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The transcription factor, Lmx1b, is necessary for the development of the principal trigeminal nucleus-based lemniscal pathway

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ABSTRACT

Little is known of transcriptional mechanisms underlying the development of the trigeminal (V) principal sensory nucleus (PrV), the brainstem nucleus responsible for the development of the whisker-to-barrel cortex pathway. Lmx1b, a LIM homeodomain transcription factor, is expressed in embryonic PrV. In Lmx1b knockout ($^{-/-}$) mice, V primary afferent projections to PrV are normal, albeit reduced in number, whereas the PrV-thalamic lemniscal pathway is sparse and develops late. Excess cell death occurs in the embryonic Lmx1b$^{-/-}$ PrV, but not in Lmx1b/Bax double null mutants. Expression of Drg11, a downstream transcription factor essential for PrV development and pattern formation, is abolished in PrV, but not in the V ganglion. Consequently, whisker patterns fail to develop in PrV by birth. Rescued PrV cells in Lmx1b/Bax double $^{-/-}$s failed to rescue whisker-related PrV pattern formation. Thus, Lmx1b and Drg11 may act in the same genetic signaling pathway that is essential for PrV pattern formation.

Introduction

The pattern of the whiskers on the face in rodents is faithfully recapitulated by neuronal aggregates in the trigeminal (V) brainstem complex, ventrobasal thalamus and cerebral cortex (reviews in Jones and Diamond, 1995). These somatotopic aggregations of cells and fibers are called barrelettes, barreloids and barrels, respectively (Woolsey and Van der Loos, 1970; Ma 1991; see Erzurumlu et al., 2006, for a recent review). In the brainstem, barrelettes occur in the V brainstem subnuclei principalis (PrV), interpolaris (SpVi) and caudalis (SpVc). The PrV is noteworthy because, during development, it is responsible for conveying the whisker-related pattern to the thalamus which, in turn, conveys and establishes the barrel pattern in layer IV of the S1 somatosensory cortex (Killackey and Fleming, 1985). The similarly patterned spinal V subnuclei subserve a "paralemniscal" function (Williams et al., 1994) and, together with the PrV, provide parallel pathways for processing and relaying whisker-related information to higher-order structures in the brain.

Within the past decade, a number of patterning mechanisms involving specific molecules and genes have been revealed in the developing whisker-barrel pathway (see Jacquin et al., 2008, for a review). However, most of these molecules and their genetic bases have been studied within the context of the developing cerebral cortex. Further study of the mechanisms controlling subcortical barrelette formation is desirable insofar as the PrV is the indispensable bridge between the periphery and the thalamocortical pathway and whisker-related patterns appear first in the PrV in development. Thus, a clear understanding of barrel formation requires an elucidation of PrV mechanisms. Recent studies of transgenic mice have begun to uncover several key molecules that are required for PrV pattern formation. Most are related to the requirement that glutamatergic neurotransmission be intact, possibly to convey requisite electrical activity-dependent competitive interactions known to serve as a patterning mechanism in other systems (Purves and Lichtman, 1985; Erzurumlu and Kind, 2001; but see Henderson et al., 1994). For example, the NMDA NR1 receptor (Li et al., 1994; Iwasoto et al., 1997) has been shown to be necessary for the development of barrel-like patterns in the PrV. The axon guidance molecule, Hoxa2, has also been implicated in PrV development (Oury et al., 2006).

Transcriptional mechanisms that regulate whisker-related pattern formation are also now being revealed. Drg11, a paired homeodomain-containing protein, is the first transcription factor that has been shown to be necessary for the development of the PrV-based lemniscal pathway (Ding et al., 2003). In Drg11$^{-/-}$ mice that survive into adulthood, whisker-related patterns fail to develop in the PrV, thalamus and cortex, but they do develop in the SpVi and SpVc. Such specificity is unprecedented in the study of the developing V system in the sense that it reveals differing genetic substrates for patterning

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different portions of the V brainstem complex. Other transcription factors, including Tlx3 and Ebf1, 2 and 3 have also been shown to be expressed in the developing PrV (Qian et al., 2002; Ding et al., 2003), but their function has yet to be ascertained.

