Expression of the Wnt signaling system in central nervous system axon guidance and regeneration

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Keywords: axon guidance, topographic mapping, spinal cord injury, axon regeneration, Wnt, Ryk, gradient

Wnt signaling is essential for axon wiring throughout the development of the nervous system in vertebrates and invertebrates. In rodents, Wnts are expressed in gradients that span the entire anterior-posterior (A–P) axis in the spinal cord and the medial-lateral axis in the superior colliculus. In the brainstem, Wnts are expressed in more complex gradients along the A–P axis. These gradients provide directional information for axon pathfinding and positional information for topographic mapping and are detected by cell polarity signaling pathways in the growth cone. The gradient expression of Wnts and the coordinated expression of Wnt signaling systems are regulated by mechanisms which are currently unknown. Injury to the adult spinal cord results in the re-induction of Wnts in multiple cell types around the lesion site and their signaling system in injured axons. The re-induced Wnts form gradients around the lesion site, with the lesion site being the peak. The re-induced Wnts may be responsible for the well-known retraction of descending motor axons through the receptor Ryk (related receptor tyrosine kinases). Wnt signaling is an appealing new therapeutic target for CNS repair. The mechanisms regulating the re-induction are unknown but will be informative for therapeutic design.

GRADED EXPRESSION OF Wnts GUIDE AXONS ALONG THE ANTERIOR–POSTERIOR AXIS IN CNS DEVELOPMENT

During development of the brain and the spinal cord, growing axons are directed by multiple guidance cues to their syncytic targets forming axon networks along the major anatomical axes, anterior–posterior (A–P), dorsal–ventral axes, and interior–superior. Axons along the A–P or rostrocaudal axis of the spinal cord establish the circuitry required for supraspinal control of motor function as well as for relaying sensory information to the brain. For long-distance projections, such as along the A–P axis, global gradients of molecular cues are necessary. Understanding the development of these long-distance connections between brain and spinal cord may provide insights for developing useful therapeutic interventions to repair these axons after injury.

The vertebrate commissural axons within the spinal cord are an excellent specimen to identify global A–P guidance cues. These axons have an initial dorsal–ventral trajectory to reach and cross the midline. Secreted attractants, Netrin-1, and Sonic Hedgehog (Shh) from the ventral midline, the floor plate, provide the commissural axons decussate and post-crossing commissural axons are mediated by both apical–basal and PCP signaling pathways (Wolf et al., 2008). In addition, planar cell polarity (PCP) signaling is required for anterior–posterior positioning of midbrain monoaminergic axons (Fenstermaker et al., 2010; Blakely et al., 2011), subpopulations of which contribute to supraspinal motor control (Holstege and Kuypers, 1987; Jordan et al., 2008). Orientation of midbrain monoaminergic axons also depends on the expression of components of the PCP signaling pathway (Fenstermaker et al., 2010).

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While work in the vertebrate spinal cord and invertebrate systems has demonstrated a role for Wnt morphogens in axon pathfinding, the vertebrate visual system utilizes Wnt expression gradients as positional cues to establish topographic connections. Wnt3 mRNA expression gradients in the developing mouse spinal cord at E11.5 and P0. At E11.5, post-crossing commissural axons (red) are attracted anteriorly up a gradient of Wnt4 (purple) through the receptor Frizzled3. At P0, descending corticospinal motor axons (red) are repelled posteriorly down a gradient of Wnt3a (purple). At P0, descending corticospinal motor axons (red) are repelled posteriorly down a gradient of Wnt3a (purple). At P0, ascending sensory and descending motor axons are guided growth of ascending sensory and descending motor axons. Wnt–Ryk signaling after spinal cord injury reduces the retraction of lesioned corticospinal axons from the injury site while concurrently promoting the sprouting of corticospinal axon collaterals within the spared spinal cord tissue (Liu et al., 2008). A re-expressed Wnt gradients form gradients that peak at the lesion sites and decreases both anteriorly and posteriorly relative to the lesion sites. In addition to Wnts, other guidance molecules have also been found to be re-induced by spinal cord injury. Class 3 semaphorins and ephrins are both re-expressed in spinal cord lesion, although the role of these guidance cues in the injury response has not been defined (Pasterkamp et al., 1999; Bundesen et al., 2003; Benson et al., 2005; Carmichael et al., 2005; Pasterkamp and Verhaagen, 2006). Wnt expression at the spinal cord injury site is coupled with re-expression of the repulsive receptor Ryk in corticospinal motor neurons where it is trafficked to the distal tip of the lesioned corticospinal axons (Liu et al., 2008). Inhibition of Wnt–Ryk signaling after spinal cord injury reduces the retraction of lesioned corticospinal axons from the injury site while concurrently promoting the sprouting of corticospinal axon collaterals within the spared spinal cord tissue (Liu et al., 2008). Another study showed similar results using a contusion model (Miyashita et al., 2009). Therefore, the re-induced Wnt gradients may be responsible for the long-range retraction of corticospinal tract axons following spinal cord injury.

