MA, Leake D, Lara MF, Contag CH, Leachman SA and Kaspar RL (2011) Use of Self-Delivery siRNAs to Inhibit Gene Expression in an Organotypic Pachyonychia Congenita. J Invest Dermatol 131: 1037–1044.

Lara MF, Gonzalez-Gonzalez E, Speaker TJ, Hickerson RP, Leake D, Milstone LM, Contag CH, and Kaspar RL (2012) Inhibition of CD44 Gene Expression in Human Skin Models, Using Self-Delivery Short Interfering RNA Administered by Dissolvable Microneedle Arrays. Hum Gene Ther 23: 816–823.

Livak KJ and Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real- Time Quantitative PCR and the 2DDCT Method. Methods 25: 402–408.

Murphy P, Topilko P, Schneider-Maunoury S, Seitanidou T, Baron-Van Evercooren A, Charnay P (1996) The regulation of Krox-20 expression reveals important steps in the control of peripheral glial cell development. Development 122:2847–2857.

Özcelik M, Cotter L, Jacob C, Pereira JA, Relvas J, Suter U, and Tricaud N (2010) Pals1 Is a Major Regulator of the Epithelial-Like Polarization and the Extension of the Myelin Sheath in Peripheral Nerves. J Neurosci 30:4120–4131.

Pereira JA, Baumann R, Norrmén C, Somandin C, Miehe M, Jacob C, Lühmann T, Hall- Bozic H, Mantei N, Meijer D, Suter U. (2010) Dicer in Schwann cells is required for myelination and axonal integrity. J Neurosci 30 : 6763-6775.

Svenningsen A.F, Shan W.S, Colman D.R, Pedraza L (2003) Rapid Method for Culturing Embryonic Neuron–Glial Cell Cocultures. J Neurosci Res 72: 565–573.

Timmerman V, De Jonghe P, Ceuterick C, De Vriendt E, Lofgren A, Nelis E, Warner LE,

Lupski JR, Martin JJ, Van Broeckhoven C (1999) Novel missense mutation in the early growth response 2 gene associated with Dejerine-Sottas syndrome phenotype. Neurology 52: 1827–1832.

Topilko P, Levi G, Merlo G, Mantero S, Desmarquet C, Mancardi G, Charnay P (1997) Differential regulation of the zinc finger genes Krox-20 and Krox-24 (Egr-1) suggests antagonistic roles in Schwann cells. J Neurosci Res 50: 702–712.

Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi AB, Seitanidou T, Babinet C, Charnay P (1994) Krox-20 controls myelination in the peripheral nervous system. Nature 371: 796 –799.

Verrier JD, Semple-Rowland S, Madorsky I, Papin JE, and Notterpek L (2010) Reduction of Dicer Impairs Schwann Cell Differentiation and Myelination. J Neurosci Res 88: 2558–2568.

Warner LE, Mancias P, Butler IJ, McDonald CM, Keppen L, Koob KG, Lupski JR (1998) Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. Nat Genet 18: 382–384.

Woodhoo A, Alonso MB, Droggiti A, Turmaine M, D’Antonio M, Parkinson DB, Wilton DK, Al-Shawi R, Simons P, Shen J, Guillemot F, Radtke F, Meijer D, Feltri ML, Wrabetz L, Mirsky R, Jessen KR (2009) Notch controls embryonic Schwann cell differentiation, postnatal myelination and adult plasticity. Nat Neurosci 12: 839-847.

Yun B, Anderegg A, Menichella D, Wrabetz L, Feltri ML, and Awatramani R. (2010) MicroRNA-Deficient Schwann Cells Display Congenital Hypomyelination. J Neurosci 30: 7722–7728.

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