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THE ROLE OF CELSR3 IN THE DEVELOPMENT OF CENTRAL SOMATOSENSORY PROJECTIONS FROM DORSAL ROOT GANGLIA

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Abstract—Dorsal root ganglion (DRG) neurons receive peripheral somatosensory information and send orderly projections to second-order relay nuclei in the spinal cord and in the brainstem. Atypical cadherin *Celsr3* is known to play a critical role in wiring of several central and peripheral axons. Although *Celsr3* mRNA is heavily expressed in DRG neurons, its role in the development of somatosensory projections remains unexplored. Here we assessed the role of *Celsr3* in DRG using conditional gene inactivation in crosses with *Wnt1-Cre* mice. Using *Celsr3-GFP* transgenic mice, we found that Celsr3 was highly expressed in different DRG cells, such as Pavalbumin-, TrkB-, and calcitonin gene-related peptide (CGRP)-positive neurons. *Wnt1-Cre; Celsr3$^{f/-}$* animals survived for a few weeks and looked smaller than littermate controls. DiI tracing showed that early DRG axons entered the spinal cord and reached spinal cord targets similarly in mutant and control mice. CGRP-positive fiber density was significantly decreased in lamina I in the mutant versus control spinal cord at postnatal day (P) 7 and P14. Furthermore, more Pavalbumin-positive fibers invaded the gray matter and made more contacts with spinal motor neurons in mutant than in control samples. Behavioral analysis showed that mutant animals were less sensitive to pain and more sensitive to mechanical stimulation than controls. In conclusion, Celsr3 is dispensable for the patterning of central DRG projections, but it regulates for the fine mapping of sensory fibers in the gray matter, which is important for somatosensory processing.

Key words: DRG, central projections, spinal cord, pain, Wnt1, development.

INTRODUCTION

Dorsal root ganglia (DRG) contain first-order somatosensory neurons that receive exteroceptive, interoceptive and proprioceptive signals, and transmit them to the central nervous system (CNS). DRG neurons are pseudo-unipolar: their peripheral axonal branches receive and convey signals, and their central branches enter the spinal cord via dorsal roots. DRG axons connect to second-order neurons in the dorsal horn, in proprioceptive spinocerebellar relay nuclei (Clarke column and external cuneate nuclei), and in brainstem gracilis and cuneate nuclei that they reach via dorsal column funiculi.

DRG neurons derive from the neural crest and differentiate into three main classes containing several subtypes of nociceptive, mechanoreceptive and proprioceptive neurons with specific morphological, electrophysiological and cellular characteristics, and modality-specific central connections (Marmigere and Ernfors, 2007; Abraira and Ginty, 2013; Usoskin et al., 2015). Large DRG neurons are generated first, with a peak at embryonic day (E) 11.5 in cervical, and at E12.5 in lumbar DRG in mice. They convey light touch and proprioception and have a soma diameter larger than 35 μm and axons that correspond to large myelinated Aβ (touch) and Ia fibers (proprioception) (Sonner et al., 2017). Light touch neurons express neurotrophin receptors TrkB- and/or TrkC, and their central axons terminate deep in the dorsal horn and in dorsal column brainstem nuclei (Yoshikawa et al., 2013); proprioceptive neurons are TrkC-positive and their axons contact motor neurons and spinocerebellar nuclei (Hong et al., 2016). The generation of small neurons starts slightly later and lasts about 48 h longer than that of large neurons (Lawson and Biscoe, 1979). They transport nociceptive and thermal afferent signals. They have a soma diameter of 15–35 μm and their axons correspond to thin unmyelinated C and poorly myelinated Aδ fibers. Small neurons express the nerve growth factor receptor neurotrophic tyrosine kinase receptor1 (TrkA)- and their axons terminate in the dorsal horn, particularly in superficial layers (Fitzgerald, 2005; Liu and Ma, 2011), Calcitonin gene-related peptide (CGRP) is specifically expressed in peptidergic nociceptive small DRG neurons (Hokfelt et al., 1992).

Axons of DRG neurons travel to their specific target in an exquisite manner, never branching into inappropriate laminae. In mice, the first axons reach the dorsal root entry zone at E12.5, and more invade the dorsolateral

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Abbreviations: CGRP, calcitonin gene-related peptide; ChAT, choline acetyltransferase; CNS, central nervous system; DRG, dorsal root ganglion; PBS, phosphate-buffered saline; PV, Parvalbumin; TrkA, neurotrophic tyrosine kinase receptor1; TrkB, neurotrophic tyrosine kinase receptor2; TrkC, neurotrophic tyrosine kinase receptor3.

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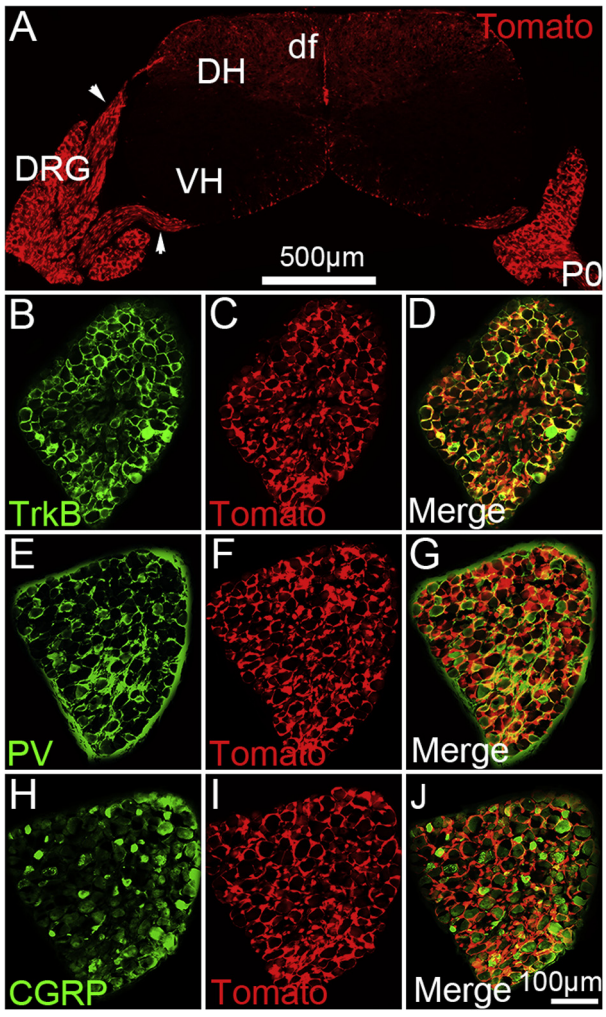

