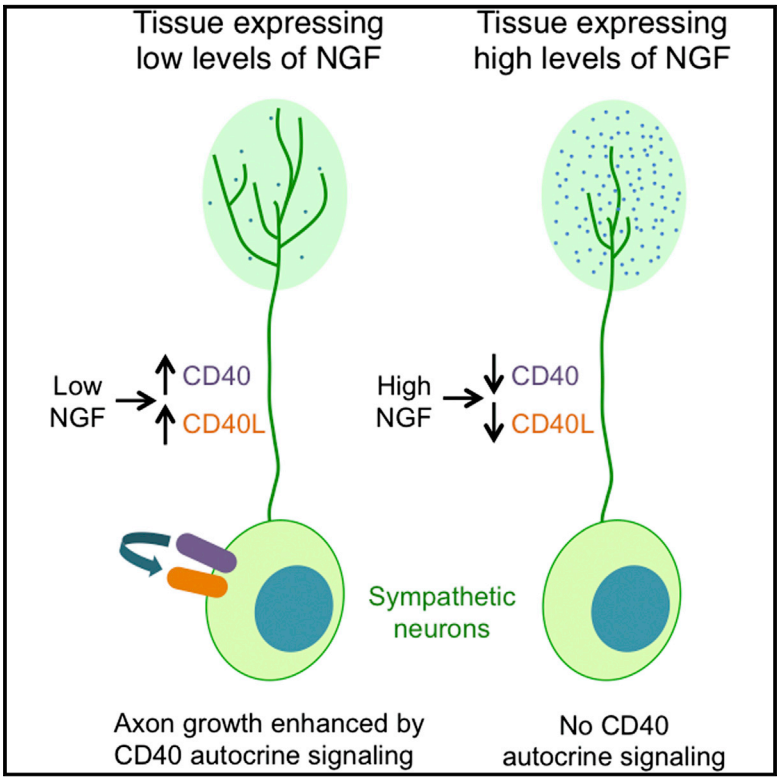
Cell Reports

Regulation of Autocrine Signaling in Subsets of Sympathetic Neurons Has Regional Effects on Tissue Innervation

Graphical Abstract



Highlights

- CD40/CD40L autocrine signaling enhances NGF-promoted sympathetic axon growth
- NGF negatively regulates CD40 and CD40L levels in developing sympathetic neurons
- Accordingly, CD40/CD40L signaling only enhances axon growth at low levels of NGF
- Innervation of tissues expressing low NGF levels is disrupted in CD40 knockout mice

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In Brief

McWilliams et al. report that CD40/CD40L autocrine signaling in developing sympathetic neurons promotes axon growth. Because NGF negatively regulates CD40/CD40L expression, this autocrine-signaling loop operates only in neurons innervating low-NGF-expressing tissues, which are hypoinnervated in CD40 knockout mice. This reveals how autocrine signaling differentially regulates tissue innervation.

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Report

Regulation of Autocrine Signaling in Subsets of Sympathetic Neurons Has Regional Effects on Tissue Innervation

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SUMMARY

The regulation of innervation by target-derived factors like nerve growth factor (NGF) is the cornerstone of neurotrophic theory. Whereas autocrine signaling in neurons affecting survival and axon growth has been described, it is difficult to reconcile autocrine signaling with the idea that targets control their innervation. Here, we report that an autocrine signaling loop in developing mouse sympathetic neurons involving CD40L (TNFSF5) and CD40 (TNFRSF5) selectively enhances NGF-promoted axon growth and branching, but not survival, via CD40L reverse signaling. Because NGF negatively regulates CD40L and CD40 expression, this signaling loop operates only in neurons exposed to low levels of NGF. Consequently, the sympathetic innervation density of tissues expressing low NGF is significantly reduced in CD40-deficient mice, whereas the innervation density of tissues expressing high levels of NGF is unaffected. Our findings reveal how differential regulation of autocrine signaling in neurons has region-specific effects on axon growth and tissue innervation.

INTRODUCTION

Neurotrophic theory provides an explanation for how the target tissues of neuronal populations in the developing peripheral nervous system control their innervation. The basic idea is that tissues synthesize just the right amount of a neurotrophic factor to support the survival of the required number of innervating neurons and promote the growth and branching of their axons within the tissue. Neurotrophic theory is endorsed by a large body of work on nerve growth factor (NGF), the first neurotrophic factor to be identified, and has been corroborated by studies of other members of the NGF family of neurotrophins and by other neurotrophic factors (Levi-Montalcini, 1987; Davies, 2003; Dekkers et al., 2013). In addition to target-derived signals, autocrine signaling in neurons involving neurotrophins and other secreted signaling molecules has been shown to affect neuronal survival, axon growth, and other aspects of neuronal development and function (Wright et al., 1992; Acheson et al., 1995; O'Keeffe et al., 2008; Cheng et al., 2011; Ryu et al., 2013). However, neuronal autocrine signaling is difficult to reconcile with neurotrophic theory because it is not clear how autonomous signaling loops in neurons could contribute to the establishment of distinctive patterns of tissue innervation.

From a PCR screen to identify novel regulators of neuronal survival and axon growth, we detected expression of transcripts encoding CD40L (TNFSF5), a member of the tumor necrosis factor superfamily (TNFSF), and CD40 (TNFRSF5), a member of the TNF receptor superfamily (TNFRSF), in the experimentally tractable sympathetic neurons of the mouse superior cervical ganglion (SCG) at the stage when the axons of these neurons are ramifying extensively in their target tissues. CD40L and CD40 are prominently expressed in the immune system, where they play a central role in the generation of immune responses and the pathogenesis of autoimmune disease (Calderhead et al., 2000; Peters et al., 2009). Whereas there is some evidence for the appearance of a neurodegenerative phenotype in aged CD40 knockout mice (Tan et al., 2002), CD40 and CD40L are not known to play any role in neural development. We demonstrate that CD40 autocrine signaling enhances NGF-promoted axonal growth and branching, is regulated by the level of NGF in targets, and exerts regional effects on innervation density in vivo. These findings resolve the long-standing conundrum of neuronal autocrine signaling by uncovering a mechanism of differential regulation of autocrine signaling within neuronal populations, resulting in specific regional effects on tissue innervation.

RESULTS

CD40 and CD40L Are Co-expressed in Perinatal SCG Neurons

Quantitative PCR revealed the expression of *Cd40* and *Cd40l* transcripts in the SCG of late fetal and early postnatal mice during the stage when sympathetic axons are ramifying extensively in their targets (Figure 1A). Compared with adult spleen, where CD40 and CD40L are expressed at very high levels, the levels