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Runx3 is required for the specification of TrkC-expressing mechanoreceptive trigeminal ganglion neurons

Kouji Senzaki [a], Shigeru Ozaki [a], Masaaki Yoshikawa [a], Yoshiaki Ito [b], Takashi Shiga [a,*]

[a] Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan
[b] Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673, Singapore

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ABSTRACT

Sensory neurons project axons to specific peripheral and central targets according to their sensory modality. Runx3 is crucially involved in proprioceptive dorsal root ganglion neuron development. Runx3 is also expressed in trigeminal ganglion (TG) neurons. The role of Runx3 in the TG, however, is largely unknown because the TG does not contain proprioceptive neurons. In *Runx3*-deficient (*Runx3*$^{-/-}$) mice, TrkB-expressing TG neurons were increased, whereas TrkC-expressing TG neurons were decreased during TG neuron development. In *Runx3*$^{-/-}$ neonatal mice, TrkC-expressing TG neurons did not project to the Merkel cells in the outer root sheath (ORS) of whisker vibrissae peripherally and the spinal trigeminal nucleus pars interpolaris (Sp5I) centrally. These findings suggest that Runx3 is required for the specification of TrkC-expressing TG neurons, conveying mechanoreceptive signals from the Merkel cells in the ORS of the whisker vibrissae to the Sp5I.

Introduction

The mammalian Runt-related (Runx) transcription factor family comprises three members: Runx1, 2 and 3. These Runxs play important roles in developmental processes of various cell types, including hematopoietic cells, osteoblasts and gastric epithelial cells (for reviews, see Coffman, 2003; Ito, 2004; 2008). Runx1 and Runx3 are also expressed in neuronal subtypes in the central and peripheral nervous systems (Simeone et al., 1995; Theriault et al., 2004; Theriault et al., 2005). In the dorsal root ganglion (DRG), Runx1 and Runx3 are expressed in a subpopulation of neurons. Runx1 controls the cell fate specification and the axonal projections of nociceptive neurons, which convey information about pain, whereas Runx3 controls those of proprioceptive neurons, which convey information regarding muscle length and tension (for reviews, see Inoue et al., 2008, Marmigère and Ernfors, 2007). Furthermore, Runx1 and Runx3 may be involved in the development of proprioceptive and nociceptive DRG neurons, respectively (Yoshikawa et al., 2007; Nakamura et al., 2008). Runx1 and Runx3 are also expressed in a subpopulation of trigeminal ganglion (TG) neurons, but their roles are not known (Levanon et al., 2002; Theriault et al., 2004).

General somatosensory information is conveyed to the central nervous system at spinal levels through the DRG and at cranial levels through the TG. Although these ganglia have a similar function, they have distinct developmental origins. The DRG contains neurons originating from neural crest cells, whereas the TG contains neurons originating from neural crest cells and placodal ectoderm cells (Baker and Bronner-Fraser, 2001; Chan and Tam, 1988). Moreover, the DRG has nociceptive, mechanoreceptive and proprioceptive neurons, whereas the TG has nociceptive and mechanoreceptive neurons. The proprioceptive neurons of the trigeminal system are located in the mesencephalic trigeminal nucleus in the brainstem (Lazarov, 2002). Although this does not mean that the proprioceptive neurons do not exist in the TG, no studies have shown proprioceptive neurons in the TG. In the developing DRG, Runx3 is necessary for the acquisition of proprioceptive neuron identities (Inoue et al., 2002; Levanon et al., 2002; Nakamura et al., 2008). The role of Runx3 in the developing TG, which lacks proprioceptive neurons, however, is largely unknown.

In the present study, to elucidate the role of Runx3 in the development of TG neurons, we analyzed cell fate and axonal projections of TG neurons of *Runx3*-deficient (*Runx3*$^{-/-}$) mice at the embryonic and neonatal stages.

Results

Changes of the expression of marker molecules in TG neurons of neonatal Runx3$^{-/-}$*mice*

Because *Runx3*$^{-/-}$ mice die soon after birth (Inoue et al., 2002), we first analyzed the TG of *Runx3*$^{+/+}$ and *Runx3*$^{-/-}$ mice at postnatal

* Corresponding author.
E-mail address: tshiga@md.tsukuba.ac.jp (T. Shiga).

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day (P) 0. We examined whether the loss of Runx3 affects the number of TG neurons using NeuN as a pan-neuronal marker. The number of NeuN-expressing ($NeuN^+$) neurons in the TG was not different between *Runx3*$^{+/+}$ and *Runx3*$^{-/-}$ mice (Figs. 1A′–A‴), suggesting that Runx3 deficiency does not affect the total number of TG neurons.

To clarify the role of Runx3 in the cell fate of TG neurons, we examined the number of neurons expressing the neurotrophin receptor family members TrkA, TrkB and TrkC (Figs. 1B–D). Compared with *Runx3*$^{+/+}$ mice, the number of $TrkB^+$ neurons in *Runx3*$^{-/-}$ mice increased to 143% (Fig. 1C‴, *Runx3*$^{+/+}$, 14475 ± 378; *Runx3*$^{-/-}$, 20653 ± 676; $p<0.0001$), while that of $TrkC^+$ neurons decreased to 38% (Fig. 1D‴, 10605 ± 714; 4075 ± 528; $p<0.0001$). The number of $TrkA^+$ neurons was not different in *Runx3*$^{-/-}$mice (Fig. 1B‴).