Fig. 1. Wnt1-Cre expression in DRG neurons. (A) Transverse section at the cervical enlargement in *Wnt1-Cre;Rosa26*^{Tomato} spinal cord at P0. Note the very strong signal in all DRG neurons as well as in dorsal and ventral roots (arrowheads), presumably associated with boundary cap cells and other neural crest derivatives. The dorsal funiculus (df) contained labeled fibers that correspond to DRG axons and almost no neurons were labeled in dorsal horns (DH); VH: ventral horn. (B–J) Colocalisation of expression. In P0 DRG sections, anti-TrkB (B), PV (E), CGRP (H) immunofluorescent staining (green) was used to identify different types of neurons, and *Wnt1-Cre; Rosa26*^{Tomato} transgene expression (red; C, F, I) was used to trace *Wnt1-Cre* activity. As seen in merged pictures, most TrkB-positive (D), PV-positive (G) and CGRP-positive neurons (J) co-expressed the red tomato protein.

margin of the spinal cord and form the primordium of the dorsal funiculus one day later. Between E12.5 and E17.5, sensory axons elaborate collateral branches and then penetrate the gray matter of the spinal cord specifically (Ozaki and Snider, 1997). Initial trajectories of DRG axons are shaped by chemorepulsive cues secreted by surrounding ‘‘non-target” tissues, such as semaphorin 3 A, chondroitin sulfate proteoglycans, and cell adhesion molecules (Masuda and Shiga, 2005). Multiple genes were shown to influence axonal pathfinding of different DRG neurons. The receptor tyrosine kinase Ret (Ret proto-oncogene) is expressed in mechanoreceptors and *Ret* null mice have early central projection deficits (Honma et al., 2010; Fleming et al., 2015). Inactivation of *Runx1* leads to increased numbers of TrkA-positive and peptidergic neurons and to altered axonal projections (Yoshikawa et al., 2007), whereas mutation of *Runx3* impairs the projections of proprioceptive DRG neurons without affecting their specification (Inoue et al., 2002). The mutation of POU homeodomain transcription factors Brn3a/Pou4f1 results in deficits of TrkA-positive nociceptive and TrkC-positive proprioceptive afferents (Zou et al., 2012). Axon pathfinding abnormalities of TrkA-positive DRG neurons are also seen in *Nrg1* mutant mice (Hancock et al., 2011). Recent studies have identified additional mechanisms involved in projections of DRG neurons. For examples, expression of *c-Maf* in the interneurons of laminae III/IV modulates afferent projections of mechanoreceptive neurons (Hu et al., 2012); a hydrophilic glycerophospholipid (LysoPtdGlc), locally synthesized and released by radial glia, regulates the targeting of nociceptive but not proprioceptive central axon projections (Guy et al., 2015).

Celsr3, an atypical cadherin, plays a crucial role in steering axonal projections (Tissir et al., 2005; Tissir and Goffinet, 2013). In the periphery, *Celsr3* inactivation impacts the formation of the enteric nervous system (Sasselli et al., 2013) and the patterning of motor nerves (Chai et al., 2014). Conditional inactivation experiments showed that Celsr3 acts either via expression in projecting neurons, such as in corticospinal motor neurons and motor axons in hindlimb, or via guidepost cells along the pathway (Zhou et al., 2008), especially in shaping reciprocal thalamocortical projections (Qu et al., 2014; Feng et al., 2016).

Celsr3 is highly expressed in the DRG, and the rostral turning of commissural spinal cord axons is defective in of E11.5 *Celsr3* mutant mice (Onishi et al., 2013). This suggests that *Celsr3* might regulate the trajectory of somatosensory axons in the spinal cord. In this work, we studied the role of *Celsr3* in the formation of DRG neuron projections and in sensory information processing, by inactivating *Celsr3* in DRG neurons *in vivo* upon expression of *Wnt1-Cre*.

EXPERIMENTAL PROCEDURES

Animals

All animal procedures were approved by the Laboratory Animal Ethics Committee of Jinan University. Animal crosses were carried out as described (Zhou et al., 2008; Feng et al., 2012; Feng et al., 2016). Briefly, we generated *Wnt1-Cre;Celsr3*$^{f/-}$ animals to inactivate *Celsr3* in neural crest cell derivatives, including DRG neurons, and used *Wnt1-Cre;Celsr3*$^{f/+}$ or *Celsr3*$^{f/-}$ as controls. *Rosa26*^{Tomato} mice (Madisen et al., 2010) were crossed with *Wnt1-Cre* mice (Danielian et al., 1998) to trace Cre-expression. Celsr3 expression profiles were studied using *Celsr3-GFP* mice (Ying et al., 2009) (kindly provided by Prof. Qiang Wu).

Immunohistochemistry

Under deep anesthesia with 2.5% tribromoethanol, mice aged P0, P7, P14 (P0 = day of birth) were perfused intracardially with 4% paraformaldehyde in 0.1 M