2. Formation of peripheral nerve pathways by chemorepulsive and chemoattractive cues with special reference to innervation of the dorsal ramus

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Abstract. Our knowledge according to the mechanism of neural network formation in higher vertebrates has been accumulated and developed over the past two decades. It is noteworthy that secreted axonal guidance cues play a crucial role in the formation of axonal trajectories in both central and peripheral nervous systems. In the peripheral nervous system, the spinal nerve consists of dorsal root ganglion (DRG) axons (sensory axons) and spinal motor axons and forms the dorsal ramus projecting to the dorsal musculature. In this review, we focus on the role of secreted cues from surrounding tissues in the early development of the spinal nerve at the trunk level. In early stages, non-target tissues including the notochord and the ventral spinal cord secrete chemorepulsive cues for DRG axons. Semaphorin 3A, chondroitin sulfate proteoglycans, and netrin-1 are known to be secreted by these non-target tissues to repel DRG or motor axons. On the other hand, the dorsal myotome (the
presumptive dorsal musculature) secretes fibroblast growth factors, which are chemoattractants for motor axons. Both chemorepulsive and chemoattractive cues for the spinal nerves may contribute to shape their trajectories in a coordinated manner.

1. Introduction

Axons navigate along pathways to reach their targets by responding to a variety of guidance cues [1, 2]. The spinal nerve consists of dorsal root ganglion (DRG) and spinal motor axons in vertebrates. Here, we review recent advances in our knowledge of the mechanisms underlying the formation of peripheral nerve pathways, with special reference to the innervation of the dorsal ramus.

2. Developmental process of the dorsal ramus fibers of the spinal nerve

The spinal nerve is known to bifurcate ventrally and dorsally from early developmental stages. Dorsally elongating spinal nerves are called the “dorsal ramus fibers.” Previously we revealed that the dorsal ramus innervation to the myotome (the presumptive dorsal muscle) at the trunk level

Figure 1. Schematic illustrations of the dorsal ramus formation in the chick embryo. (A) At HH22, presumptive dorsal ramus (dr) fibers run diffusely without forming bundles. (B) At HH29, axons in the dorsal ramus become fasciculated. The medial branch (mb) of the dorsal ramus grows many collaterals into the presumptive dorsal muscle (dm). The lateral branch (lb) goes through the presumptive dorsal muscle and spreads widely throughout the dermis. drg; dorsal root ganglion, m; myotome, mn; motor neuron, nc; notochord.
is delayed owing to passing a “waiting period” of approximately 2 days (between embryonic day [E] 9.5 and 11.5 in the mouse embryo and between Hamburger-Hamilton [HH] stages 20 and 25 in the chick embryo) [3-5] (Fig. 1A). Similar phenomena were observed in other regions of the chick embryo. For instance, ventral ramus peripheral fibers, which also consist of both DRG and motor axons, pause in the plexus region for about 1 day before innervating the hindlimb [6, 7]. After passing this period, the dorsal ramus divides into its lateral and medial branches. The medial branch extends many collaterals into the presumptive dorsal muscle, whereas the lateral branch goes through the presumptive dorsal muscle and displays a broad distribution throughout the dermis of the skin (Fig. 1B).

3. Sensory axonal guidance of the dorsal ramus in the trunk region at early stages

During early development, peripherally projecting DRG axons (sensory axons) never orient themselves toward the notochord, the dermamyotome (dermatome/myotome) or the ventral spinal cord (Fig. 2). Several studies previously showed that the notochord and the dermamyotome secrete semaphorin 3A (Sema3A), known as a chemorepellent for DRG axons.

Figure 2. Surrounding non-target tissues secrete chemorepellents for DRG axons. A schematic transverse section of the chick embryo at HH22. The notochord (nc) and the myotome (m) secrete Sema3A. The floor plate of the ventral spinal cord (vsc) produces netrin-1. Additionally, the notochord also produces CSPGs.
Indeed, in vitro studies also indicated that DRG axons respond to the repulsive activity of Sema3A via their neuropilin-1 receptors [10, 11]. This is the reason why DRG axons never project toward these tissues and never join the dorsal ramus fibers at the early developmental stages.

Along the same line, several observations strongly suggest that the axonal guidance molecule netrin-1 also plays an important role in the ventral spinal cord-derived repulsive activity [12] (Fig. 2). First, netrin-1 is strongly expressed in the floor plate of the ventral spinal cord when many DRG axons are orienting themselves to reach the dorsal root entry zone of the dorsal spinal cord [12]. Simultaneously, repulsive netrin-1 receptor Unc5 is expressed in DRG neurons during development [12, 13]. Second, in experiments using cell and tissue cultures combined with tissues from netrin-1-deficient mouse embryos, DRG axons have been shown to respond to the repulsive activity of netrin-1 in vitro [12]. Third, misrouted DRG axonal trajectories can be observed in netrin-1-deficient mouse embryos. In netrin-1-deficient embryos at E10, some DRG axons become misoriented toward the ventral spinal cord where netrin-1 proteins are absent [12]. In addition to this loss-of-function experiment, gain-of-function studies further confirmed the repulsive activity of netrin-1 toward DRG axons in vivo [12]. These lines of evidence lead us to the conclusion that netrin-1 from the ventral spinal cord prevents DRG axons from approaching the ventral spinal cord and helps them to form the ventral ramus.