To gain further insight into the role of Runx3 in TG neuron subtype specification, we examined the number of neurons that express calcium-binding proteins (PV, Calr and CB) and neuropeptides (SOM, CGRP and SubP). A similar number of PV^+ neurons was observed in the TG of *Runx3*$^{+/+}$(2810 ± 164) and *Runx3*$^{-/-}$ (2794 ± 114) mice (Fig. 1E‴), which is in great contrast to the lack of PV^+ neurons observed in *Runx3*$^{-/-}$DRG (Nakamura et al., 2008). Compared with *Runx3*$^{+/+}$ mice, SOM^+ TG neurons were dramatically decreased to 16% in *Runx3*$^{-/-}$mice (Fig. 1F‴, 395 ± 50; 63 ± 24; $p<0.0001$), suggesting that SOM expression is downregulated in the absence of Runx3. The numbers of $Calr^+$, CB^+, $CGRP^+$ and $SubP^+$ TG neurons were not different between *Runx3*$^{+/+}$ and *Runx3*$^{-/-}$ mice (Fig. S1). These findings suggest that Runx3 negatively regulates the expression of TrkB and positively regulates the expression of TrkC and SOM in the TG at P0.

Correlation between the expression of Runx3 and marker molecules at the neonatal stage

$TrkB^+$ neurons were increased and $TrkC^+$ neurons decreased in *Runx3*$^{-/-}$ TG at P0 (Fig. 1). We next examined the expression of TrkB, TrkC and Runx3 by triple immunostaining in order to clarify the correlation between the expression of TrkB and TrkC and the involvement of Runx3 in the regulation of these expressions in the TG. Runx3 was expressed in a subpopulation of $TrkB^+/TrkC^-$ neurons, $TrkB^-/TrkC^+$ neurons and $TrkB^+/TrkC^+$ neurons in *Runx3*$^{+/+}$ mice (Figs. 2A′, A″). In *Runx3*$^{-/-}$ mice, the number of $TrkB^-/TrkC^+$ neurons was decreased (Fig. 2G, *Runx3*$^{+/+}$, 6643 ± 593; *Runx3*$^{-/-}$, 2249 ± 345; $p<0.001$) and that of $TrkB^+/TrkC^-$ neurons was increased (Fig. 2G, 13952 ± 524; 16293 ± 671; $p<0.05$) compared with that in *Runx3*$^{+/+}$ mice. The number of $TrkB^+/TrkC^+$ neurons, however, was not significantly different between these mice (3055 ± 509; 3649 ± 455; Figs. 2A″, B″, G). These findings suggest that Runx3 promotes the cell type specification of $TrkB^-/TrkC^+$ neurons and suppresses the cell type specification of $TrkB^+/TrkC^-$ neurons in the TG.

We also examined the relationship of the co-expression of SOM or PV with Runx3 and TrkC to elucidate the role of Runx3 in the expression of SOM and PV. In *Runx3*$^{+/+}$ mice, TrkC and Runx3 were expressed in approximately 82% and 76% of SOM^+ neurons, respectively (Figs. 2C″, H). In *Runx3*$^{+/+}$ mice, approximately 280 neurons co-expressed SOM and Runx3 (278 ± 49). The reduced number of SOM^+ neurons (281 ± 42) in *Runx3*$^{-/-}$TG was comparable to the number of $Runx3^+/SOM^+$ neurons (Figs. 2C″, D″, H). Taken together, these findings suggest that most SOM^+ TG neurons co-express TrkC and Runx3, and Runx3 may positively regulate the expression of SOM cell autonomously.

TrkC was expressed in approximately 69% of PV^+ neurons, but Runx3 was expressed in only 9% of PV^+ neurons (Figs. 2E″, I). In addition, the number of $PV^+/TrkC^+$ neurons was not significantly different between *Runx3*$^{+/+}$(1705 ± 252) and *Runx3*$^{-/-}$ (1572 ± 174) mice. In the DRG, most PV^+ neurons co-express TrkC and Runx3 in *Runx3*$^{+/+}$ mice, and PV is not expressed in *Runx3*$^{-/-}$mice (Nakamura et al., 2008). These findings suggest that Runx3 is not involved in the expression of PV in the TG, although the majority of PV^+ neurons co-express TrkC, like in the DRG.

