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PERIPHERAL AXON REGROWTH: NEW MOLECULAR APPROACHES

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Abstract—Peripheral nerves, essential connections between the brain, spinal cord and body, do not regenerate as well as generally reported. Identifying new strategies to facilitate regeneration is essential to reversing neurological deficits from nerve injuries or disease. This review will discuss several selected and novel molecular insights into peripheral nerve trunk repair and axon regrowth that have the potential to improve regenerative success. Of particular interest is the phosphatidylinositol 3-kinase (PI3K)-Akt pathway in peripheral neurons, inhibited by the constitutively expressed phosphatase tumor suppressor PTEN. Knockdown or inhibition of PTEN is associated with robust sprouting of adult sensory neurons *in vitro* and *in vivo*, additive to the accelerated outgrowth offered by the preconditioning effect. This sprouting response, if spatially and temporally constrained, may provide potent regrowth initiation, of interest in otherwise untreatable nerve damage.

Key words: peripheral nerve regeneration, PTEN, nerve injury.

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INTRODUCTION

Peripheral nerve injury is an under-appreciated clinical problem, even though it is more common than injury to the central nervous system (CNS). For example, 5% of multiple trauma patients have root, plexus or peripheral nerve injuries, often involving more than one territory in the context of multiple trauma, compared to 1.8% with spinal cord injury (Noble et al., 1998; Robinson, 2000; Hasler et al., 2011). This prevalence does not include the large numbers of peripheral nerve lesions repaired in emergency wards in patients without multiple trauma. Unfortunately, despite 150 years of experience in surgical repair of peripheral nerves, functional recovery afterward is disappointing (Johnson et al., 2005). Peripheral nerve regeneration following trauma or nerve disease is not as successful as generally considered, even when compared to that of the CNS (Smith et al., 2009; Tuszynski and Steward, 2012). For example, patients with severe axonal Guillain–Barre syndrome may not experience recovery because of extensive and proximal axonal damage, despite intact nerve continuity. In this context, investigation into methods of facilitating regeneration by peripheral axons is critical.

Lacerations (transections), stretch and compression (crush) injuries of peripheral axons are the most common types of injury (Burnett and Zager, 2004). To repair these lesions, rapid initiation of regeneration, allowing target reinnervation, is essential. If not accomplished, prolonged denervation of end organ

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Abbreviations: BDNF, brain-derived growth factor; cAMP, cyclic adenosine monophosphate; CGRP, calcitonin gene-related peptide; CNS, central nervous system; CREB, cAMP response element-binding protein; CSPG, chondroitin sulfate proteoglycan; DRG, dorsal root ganglia; ECM, extracellular matrix; ERKs, extracellular signal-regulated protein kinases; GAP-43, growth-associated protein-43; GSK3, glycogen synthase kinase 3; IL-6, interleukin-6; JAK-STAT, Janus kinase/signal transducers and activators of transcription; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; MAGs, myelin-associated glycoproteins; MCP-1, monocyte chemoattractant protein-1; MMPs, matrix metalloproteinases; NAD, nicotinamide adenine dinucleotide; Nedd4, neural precursor cell-expressed developmentally down-regulated gene 4; NGF, nerve growth factor; Nmnat, nicotinamide mononucleotide adenylyltransferase; Nmnat-1, nicotinamide mononucleotide adenylyl transferase 1; NT, neurotrophin; NTR, neurotrophin receptor; NRGs, neuregulins; PI3K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol 3,4,5 triphosphate; PKC, protein kinase C; PLC-γ, phospholipase Cγ; RAGs, regeneration-associated genes; RGCs, retinal ganglion cells; ROK, RHO kinase; SC, Schwann cell; SOCS3, suppressor of cytokine signaling 3; Trk, tropomyosin-related kinase; UPS, ubiquitin–proteasome system.

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targets and of the distal denervated nerve stumps may be associated with structural and molecular changes that hinder appropriate regrowth of axons (Fu and Gordon, 1997; Burnett and Zager, 2004). Regrettably, despite modern improvements in nerve trunk reconstruction, motor, sensory and autonomic system recovery is often incomplete, from failed reinnervation of targets or incomplete collateral sprouting of uninjured neighboring axons.

A number of neurobiological events are required to prepare axons to re-enter denervated nerve trunks. Prior to regeneration of axons through to the distal stump, breakdown of myelin, a potential regenerative inhibitor, and proliferation of migrating Schwann cells are required, all part of a process known as Wallerian or Wallerian-like degeneration. Ideal axonal regeneration proceeds at approximately one mm per day but this rate, albeit slow to begin with, is rarely achieved in humans or after severe transection injuries (Brushart, 2011). Among the many sequential steps that ensue during nerve regrowth, maturation of new axons is essential. Remyelination of new axons with shortened internodes develops within 2 weeks of regeneration (Burnett and Zager, 2004). Subsequent reinnervation of target organs may have its own distinct challenges. Overall, while there are many facets of the biology of peripheral axon regrowth, this review will highlight only a few, exploring the potential role of some exciting but hitherto unexplored molecules and their pathways that may have a significant bearing on regenerative success.