Lmx1b is a LIM homeodomain transcription factor that has been implicated in the development of several neuronal systems, such as the serotonin-containing raphe nuclei (e.g. Zhao et al., 2006). Here, we show that in mouse embryonic development, Lmx1b is also abundantly expressed in the PrV. But, unlike the case for Drg11, Lmx1b is not expressed in the V ganglion. Therefore, genetic manipulations targeting Lmx1b have implications for PrV development exclusive of any confounding effects upon its presynaptic V primary afferent inputs. Thus, we report on the development of the PrV-based lemniscal pathway in Lmx1b$^{-/-}$ embryos. Similarities between the latter and Drg11$^{-/-}$ mice, along with known actions of Lmx1b upon Drg11 expression, suggest that these two genes function within the same genetic signaling cascade to form barrelettes within the developing PrV.

Results

Lmx1b is expressed in postmitotic PrV neurons, but not in the V ganglion

The spatiotemporal expression patterns of Lmx1b were studied at varied ages in the embryonic PrV by the use of in situ hybridization. At E12.5, many neurons that have completed their ventrolateral migration from the ventricular zone and are beginning to form the nascent PrV display intense Lmx1b expression (Fig. 1A). This continues through E15.5 when Lmx1b staining becomes more widespread throughout the PrV and appears to also extend dorsomedially into the supratrigeminal region abutting the PrV (Fig. 1B). In the SpVi, Lmx1b is only weakly expressed and this occurs in SpVi's dorsomedial extent (Fig. 1C). Lmx1b staining was never observed in the V ganglion or motor nucleus (Figs. 1A–C). To assess the mitotic status of Lmx1b expressing cells, BrdU pulse-labeling was followed by Lmx1b immunohistochemistry. BrdU/Lmx1b double-labeled cells were never observed in the PrV at E11.5 (Fig. 1D), indicating that Lmx1b is only expressed in postmitotic PrV neurons.