Runx3 controls TrkB and TrkC expression from the early stage of TG neuron development

To determine the embryonic stage at which Runx3 begins to control the expression of TrkB and TrkC, we estimated the number of $TrkB^+$ and $TrkC^+$ neurons from E10.5 to E13.5 (Fig. 3). Similar numbers of $TrkB^+$(*Runx3*$^{+/+}$, 1982 ± 99; *Runx3*$^{-/-}$, 1683 ± 39) and $TrkC^+$(4443 ± 88; 4226 ± 12) neurons were observed in *Runx3*$^{+/+}$ and *Runx3*$^{-/-}$mice at E10.5 (Figs. 3A′–D′). Compared with *Runx3*$^{+/+}$ mice, in *Runx3*$^{-/-}$mice, the number of $TrkB^+$ neurons was increased to 164% (8386 ± 534; 13775 ± 979; $p<0.01$), 145% (4424 ± 236; 6436 ± 616; $p<0.05$) and 164% (5371 ± 599; 8810 ± 668; $p<0.01$) at E11.5, E12.5 and E13.5, respectively (Figs. 3A″–A‴, B″–B‴, E), whereas that of $TrkC^+$ neurons was decreased to 56% (8582 ± 88; 4808 ± 237; $p<0.01$), 70% (5856 ± 357; 4095 ± 485; $p<0.05$) and 39% (6479 ± 368; 2548 ± 195; $p<0.001$) at E11.5, E12.5 and E13.5, respectively (Figs. 3C″–C‴, D″–D‴, F). We also examined $TrkA^+$ and $NeuN^+$ neurons at E11.5 and E13.5. The number of $TrkA^+$ neurons was not different between *Runx3*$^{+/+}$ and *Runx3*$^{-/-}$ mice at either embryonic stage. The number of $NeuN^+$ neurons was not different at E11.5 but was slightly decreased at E13.5 in *Runx3*$^{-/-}$mice (Fig. S2). These results suggest that Runx3 may begin to control the expression of TrkB and TrkC in TG neurons at E11.5.

We then investigated the co-expression of TrkB, TrkC and Runx3 in the TG at E10.5 and E13.5 (Fig. 4). At E10.5, Runx3, together with TrkB and TrkC, appeared in the TG of *Runx3*$^{+/+}$ mice. Most of the $TrkB^+$ neurons (91%) expressed TrkC in both *Runx3*$^{+/+}$ and *Runx3*$^{-/-}$ TG (Figs. 4A″, C″, E), and most of the $TrkB^+/TrkC^+$ neurons (88%) expressed Runx3 in *Runx3*$^{+/+}$ TG (Figs. 4A″, E). The numbers of $TrkB^-/TrkC^+$(2809 ± 407; 2701 ± 37), $TrkB^+/TrkC^-$ (173 ± 26; 159 ± 64) and $TrkB^+/TrkC^+$(1719 ± 96; 1623 ± 136) neurons were not different between *Runx3*$^{+/+}$ and *Runx3*$^{-/-}$ mice at E10.5, suggesting that Runx3 is not involved in regulating the expression of TrkB and TrkC at E10.5, despite the fact that Runx3 was expressed in the majority of $TrkB^+/TrkC^+$ neurons (Fig. 4E).

At E13.5, TrkC was expressed in few $TrkB^+$ neurons (1.3%) in *Runx3*$^{+/+}$ mice. In *Runx3*$^{+/+}$ mice, virtually all $TrkC^+$ neurons (92%) and only a few $TrkB^+$ neurons (3.7%) expressed Runx3 (Figs. 4B″, F). In sharp contrast to *Runx3*$^{+/+}$ mice, TrkC was expressed in most $TrkB^+$ neurons (91%) in *Runx3*$^{-/-}$ mice, and the number of $TrkB^+/TrkC^+$ neurons increased by 35-fold (97 ± 3; 3436 ± 71; $p<0.0001$, Figs. 4B″, D″, F). In addition, the number of $TrkB^-/TrkC^+$ neurons was decreased to 4.9% (7001 ± 180; 341 ± 7; $p<0.0001$), whereas that of $TrkB^+/TrkC^-$ neurons was not significantly different between *Runx3*$^{+/+}$(5491 ± 76) and *Runx3*$^{-/-}$ (5146 ± 428) mice at E13.5 (Figs. 4B″, D″, F).

These findings suggest that Runx3 positively regulates the expression of TrkC and negatively regulates the expression of TrkB in a cell-autonomous manner and that Runx3 has a role in specifying TrkB/TrkC co-expressing TG neurons as single-expressing TrkB or TrkC TG neurons.

Axonal projection of $TrkB^+$ and $TrkC^+$ TG neurons in Runx3$^{-/-}$ *mice*

We next analyzed the axonal projections of TG neurons at E13.5 and P0. At E13.5, in *Runx3*$^{+/+}$ mice, TG afferents reached the peripheral and central targets (Figs. 5 and 6). In the peripheral projection of TG neurons of E13.5 *Runx3*$^{+/+}$ mice, $TrkB^+$ and $TrkC^+$ afferents innervated the facial areas, including the large and small whisker vibrissae (Figs. 5A, C, E). In *Runx3*$^{-/-}$ mice, $TrkB^+$ afferents projected to these areas, but few $TrkC^+$ afferents were observed (Figs. 5B, D, F), despite the fact that a substantial number of TG neurons expressed TrkC (Figs. 3D‴, F). In the central projection, in *Runx3*$^{+/+}$ mice, $TrkB^+$ and $TrkC^+$ afferents projected to the medial and lateral portion of the spinal trigeminal tract (Sp5t),