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BRAIN RESEARCH

Research Report

Expression and function of *Nkx6.3* in vertebrate hindbrain

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ARTICLE INFO

Article history:
Accepted 18 April 2008
Available online 6 May 2008

Keywords:
Homeobox transcription factor
CNS development
V2 interneuron
Nkx6.3
Nkx6.1
Rhombomere
Medullary reticular formation

ABSTRACT

Homeodomain transcription factors serve important functions in organogenesis and tissue differentiation, particularly with respect to the positional identity of individual cells. The Nkx6 subfamily controls tissue differentiation in the developing central nervous system where they function as transcriptional repressor proteins. Recent work indicates that *Nkx6.3* is expressed in hindbrain V2 interneurons that co-express *Nkx6.1*, suggesting the possibility of functional redundancy. Here, we report that *Nkx6.3* expression is specific to Chx10^{+} V2a interneurons but not to Gata3^{+} V2b interneurons of the hindbrain, and that *Nkx6.3* expression appears to mark cells of the prospective medullary reticular formation. Molecular analysis of *Nkx6.3* null embryonic mouse hindbrain did not reveal detectable defects in progenitor markers, motor neuron or V2 interneuron sub-types. Forced expression of *Nkx6.3* and *Nkx6.1* promote V2 interneuron differentiation in the developing chick hindbrain. These findings indicate *Nkx6.3* function is dispensable for CNS development and lead to the proposal that absence of overt defects is due to functional compensation from a related homeodomain transcription factor.

1. Introduction

The "reticular formation" is located in the central brainstem running through the mid-brain, pons and medulla. The ascending reticular activating system connects to areas in the thalamus, hypothalamus, and cortex, while the descending reticular activating system connects to the cerebellum and sensory nerves. The reticular formation is an important regulator in the autonomic nervous system for such processes as respiration rate, heart rate and gastrointestinal activity, and it is proposed to have further roles in sleep and consciousness, modulation of pain and other behaviors. Development of this system is poorly understood.

The Nkx6 subfamily is part of the Nkx class of transcription factors. Its 18 members have diverse functions in precise regional specification of precursor cells in a variety of organ systems including the central nervous system (CNS) (Kimura et al., 1996; Lyons et al., 1995; Pabst et al., 1999; Stanfel et al., 2005; Sussel et al., 1998). Two Nkx6 subfamily members, *Nkx6.2*, previously called *Gtx* (Komuro et al., 1993), and *Nkx6.1* (Rudnick et al., 1994) share DNA-binding preference, repress transcription, and show features of dynamic regulation in

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doi:10.1016/j.brainres.2008.04.072

broad areas of the developing ventral hindbrain and spinal cord (Mirmira et al., 2000; Muhr et al., 2001; Qiu et al., 1998). Sonic hedgehog (Shh) signaling induces *Nkx6.1* and *Nkx6.2* expression in ventral neural tube progenitors where they exhibit redundant function in neuron and oligodendrocyte specification (Briscoe et al., 2000; Cai et al., 2005; Vallstedt et al., 2001).

Absence of Nkx6.1 results in a substantial decrease in the number of V2 interneurons and somatic motor neurons with significant cell loss along the murine CNS anterior–posterior (A-P) axis (Sander et al., 2000a); oligodendrocyte differentiation is delayed in the spinal cord but not in the hindbrain (Liu et al., 2003). Isolated Nkx6.2 loss causes an approximately 50% decrease in V1 interneurons and a corresponding increase in V0 neurons without affecting the number of somatic motor neurons or V2 neurons (Vallstedt et al., 2001). However, combined loss of Nkx6.1 and Nkx6.2 reduces the number of motor neuron by 90% throughout the spinal cord (Vallstedt et al., 2001) and interferes with proper differentiation, migration and projection of visceral motor neurons in the caudal hindbrain (Pattyn

