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Schwann Cell Plasticity is Regulated by a Weakened Intrinsic Antioxidant Defense System in Acute Peripheral Nerve Injury

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Abstract—The biological effects of the transcription factor NF-E2-related factor 2 (Nrf2) in acute peripheral nervous system (PNS) injury have not been adequately elucidated. By analyzing the results of Nrf2 knockout and Nrf2 activation experiments, we found the following: (1) the antioxidant system was rapidly inactivated after acute PNS injury in a partly Nrf2-dependent manner, giving rise to a temporary state of oxidative stress, and then slowly and partially recovered following regeneration. (2) Nrf2 knockout promoted the reprogramming and proliferation of Schwann cells and inhibited myelination, as well as the redifferentiation of repair Schwann cells. (3) Dimethyl fumarate had no influence on the myelination of regenerated nerves. (4) Nrf2 functional regulation was able to regulate the redox status of nerves by changing the levels of target antioxidants and reactive oxygen species (ROS) at the same time, without altering the balance between them. In conclusion, the Nrf2-antioxidant system was temporarily inactivated in injured nerves, promoting Schwann cell reprogramming and proliferation, and its functional recovery was essential for Schwann cell redifferentiation and myelination.

Key words: transcription factor NF-E2-related factor 2, acute peripheral nerve injury, Schwann cell, dimethyl fumarate.

INTRODUCTION

Peripheral nervous system (PNS) axons regenerate following axonal degeneration, proliferation of Schwann cells and activation of macrophages after injury (Jessen and Mirsky, 2008). Schwann cells constitute over 80% of the cells in the adult PNS (Kim et al., 2013). Injury induces Schwann cells to temporarily proliferate and transdifferentiate into specialized repair cells, which guide the growth of the injured axons and recruit macrophages to support PNS axon regeneration (Kim et al., 2013). These repair cells achieve maximum proliferation at approximately the 5th day after nerve crush (Gaudet et al., 2011), and are the major contributor to myelin breakdown, responsible for the clearance of 40%-50% of the myelin debris during the first 5–7 days after injury (Perry et al., 1995).

The redox state in cells and its relation to cell differentiation form the core of an emerging research field in the study of nervous system development, aging, demyelinating diseases and nerve injury (Ravera et al., 2015; Xiong et al., 2015; Olguin-Albuerne and Moran, 2017; Vasilaki et al., 2017). Under physiological conditions, reactive oxygen species (ROS) and counteracting antioxidants maintain an equilibrium, which is termed "redox homeostasis", and any type of tissue injury can switch on redox signals either temporarily or permanently; an injury can switch on oxidative stress by activating ROS and/or inactivating antioxidants, or it can switch on reductive stress by inactivating ROS and/or providing an uninterrupted supply of reductive molecules (Narasimhan and Rajasekaran, 2015).

Antioxidants, including catalase (Cat), NAD(P)H quinone dehydrogenase 1 (Nqo1), glutathione synthetase (Gss), glutamate-cysteine ligase catalytic subunit and modulatory subunit (Gclc, Gclm),

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Abbreviations: Cat, catalase; DMF, dimethyl fumarate; Gclc, Gclm, glutamate-cysteine ligase catalytic subunit and modulatory subunit; Gss, glutathione synthetase; IHC, immunohistochemistry; Keap1, Kelch-like ECH-associated protein-1; MBP, myelin basic protein; Meth, Methocel; Nqo1, NAD(P)H quinone dehydrogenase 1; Nrf2, the transcription factor NF-E2-related factor 2; PBS, phosphate-buffered saline; PNS, peripheral nervous system; ROS, reactive oxygen species; Sod, superoxide dismutase; Srxn1, sulfiredoxin-1; WT, wild-type.

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superoxide dismutase (Sod), and sulfiredoxin-1 (Srxn1), are the first line of defense against ROS (Bell et al., 2015). The transcription factor NF-E2-related factor 2 (Nrf2) is a master regulator of antioxidant defenses and is ubiquitously expressed; Ser40 phosphorylation is important for Nrf2 activation and translocation into the nucleus (Dodson et al., 2015). The Nrf2 system acts as a cellular sensor for damage caused by ROS (Kumari et al., 2018). Changes in redox status can activate or inactivate Nrf2, promoting a reductive or oxidative environment (Handy and Loscalzo, 2017). Deletion of Nrf2 impaired functional recovery and decreased axonal remyelination after peripheral nerve injury (Zhang et al., 2013), but the underlying molecular mechanism is unclear. Exploring the role of ROS and antioxidants in nerve injury may provide a deeper understanding of the regenerative ability of peripheral nerves and provide potential therapeutic targets to treat nerve injury.