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**Wnt GRADIENTS IN TOPOGRAPHIC MAPPING**

While work in the vertebrate spinal cord and invertebrate systems has demonstrated a role for Wnt morphogens in axon pathfinding, the vertebrate visual system utilizes Wnt expression gradients as positional cues to establish topographic connections. Wnt3 mRNA and protein are both expressed in a decreasing gradient along the medial (high) to lateral (low) axis within the developing chick tectum and mouse superior colliculus (Schmitt et al., 2006). This gradient of Wnt3 is used to establish a retinotopic map through the expression of the repulsive Wnt receptor Ryk in a ventral to dorsal decreasing gradient in retinal ganglion cells (Schmitt et al., 2006). The Wnt3 gradient was proposed to counterbalance the expression of Wnt5a (purple). The Wnt3 gradient was proposed to counterbalance the expression of Wnt5a (purple). Therefore, Wnt signaling may have a conserved role in topographic map formation. How Wnt expression gradients are established in these brain areas are currently unknown.

**RE-INDUCED Wnts FORM DIFFERENT GRADIENTS AFTER INJURY**

In the intact adult spinal cord, Wnt mRNA expression is undetectable, however after spinal cord injury, re-induction of Wnts 1, 4, and 5a occurs as evidenced by expression of mRNA in the cells immediately surrounding the lesion (Liu et al., 2008). The re-induced Wnts form gradients that peak at the lesion sites and decrease both anteriorly and posteriorly relative to the lesion sites. In addition to Wnt3, other guidance molecules have also been found to be re-induced by spinal cord injury. Class 3 semaphorins and ephrins are both re-expressed in spinal cord lesion, although the role of these guidance cues in the injury response has not been defined (Pasterkamp et al., 1999; Bundesen et al., 2003; Benson et al., 2005; Carmichael et al., 2005; Pasterkamp and Verhaagen, 2006). Wnt expression at the spinal cord injury site is coupled with re-expression of the repulsive receptor Ryk in corticospinal motor neurons where it is trafficked to the distal tip of the lesioned corticospinal axons (Liu et al., 2008). Inhibition of Wnt–Ryk signaling after spinal cord injury reduces the retraction of lesioned corticospinal axons from the injury site while concurrently promoting the sprouting of corticospinal axon collaterals within the spared spinal cord tissue (Liu et al., 2008). Another study showed similar results using a contusion model (Miyashita et al., 2009). Therefore, the re-induced Wnt gradients may be responsible for the long-range retraction of corticospinal tract axons following spinal cord injury.
of the regenerating axonal marker growth-associated protein 43 (GAP-43) near the lesion site (Suh et al., 2011). In corticospinal motor neurons, Wnt-Ryk signaling is able to promote both axon outgrowth as well as repulsive guidance through distinct signaling cascades (Li et al., 2009). This bi-functionality of Ryk may be active in DRG neurons as well, though it is currently unknown which neurons express Ryk after injury and what role the increased expression of the repulsive Wnt receptor Ryk may play in the peripheral conditioning lesion, if any.

OTHER MORPHOGENS AFTER INJURY
Wnts are not the only morphogens that are re-induced after spinal cord injury. Motor nuclei have been demonstrated to increase bone morphogenic protein (BMP) production following peripheral axotomy and potentially respond to BMP-2 protein infusion (Jin et al., 2003; Wang et al., 2007). Downstream of BMP-2 and 4 activation, BMP type I receptor mediates signaling through Smad1, 5, and 8 (Babitt et al., 2005). Smad1 activation in DRG neurons following peripheral injury is necessary for conditioning lesion mediated neurite outgrowth in vitro (Zou et al., 2009). Intragonadal injection of BMP-2 or 4 had similar effects in vitro, mediating neurite outgrowth through phospho-Smad nuclear translocation, while AA-83-BMP4 delivery to DRG neurons has been shown to reduce sensory axon retraction from an injury site and promote limited regeneration (Zou et al., 2009; Parikh et al., 2011). BMP-2 signaling mediates trkC expression in developing sympathetic superior cervical ganglia neurons (Zhang et al., 1998), though it remains to be seen whether increased Smad activation corresponds to increased capacity for central regeneration of large-diameter proprioceptive axons demonstrated in response to NT-3 gradients following peripheral conditioning lesion (Alto et al., 2009).

Conversely, BMP-2 following spinal cord injury has been proposed to inhibit regeneration of CNS axons (Setoguchi et al., 2004; Iliciho et al., 2008). In experiments delivering noggin producing neural precursor cells following compression injury to thoracic spinal cord, noggin was found to reduce astroglial cell fate and promote both axon outgrowth as well as repulsive guidance through distinct signaling cascades (Li et al., 2009). This induced axonal plasticity may provide a substrate for the formation of novel supraspinal motor circuits and improved functional recovery after injury. Understanding how Wnt expression is regulated will provide additional therapeutic tools.
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