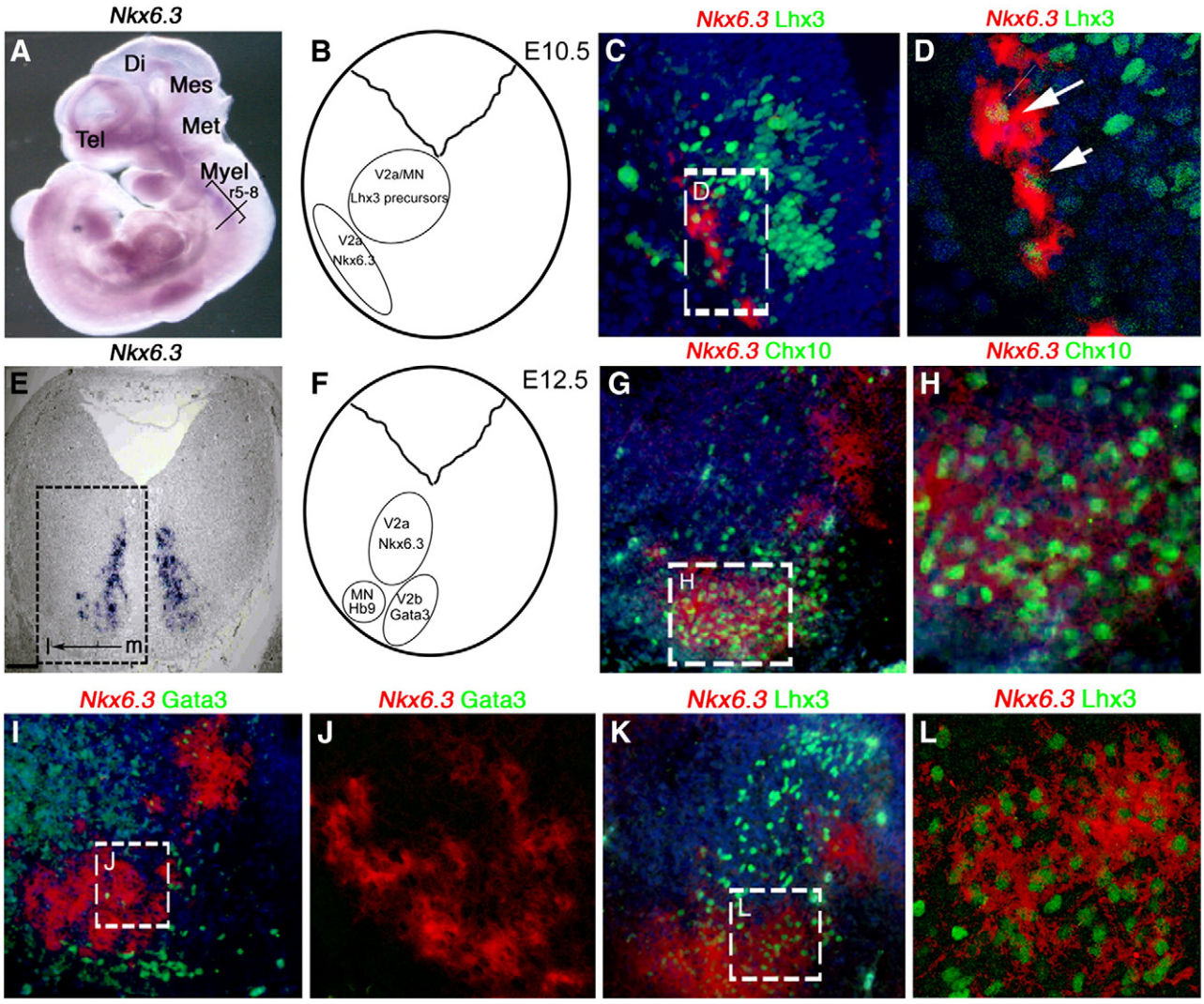

Fig. 1 – Expression of *Nkx6.3* in V2a interneurons of the embryonic CNS. (A–D) E10.5 embryo. (A) Whole mount *in situ* hybridization confirms *Nkx6.3* expression between rhombomere 5 and rhombomere 8 (r5–8). A-P level of analysis (transverse black line at r7), telencephalon (tel), diencephalon (di), mesencephalon (mes), metencephalon (met), and myelencephalon (myel) are indicated. (B) Cartoon of transverse section at level of analysis showing prospective V2a/b and MN populations. (C) Combined double-label ISH for *Nkx6.3* mRNA (pseudocolored red, cytoplasmic) and immunohistochemistry (IHC) for Lhx3 proteins (green, nuclear); (D) confocal photomicrograph showing overlap of *Nkx6.3* and Lhx3 in some ventrolateral cells. (E–L) E12.5 embryo. (E) *Nkx6.3* ISH in a transverse section from E12.5 caudal hindbrain. (F) Cartoon showing relative location of *Nkx6.3* expression with V2a/b interneuron and motor neuron populations in the E12.5 caudal hindbrain at the level of analysis. (G–L) Double-label ISH for *Nkx6.3* (red pseudocolor) and IHC with against Chx10, Gata3 and Lhx3 (green). (H, J, and L) High-resolution confocal images from areas indicated within (G, I, and K). (G and H) Note that (G and H) the V2a marker Chx10 and (K and L) Lhx3 are co-expressed in most *Nkx6.3*$^{+}$ cells, whereas the V2b marker Gata3 is not co-expressed. In all panels except (A) dorsal is top, ventral is bottom, medial (m) is right and lateral (l) is to left. Scale bars: C, G, I, and K = 10 μM, E = 100 μM, D, H, J, and L = 25 μM. Original magnification A = ×2.5, C, G, I, and K = ×40, E = 10×, D, H, J, and L = ×80.