WALLERIAN AND WALLERIAN-LIKE DEGENERATION

In 1850, Augustus Waller described the morphological changes associated with degeneration of axons separated from their cell body following nerve injury (Waller, 1850). This process, termed Wallerian degeneration, refers to the sequence of events following nerve transection. Similar events that damage axons after blunt or crush injury are termed Wallerian-like degeneration. The process was further elucidated by RamonY Cajal and others (Ramon y Cajal, 1928; Fenrich and Gordon, 2004). Following injury, calcium influx into the axon activates axonal proteases necessary for the degeneration of the axoplasm and axolemma (Schlaepfer and Bunge, 1973; Stoll et al., 1989, 2002a). The ubiquitin–proteasome system (UPS) is also important for this process as it has been shown that inhibitors to this pathway can delay Wallerian degeneration (Mack et al., 2001). The Wlds mutant mouse is a unique phenotype that exhibits delayed Wallerian degeneration, associated with a genetic alteration in the synthesis of proteins that regulate axon breakdown (Mack et al., 2001; Ehlers, 2004). The mechanism involves the actions of a fusion protein that elevates nicotinamide mononucleotide adenylyltransferase (Nmnat) and downstream nicotinamide adenine dinucleotide (NAD) and SIRT1 (Araki et al., 2004; Luo and O'Leary, 2005; Sasaki et al., 2006; Conforti et al., 2009; Sasaki and Milbrandt, 2010). Calcium influx, a key feature of degeneration, is also altered in the slow Wallerian response phenotype (Adalbert et al., 2012). Two Nmnat isoforms impact Wallerian degeneration in different ways. For example, in the Wlds mouse, the chimeric protein fuses an N-terminal sequence from the Ube4b multiubiquitination factor with the NAD-synthesizing enzyme nicotinamide mononucleotide adenylyltransferase 1, Nmnat-1. Both the Ube-4b VCP-binding sequence and Nmnat enzymatic action are required to protect axons from degeneration after a nerve section (Conforti et al., 2009). In contrast, in uninjured wild-type axons, loss of Nmnat-2 leads to Wallerian degeneration, an outcome that is not rescued by Nmnat-1 or 3, indicating a requirement for axon maintenance (Gilley and Coleman, 2010).

Wallerian and Wallerian-like degeneration critically depend on Schwann cell (SC) participation. A change in gene expression is initiated: the SCs alter their behavior from synthesizing myelin to a more plastic and regenerative phenotype that instead initiates myelin breakdown. Proliferating plastic SCs dedifferentiate and upregulate a cascade of regeneration-related genes that include growth associated protein-43 (GAP-43), that mediates SC proliferation, and genes for neurotrophic factors and receptors (LeBlanc and Poduslo, 1990; Fu and Gordon, 1997; Boyd and Gordon, 2003; Chen et al., 2005). Dedifferentiated SCs also scavenge or self-digest myelin debris and proliferate along the basal lamina of degenerating axons, forming bands of Bungner that support and guide the axons that are regenerating from the proximal stump (Stoll et al., 1989; Liu et al., 1995). Activation of p38 MAPK is sufficient to induce myelin breakdown and promote dedifferentiation of SCs by regulating Krox 20 and c-Jun (Hossain et al., 2012; Yang et al., 2012). The proliferation of SCs across the injury site also appears to be aided by nerve growth factor (NGF) (Anton et al., 1994). Recently, the transcription factor c-Jun has been shown to be a global transcriptional regulator in the SC response to injury (Arthur-Farraj et al., 2012). When c-Jun is inactivated the regeneration response is significantly impaired; specifically its role involves the activation of repair programs in SCs and the inhibition of myelin synthesis. Following injury, neuregulins (NRGs) released from axons bind to ERBB2 receptors on SCs (Michailov et al., 2004). While not required for SC proliferation, NRG1 type III is a key signal for later differentiation and remyelination of axons following injury in adults. Recent work has also identified an isoform released from SCs, NRG1 type I that acts locally in the absence of axons and is also essential for SC differentiation and efficient remyelination (Stassart et al., 2013). Thus, both axonally derived and SC synthesized NRGs contribute to remyelination following injury.

A limited inflammatory response also plays a role in axonal degeneration of the nerve stump; macrophages infiltrate the lesion site by 48 h after injury and by day 4 are in the distal stump (Bruck, 1997). This is in response to cytokines and chemokines released from SCs (Perry et al., 1987; Tofaris et al., 2002). The macrophages remove the majority of the myelin debris