Our study provides a molecular mechanism for Schwann cell plasticity in PNS injury and tests the therapeutic effect of an Nrf2 activator, dimethyl fumarate (DMF). We show that activity of the Nrf2-antioxidant pathway after injury was rapidly decreased to promote the reprogramming of Schwann cells into repair cells and was then slowly and partially recovered to promote the redifferentiation and myelination of repair cells. Persistent shutdown of Nrf2 activity impeded Schwann cell redifferentiation and myelination.

EXPERIMENTAL PROCEDURES

Mouse line preparation

$Nrf2^{-/-}$ and $Nrf2^{+/+}$ CD1/ICR mice were obtained from Dr. Thomas W. Kensler (Johns Hopkins University, Baltimore, MD). The wild-type (WT) mice were from the C57BL/6 strain background and were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd.

The genotypes of $Nrf2^{-/-}$ and $Nrf2^{+/+}$ mice were confirmed by PCR analysis of DNA from tail biopsies; the primers used for genotype detection are listed in Table 1. All experimental animals were 8- to 10-month-old female mice and were bred in a specific-pathogen-free animal room. All animal experiments were carried out in compliance with local and international guidelines.

Animal surgery and drug administration

The animal surgery was conducted as described previously with some modifications (Homs et al., 2011). Mice were anesthetized by intraperitoneal injection of 2% pentobarbital sodium (3.3 μL/g). The sciatic nerve was exposed at the mid-thigh and crushed once for 30 s using forceps after skin and muscle dissection.

The WT mice that received surgery were given DMF suspended in 1.5% Methocel at a dosage of 75 mg/kg twice a day via oral gavage from Day 6 to Day 22 after injury, when measurements and analyses were performed, and the control group was given 1.5% Methocel at the same time (Fig. 8A).

Antibodies and chemicals

The primary antibodies included anti-NRF2 (Abcam, ab62352 for immunohistochemistry (IHC) staining, ab31163 for Western blot), anti-NQO1 (Abcam, ab2346), anti-GSS (Abcam, ab91591), anti-GCLC (Abcam, ab53179), anti-GCLM (Proteintech Group, 14241-1-AP), anti-SOD1 (Santa Cruz, sc-8637), anti-β-

Table 1. Primer sequences

qRT-PCR Primer	Forward	Reverse
P0	CTGGTCCAGTGAATGGGTCT	CATGTGAAAGTGCCGTTGTC
Periaxin	AGGAGCTCTGGAGGTGTCTGG	TCTTGAGTGATGGCCTTTTC
Mbp	AATCGGCTCACAAGGGATTCA	TCCTCCCAGCTTAAAGATTTTGG
Krox24	CAGCGCCTTCAATCCTCAAG	AGCGGCCAGTATAGGTGATG
P75	CAACCAGACCGTGTGTGAAC	GGAGAACACGAGTCCTGAGC
CyclinD1	GCGTACCCTGACACCAATCT	CACAACTTCTCGGCAGTCAA
Shh	AAAGCTGACCCCTTTAGCCTA	TTCGGAGTTTCTTGTGATCTTCC
Gdnf	GATATTGCAGCGGTTCCTGT	AACATGCCTGGCCTACTTTG
Il1	AGTTGACGGACCCCAAAAG	CTTCTCCACAGCCACAATGA
Tnfa	ACGGCATGGATCTCAAAGAC	GTGGGTGAGGAGCACGTAGT
Mcp1	AGGTCCCTGTCATGCTTCTG	GCTGCTGGTGATCCTCTTGT
Cd68	GGATTGGATTGAGGAAGGAACTG	GCCGCATGGCAGAGATG
Nrf2	GTTCTCCGCTGCTCGGACTA	GGTGGCAACTCCAAGTCCAT
Srxn1	GACGTCCTCTGGATCAAAG	GCAGGAATGGTCTCTCTCTG
Cat	GGCACATGAATGGCTATGGA	CTTCCTGCCTCTCCAACAGG
Gclm	GCTGTGTGATGCCACCAGAT	CGAGTACCTCAGCAGCCACA
Gss	TGGAGCAGCTGAAGGACAGT	TACACTGGACCACTTGGGCA
Gclc	CCAACCATCCGACCCTCTG	TGTTCTGGCAGTGTGAATCC
Nqo1	CATCCTGCGTTTCTGTGGCT	TCTCCTCCCAGACGGTTTCC
Sod1	GCAGGGAACCATCCACTTCG	CCTGCACTGGTACAGCCTTG
Gapdh	ATGACATCAAGAAGGTGGTG	CATACCAGGAAATGAGCTTG
Actb	GTGCTATGTTGCTCTAGACTTCG	ATGCCACAGGATTCCATACC
Genotype detection PCR Primer	NRF5 TGGACGGGACTATTGAAGGCTG (in *Nrf2* gene)	
	NAS GCCGCCTTTTCAGTAGTAGGAGG (in *Nrf2* gene)	
	NLACZ GCGGATTGACCGTAATGGGATAGG (in *LacZ* gene)	

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