In Sema3A-deficient mouse embryos, DRG axons exhibit no misrouted trajectories in relation to the notochord, suggesting the possibility that repulsive activities other than Sema3A may play a pivotal role in the notochord-derived chemorepulsion for DRG axons [14]. The notochord secretes chondroitin sulfate proteoglycans (CSPGs) including aggrecan and PG-M/versican at early stages [15-20]. In vitro studies have revealed that aggrecan can inhibit DRG axonal growth [21]. Furthermore, chondroitinase ABC treatment, which digests chondroitin sulfate (CS) chains, clearly demonstrated that notochord-derived chemorepulsion for DRG axons is reduced in the absence of CS chains, suggesting that residual axon repulsive activity of the notochord may be due to CSPGs [21, 22] (Fig. 2).

4. Motor axonal guidance of the dorsal ramus in the trunk region at early stages

The development of trunk motor fibers in the dorsal ramus is well documented in two species, the chick and the mouse [4, 5, 23]. Shirasaki et al. [23] clearly demonstrated the movement of motor fibers in the dorsal ramus by using Hb9::gfp transgenic mice whose motor cell bodies and axons were
labeled by GFP. In the chick embryo, we showed the detailed formation of motor fibers in the dorsal ramus by using specific antibodies against β-tubulin for the detection of all axons, against axonin-1/SC2 for the detection of DRG axons, and against an early muscle myosin heavy chain for the detection of muscle cells [4, 24, 25]. Furthermore, we confirmed their time course and patterning of innervation by anterograde DiI and DiO labelings [4]. During early development, motor axons grow out from the ventral spinal cord into the sclerotome. Then they diverge to form a fan-like projection and reach just beneath the medial surface of the myotome, but never project beyond its edge, resulting in the formation of a “waiting period” [4]. This phenomenon may be induced by the axon guidance activity of Sema3A (Fig. 3). Motor neurons express the Sema3A receptor neuropilin-1 [26]. Motor axons actually respond to Sema3A-induced repulsion in vitro [4]. Furthermore, in Sema3A-deficient mouse embryos, many dorsal ramus fibers reach the myotome and pass beyond its edge, showing the complete loss of this “waiting period” in the absence of Sema3A [5]. Although both DRG and motor axons respond to the Sema3A-induced repulsion, motor axons can form an early dorsal ramus and reach nearby the myotome, whereas DRG axons cannot. What is the reason for this discrepancy between DRG and motor axons? How do motor axons reach nearby the myotome?

**Figure 3. Motor axons are under dual control by Sema3A and FGFs.** A schematic transverse section of the chick embryo at HH22. The myotome (m) secretes FGFs to attract early motor axons, resulting in the formation of the dorsal ramus (dr). On the other hand, the myotome also secretes Sema3A to repel motor axons, resulting in the formation of the “waiting period.” drg; dorsal root ganglion, mn; motor neuron.
The presence of the attractant molecule for motor axons explains this discrepancy. Previous studies have revealed that fibroblast growth factors (FGFs) exert an attractive activity toward motor axons in vitro [4, 23]. FGF2, FGF4, FGF8, and FGF9 are expressed in the myotome [27-30]. In addition, their receptor FGFR1 is expressed in motor neurons, which extend their axons into the dorsal ramus. These lines of evidence suggest that myotome-derived FGFs may attract motor axons [23]. Genetic analyses using FGFR1-deficient mouse embryos further confirmed that FGFs are responsible for the development of the dorsal ramus. Thus, stop and attractive signals derived from the myotome may lead motor axons to reach just beneath the myotome and stop at its edge (Fig. 3).

5. Guidance process of mixed fibers in the dorsal ramus at later stages

At later stages (HH26 of the chick embryo and E12 of the mouse embryo), Sema3A expression disappears from the medial part of the presumptive dorsal muscle where the lateral branch of dorsal ramus fibers invades [4, 5]. Together with the disappearance of Sema3A stop signals from the medial presumptive dorsal muscle, DRG axons begin to appear in the dorsal ramus. Subsequently, mixed dorsal ramus fibers (sensory and motor

Figure 4. Mixed fibers in the dorsal ramus become fasciculated at later stages. After the disappearance of Sema3A expression in the medial part of the presumptive dorsal muscle (m), the lateral branch (lb) of the dorsal ramus runs through the presumptive dorsal muscle. drg; dorsal root ganglion, mb; medial branch, mn; motor neuron.
axons) appear in the lateral branch of the dorsal ramus at HH27 and E12.5 because of the absence of Sema3A expression in the dorsal muscle [4, 5]. At HH29, mixed fibers develop in the medial and lateral branches of the dorsal ramus [4] (Fig. 4).

In summary, we reviewed the formation of peripheral nerve pathways. Mounting evidence suggests that both chemoattractive and chemorepulsive signals are required for the proper formation of the dorsal ramus.

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References