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Commentary

Pathways regulating modality-specific axonal regeneration in peripheral nerve

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ABSTRACT

Following peripheral nerve injury, the distal nerve is primed for regenerating axons by generating a permissive environment replete with glial cells, cytokines, and neurotrophic factors to encourage axonal growth. However, increasing evidence demonstrates that regenerating axons within peripheral nerves still encounter axonal-growth inhibitors, such as chondroitin sulfate proteoglycans. Given the generally poor clinical outcomes following peripheral nerve injury and reconstruction, the use of pharmacological therapies to augment axonal regeneration and overcome inhibitory signals has gained considerable interest. Joshi et al. (2014) have provided evidence for preferential or modality-specific (motor versus sensory) axonal growth and regeneration due to inhibitory signaling from Rho-associated kinase (ROCK) pathway regulation. By providing inhibition to the ROCK signaling pathway through Y-27632, they demonstrate that motor neurons regenerating their axons are impacted to a greater extent compared to sensory neurons. In light of this evidence, we briefly review the literature regarding modality-specific axonal regeneration to provide context to their findings. We also describe potential and novel barriers, such as senescent Schwann cells, which provide additional axonal-growth inhibitory factors for future consideration following peripheral nerve injury.

Peripheral nerve injury, axonal regeneration, and functional recovery

Functional recovery following general nerve reconstruction is often associated with poor results. Recovery following axonal regeneration is largely driven by the ability of neurons to regenerate their axons through the nerve "pathway" to reinnervate their end-organ "target". Axonal regeneration following injury is promoted by neurotrophic factors, cytokines, and axon adhesion molecules produced by glial cells within nerve and muscle, as well as axonal regenerative programs initiated by peripheral neurons. In addition, sensory and motor axons are guided to their specific end-organ target (specificity) through both pathway and target-derived factors. These factors are spatially and temporally produced and regulated following injury (Boyd and Gordon, 2003). *Recovery, then, is dependent upon the number of motor and sensory axons that successfully regenerate through the pathway and are properly matched with their respective motor endplates and sensory receptors in a timely manner.*

Following injury, damaged peripheral nerve undergoes a process described by Wallerian degeneration, which prepares the distal nerve facilitating axonal regeneration. The blood–nerve barrier breaks down uniformly along the nerve within days of injury allowing large molecules to cross and enter the endoneurial space containing axons and Schwann cells (SCs) (Bruck, 1997; Olsson, 1966; Rotshenker, 2003; Seitz et al., 1985, 1989). Concurrently, SCs dedifferentiate or transdifferentiate into a pro-regenerative phenotype within days. This trans-differentiation is characterized by changes in mitogen and neurotrophic factor expression and phagocytic activity (Fu and Gordon, 1997; Jessen and Mirsky, 2002, 2005; Xu et al., 2008; You et al., 1997).

In contrast to the central nervous system, where glial cells direct scarring and the persistence of myelin-based inhibitory proteins, SCs phagocytose myelin debris (Kazakova et al., 2006; Lai, 2005; Lyons et al., 2005). This dramatic change in the nerve pathway for regenerating axons is a major component responsible for facilitating axonal growth after injury in the peripheral nervous system compared to the central nervous system. However, increasing evidence demonstrates that this pathway still contains, and actively expresses, inhibitory components to axonal growth, such as chondroitin sulfate proteoglycans (CSPGs) (Zuo et al., 1998a, 1998b, 1998c, 2002). A recent study by Joshi et al. considered the role of CSPGs on modality-specific (motor vs sensory) axonal regeneration. They demonstrated differential

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axonal regeneration of motor axons through the nerve pathway due to CSPG signaling (Joshi et al., 2014). Their evidence suggests a role for CSPGs in axonal specificity for end-organ targets and modality-specific barriers to successful axonal regeneration through the nerve pathway. We present a general background on factors influencing modality-specific axonal regeneration to give context to their study and future directions.

Specificity of axonal regeneration

Regenerating axons are significantly influenced by regenerative pathway cues and their innate signaling pathways. Sensory and motor neurons express different levels of a wide range of receptors (for example, tyrosine kinases receptors) resulting in modality-specific regulation of ligand-induced signaling (Boyd and Gordon, 2003). This signaling provides a mechanism to regulate axon growth to appropriate targets. The choice axons make to grow into a motor or sensory pathway and end-organ target is generally referred to as axonal specificity.

Much of what is known regarding modality-specific axonal regeneration is provided by animal models. The femoral nerve injury model is ideally suited for studying modality-specific axonal regeneration. In the femoral nerve model, motor and sensory fibers demonstrate a predictable topography distally with branches into the cutaneous saphenous nerve and the quadriceps motor branch (Brushart, 1988, 1993; Brushart et al., 1998; Brushart and Seiler, 1987; Madison et al., 1996; Martini et al., 1994). When given equal access to motor and sensory pathways, regenerating motor axons preferentially regenerate down a terminal motor pathway and reinnervate its muscle target in what has been termed preferential motor reinnervation (PMR) (Brushart, 1988, 1993). In a series of studies, Brushart, Madison, and colleagues have demonstrated that regenerating motor axons (Brushart, 1988, 1993; Madison et al., 1996), as well as afferents from the muscle spindle, preferentially regenerate down the quadriceps' motor pathway even when deliberate attempts at mismatching sensory and motor paths are made (Madison et al., 1996). These studies have provided a major framework to elucidate the impact of principal components guiding axons to their specific targets (Fig. 1). The components involved have included specific glial cells intrinsic to the nerve pathways (sensory and motor SCs) (Hoke et al., 2006), tropic influence from end-organs (Robinson and Madison, 2004), and basal lamina proteins and architecture inherent to sensory and motor nerves (Nichols et al., 2004). While axonal guidance is directed, to a degree, by all these factors, it is of great interest to identify a potentially predominant mechanism to establish translational efforts to improve functional recovery.

