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Mini-Review

Axial Level-Specific Regulation of Neuronal Development: Lessons From PITX2

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Transcriptional regulation of gene expression is vital for proper control of proliferation, migration, differentiation, and survival of developing neurons. *Pitx2* encodes a homeodomain transcription factor that is highly expressed in the developing and adult mammalian brain. In humans, mutations in *PITX2* result in Rieger syndrome, characterized by defects in the development of the eyes, umbilicus, and teeth and variable abnormalities in the brain, including hydrocephalus and cerebellar hypoplasia. Alternative splicing of *Pitx2* in the mouse results in three isoforms, *Pitx2a*, *Pitx2b*, and *Pitx2c*, each of which is expressed symmetrically along the left–right axis of the brain throughout development. Here, we review recent evidence for axial and brain region-specific requirements for *Pitx2* during neuronal migration and differentiation, highlighting known isoform contributions.

Key words: brain; migration; differentiation; spinal cord

In humans, heterozygosity for mutations in the homeodomain transcription factor gene *PITX2* results in Rieger syndrome, characterized by ocular defects, dental malformations/hypodontia, craniofacial abnormalities, and failure of periumbilical involution (Semina et al., 1996). Some individuals with Rieger syndrome have structural brain defects that include cerebellar hypoplasia, enlargement of the cisterna magna, and hydrocephalus, but the underlying mechanisms contributing to these defects are not known (Idrees et al., 2006). In humans, *PITX2* is expressed as four distinct isoforms, *PITX2A*, *PITX2B*, *PITX2C*, and *PITX2D*, through alternative splicing and promoter usage (Cox et al., 2002); however, the specific expression patterns of each isoform in the human brain have not been fully explored.

In mice, *Pitx2* has a broad range of expression and is required for development of the eyes, teeth, heart, lungs, gut, umbilicus, and central nervous system (CNS; Ryan et al., 1998; Gage et al., 1999; Kitamura et al., 1999; Lin et al., 1999; Hjalt et al., 2000; Suh et al., 2002; Martin et al., 2004). In the CNS, *Pitx2* is expressed in the forebrain (subthalamic nucleus, mammillary region, and zona limitans intrathalamica), midbrain (superior colliculus and red nucleus), hindbrain (ventral rhombomere 1), and spinal cord (Fig. 1; Mucchielli et al., 1997; Lindberg et al., 1998; Martin et al., 2002; Zagoraiou et al., 2009; Waite et al., 2011, 2012; Matsui et al., 2013). *Pitx2* is expressed in mice as three distinct isoforms, *Pitx2a*, *Pitx2b*, and *Pitx2c*. In the mouse, all three isoforms are expressed in the developing craniofacial tissue, brain, hematopoietic stem cells in the liver, pituitary, eyes, and teeth (Gage and Camper; 1997; Smidt et al., 2000; C. Liu et al., 2001; W. Liu et al., 2003; Kieusseian et al., 2006; Ai et al., 2007; Waite et al., 2013), whereas only Pitx2c is expressed in the lateral plate mesoderm, heart, lungs, and gut (Kitamura et al., 1999; Schweickert et al., 2000; Yu et al., 2001).

All three isoforms are present by E9.25–E9.5 in the developing brain (Gage and Camper, 1997; Smidt et al., 2000; C. Liu et al., 2001; W. Liu et al., 2003; Kieusseian et al., 2006; Ai et al., 2007; Waite et al., 2011). In the developing zebrafish brain, *Pitx2c* is expressed asymmetrically, and *Pitx2a* and *Pitx2b* are not present (Essner et al., 2000), suggesting that the requirements and roles for *Pitx2* isoforms are species specific.

PROLIFERATION AND MIGRATION

Pitx2 is expressed primarily in postmitotic neurons (Smidt et al., 2000; Martin et al., 2002) and has no known role in neural progenitor proliferation. Global and neural-specific loss of *Pitx2* results in arrested or delayed migration of

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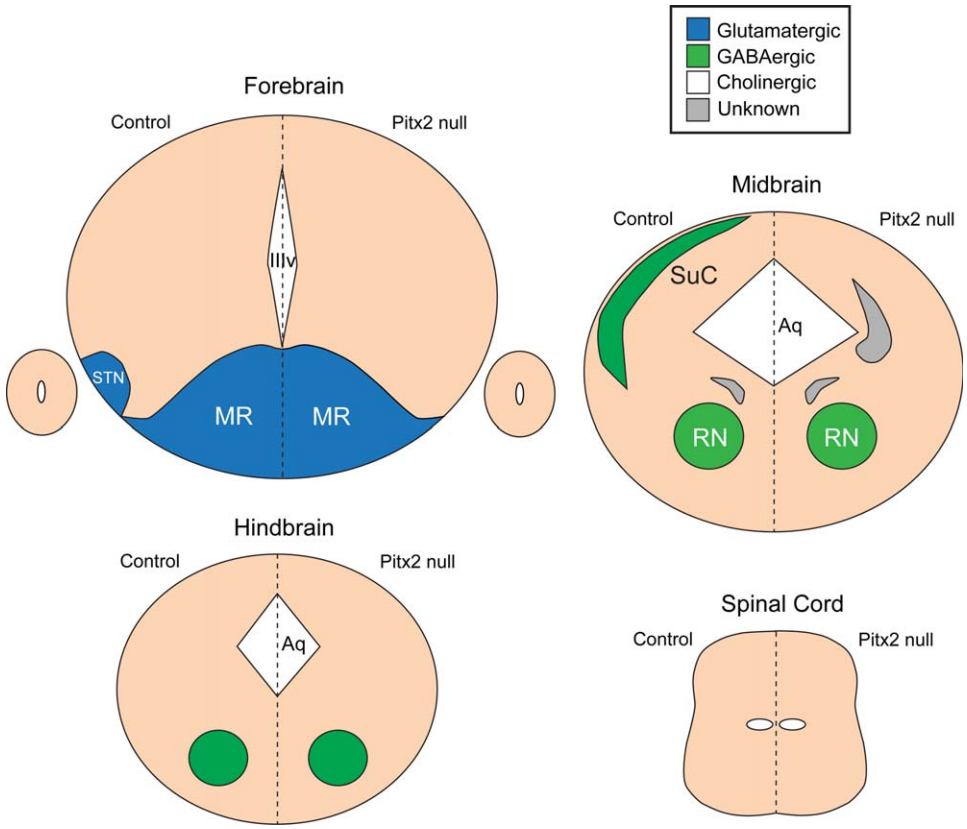