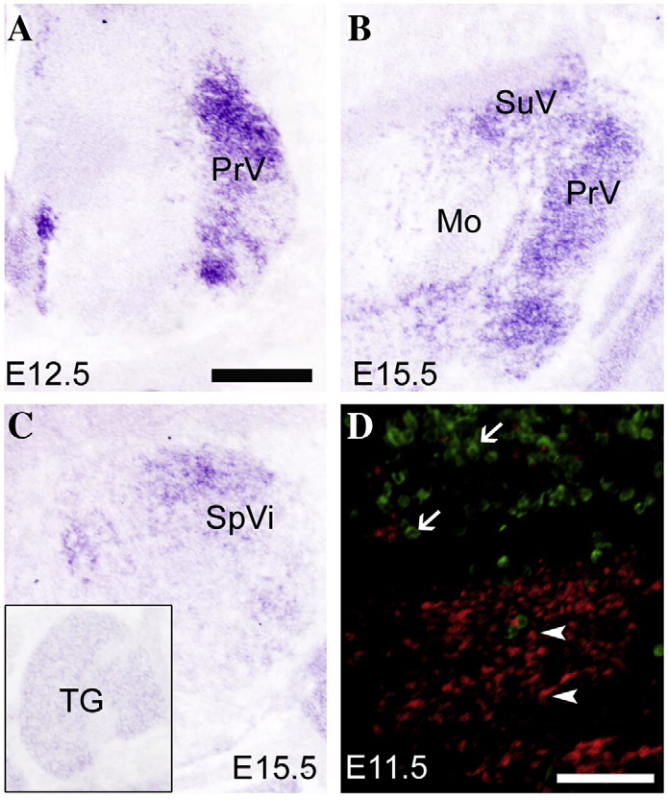

Fig. 1. Expression of Lmx1b in wild-type (WT) developing trigeminal nuclei detected by in situ hybridization (A–C) and immunocytochemical staining (D). A. At E12.5, Lmx1b is detected in the presumptive PrV. B. At E15.5, Lmx1b expression has expanded concurrent with the increase in the size of PrV. It is also present in the supratrigeminal nucleus (SuV), but not in the trigeminal motor nucleus (Mo). C. Lmx1b is also expressed in SpVi , but much weaker compared to PrV. Lmx1b is not expressed in TG (small frame). D. Lmx1b (red, arrowheads) is not colocalized with BrdU staining (arrows, green). Scale bar: 100 μm (A–C); 50 μm (D).

PrV cell survival in Lmx1b mutant mice

The preceding section revealed Lmx1b expression coinciding with PrV morphogenesis. To assess whether Lmx1b impacts PrV cell morphogenesis, PrV integrity was evaluated in Lmx1b$^{-/-}$ embryos. Until E17.5 there were no obvious cytoarchitectural differences between the mutant and wild-type PrV (Figs. 2A, B). However, at E18.5, the Lmx1b$^{-/-}$ PrV as a whole was narrower and less densely populated than the PrV of littermate controls (Figs. 2C, D). This could reflect a reduced genesis of postmitotic neurons. However, BrdU pulse-labeling produced equal numbers and distributions of labeled PrV cells in mutant and wild-type embryos (Figs. 2E–G). The reduced size of, and decreased cell density within, the Lmx1b$^{-/-}$ PrV might then reflect increased cell death there. At E16.5, the spatial distributions and numbers of TUNEL+PrV cells were indistinguishable between mutant experimental and control cases (Figs. 3A–C). However, by E18.5, significantly increased PrV cell death was detected in the mutant group (Figs. 3D–F). This was reflected in a dramatically reduced total number of PrV cells on the day of birth. The mutant PrV contained 21,734 +/− 2303 cells (mean +/− standard deviation; $N=6$), which is significantly lower (one-tailed t-test, $p<.05$) than the 48,439 +/− 6515 PrV cells estimated in wild-type littermate controls ($N=8$). Consequently, the total number of V ganglion cells (most of which project to PrV) was significantly reduced ($p<.05$) in these same Lmx1b $^{-/-}$s at birth (23,350 +/− 2563) vs. wild-type controls (40,829 +/− 3755).

PrV cell death mechanism in Lmx1b mutant mice

The preceding section revealed extensive PrV cell death that might be attributed to Lmx1b deletion-induced activation of an apoptotic pathway. To test this hypothesis, mice lacking the proapoptotic gene, Bax, were crossed with Lmx1b heterozygotes. On the day of birth (P0), the Lmx1b/Bax$^{-/-}$ PrV had a normal shape, size and cell density, unlike the Lmx1b single null PrV (Figs. 4A–D). Cell counts in random transverse sections taken through the single null PrV on the day of birth (Fig. 4E) revealed significant cell loss that was not observed in the Lmx1b/Bax$^{-/-}$ cases. Such PrV cell counts did not differ between Bax single $^{-/-}$ and wild-type mice in this non-stereological assessment. To provide independent validation of Bax deletion-induced rescue of V neurons based upon stereological estimates, total numbers of V ganglion cells were estimated. In 3 Lmx1b/Bax double null mutants, total ganglion cell numbers (31,451 +/− 1460) fell at a midpoint between above-listed totals obtained from Lmx1b single nulls and wild-type controls (23,350 and 40,829, respectively).

Normal primary afferent projections in Lmx1b mutant mice

Bulk labeling of the peripheral projections of the Lmx1b$^{-/-}$ V ganglion revealed a qualitatively normal innervation pattern of the whiskers (Figs. 5A–D). DiI-labeled axons displayed the characteristic and dense circumferential projections to individual whisker follicles when viewed either in tangential or transverse sections. No differences were observed between mutant and wild-type cases at this rather gross level of analysis.