Schwann cells

As Schwann cells (SCs) are the primary intrinsic mediators of nerve regeneration in the peripheral nervous system, they play an extensive role in regulation of axonal regeneration. In fact, the absence of SCs following nerve injury and during regeneration severely limits the quality and extent of axonal regeneration (Hall, 1986a, 1986b). The protein expression of SCs supports axons through the deposition of basal lamina, excretion of trophic factors, and adhesion molecules that facilitate regeneration after nerve injury (Araki and Milbrandt, 1996; Bunge, 1994; Bunge et al., 1986; Friedman et al., 1992; Levi and Bunge, 1994). While SCs are usually described as myelinating and non-myelinating (Jessen and Mirsky, 2002), there is strong evidence for the existence of SC phenotypes that are distinct in motor and sensory nerves (Hoke et al., 2006; Jesuraj et al., 2012).

The concept of modality-specific SC phenotypes resulted from the original experiments considering PMR (Brushart, 1993). These sensory- and motor-derived SCs were first described based on gene expression differences between cutaneous nerve and ventral root SCs. The variations in gene and protein expression between these SC phenotypes directly support modality-matched axonal growth and regeneration (i.e. sensory neurons extend longer and more axons in sensory nerve-derived SC environments) (Hoke et al., 2006; Marquardt and Sakiyama-Elbert, 2014). In fact, the delivery of neurotrophic factors, such as glial cell-line derived neurotrophic factor (GDNF), guides and regulates the differentiation and phenotype of sensory- and motor-derived SCs (Jesuraj et al., 2014). The delineation of sensory- and motor-derived SCs has also been further refined by spatial arrangement, where SC phenotypes defined by growth factor expression vary according to a central–peripheral location (Brushart et al., 2013).

Beyond SC phenotype, the "state" of SCs can greatly affect regenerative potential. Prolonged axonal denervation of nerve leads SCs to no longer divide and possibly enter a quiescent state. This proposed state of quiescence in SCs has not been specifically characterized in detail, as there are no specific markers to identify this state, but is generally described to result in less axonal growth support and cell division (Sulaiman and Gordon, 2000, 2002). In addition to the state of *quiescence*, aging and stressful environments can significantly alter SCs' state inducing *senescence*. Our lab recently described the accumulation of senescent SCs, defined by expression of specific senescence markers (Ben-Porath and Weinberg, 2005), following long nerve graft reconstruction (Saheb-Al-Zamani et al., 2013). Cellular senescence is a state of permanent growth arrest distinct from *quiescence*, which is an exit from the cell cycle and reversible. Senescent cells not only cease to proliferate, they also undergo a change in protein expression called the senescence associated secretory phenotype (SASP). The SASP of senescent cells is replete with altered expression of chemokines, cytokines, growth factors, and extracellular remodeling enzymes that radically alter the tissue microenvironment (Coppe et al., 2010; Pazolli and Stewart, 2008). Given this radical change in protein expression, SC senescence may play a pivotal role in axonal regeneration after peripheral nerve injury. Due to the known association of limited axonal regeneration with senescent SC accumulation, increased production of axonal-growth inhibitors could drive this axonal arrest (Saheb-Al-Zamani et al., 2013). It is yet to be determined if senescence can result in differences in axonal regeneration modalities.

End-organ

While SC phenotype provides excellent intrinsic evidence to support regenerative modality regulation (Redett et al., 2005), other work suggests that tropic support derived from nerves' direct connection with muscle ("target-derived") is predominant (Madison et al., 2009; Robinson and Madison, 2004). Using the femoral nerve model, Madison and Robinson have shown that motor axons lose PMR and regenerate increasing numbers of motor axons into the sensory saphenous branch when the motor quadriceps branch is severed from its muscle end-organ or the regenerative distance to muscle increased (Robinson and Madison, 2004). Additionally, selectively removing Schwann cells distal to a nerve lesion while maintaining end-organ contact results in a maintenance of PMR (Madison et al., 2009). These studies provide significant evidence that tropic support from end-organs determine the specificity of axonal regeneration. However, until recently understanding the form

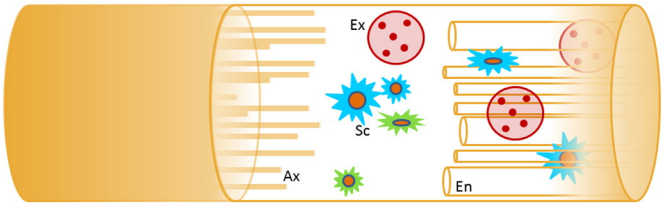

Fig. 1. Pathways influencing modality-specific axonal regeneration. Within the regenerative pathway, axons (Ax) encounter glial cells (Schwann cells (Sc)), tropic factors derived from end-organs (exosomes (Ex)), and endoneurial architecture (En). These components modulate the growth response of sensory and motor neurons and their axons as they respond to this environment.