Fig. 1. *Pitx2* expression throughout the brain and spinal cord and effects of loss of *Pitx2* on various neuronal populations. Schematic of *Pitx2* expression in coronal sections of the forebrain, midbrain, hindbrain, and spinal cord. Left side of the coronal section represents control genotype, and right side represents the *Pitx2* null genotype. *Pitx2*-positive populations are color coded based on their neurotransmitter identity. IIIv, third ventricle; Aq, aqueduct; MR, mammillary region; RN, red nucleus; SuC, superior colliculus; STN, subthalamic nucleus.

neurons in the subthalamic nucleus and midbrain in a cell-autonomous manner (Martin et al., 2004; Skidmore et al., 2008; Waite et al., 2011). Effects of *Pitx2* deficiency on migration have not been observed at other axial levels, and it is not known whether *Pitx2* regulates proliferation or neuronal migration in other brain regions.

GABAergic DIFFERENTIATION

In the dorsal midbrain, *Pitx2* is required for the development of GABAergic neurons via a molecular cascade that begins with *Helt*, a basic helix-loop-helix (bHLH) transcription factor expressed in, and required for, GABAergic midbrain progenitor neuronal differentiation (Cazorla et al., 2000; Miyoshi et al., 2004; Nakatani et al., 2004). *Helt* cooperates with *Ascl1* to promote GABAergic differentiation, and *Helt* is required for expression of the transcription factor *Gata2* in neural progenitors as they exit the cell cycle (Kala et al., 2009). In turn, *Gata2* is required in neuronal GABAergic precursors to promote GABAergic over glutamatergic fates and for downstream *Pitx2* expression. *Pitx2* null mice exhibit loss of GABA in midbrain *Pitx2*-expressing neurons, suggesting that *Pitx2* is required for GABAergic differentiation of a midbrain neuronal subpopulation (Waite et al., 2011). Finally, *Pitx2* is known to activate the *Gad1* promoter (Westmoreland et al., 2001) and might therefore function as a GABAergic terminal differentiation factor in the midbrain *Helt* cascade.

Although *Pitx2* appears to be required for the differentiation of some GABAergic subpopulations, little is known about human diseases resulting from GABAergic neuron loss in these regions. Additionally, genes required for GABAergic subpopulation differentiation, such as *Ascl1*, *Helt*, and *Gata2*, are critical for mouse survival, and detailed studies of conditional deletions are required to determine the downstream impact of GABAergic neuronal loss (Guillemot et al., 1993; Tsai et al., 1994; Guimera et al., 2006). Mice lacking *Helt* die at 5 weeks old, possibly as a result of neurological impairment, suggesting that the loss of specific GABAergic subpopulations might be fatal. Studies of fetal valproate syndrome suggest that early loss of specific GABAergic subpopulations in the superior colliculus might result in decreased startle responses and

prepulse inhibition as well as increased sensitivity to nociception (Dendrinos et al., 2011). Future studies will identify additional functional roles for specific *Pitx2*-expressing GABAergic subpopulations and how they impact behavioral and health disorders.

AXON FORMATION

In addition to its roles in neural progenitor proliferation, differentiation, and migration, *Pitx2* has been shown to regulate axon formation of the mammillothalamic tract (MTT), an axonal projection that is involved in self-movement cue processing and spatial memory (Vann and Aggleton, 2003, 2004; Kim et al., 2009; Winter et al., 2011). Normally, the MTT is detectable in mice at E18 as it bifurcates rostrally from the principal mammillary tract (PMT; Valverde et al., 2000; Skidmore et al., 2012). In *Nestin-Cre Pitx2* conditional null mice, the PMT appears normal in size and location, whereas the MTT is absent. Because *Pitx2* is highly expressed in the mammillary area and in the cells surrounding the MTT, these data suggest a noncell-autonomous requirement for *Pitx2* in the establishment of this important neural projection (Skidmore et al., 2012).

NEURONAL SURVIVAL AND MAINTENANCE

Pitx2 expression persists in the brain from embryonic development through adulthood, suggesting that it might also have important roles in neuronal maintenance or ongoing signaling to neighboring cells, although this has not been formally tested (Smidt et al., 2000). *Pitx2* does not appear to be required for cell survival, in that loss of *Pitx2* does not promote neuronal death (Martin et al., 2004; Waite et al., 2011). It is not known whether *Pitx2* is required for maintenance of mature neuronal identity.

SUMMARY

In the CNS, *Pitx2* is expressed in distinct neuronal populations in the forebrain, midbrain, hindbrain, and spinal cord. *Pitx2* is required in an isoform-specific manner for formation of the MTT in the forebrain and migration and differentiation of a midbrain GABAergic subpopulation. The specific requirements for *Pitx2* in the hindbrain and spinal cord are still being explored, but early studies point to functions in cellular differentiation and fate establishment. Further characterization of the mechanisms by which *Pitx2* functions should lead to improvements in our understanding of axial level-specific contexts that influence neuronal development.

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