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The transcription factor *Zfp423/OAZ* is required for cerebellar development and CNS midline patterning

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Abstract

The dorsal midline structure is critical for patterning the developing central nervous system (CNS). We show here that *Zfp423/OAZ,* a multiple zinc-finger transcription factor involved in both OE/EBF and BMP-signaling pathways, is required for the proper formation of forebrain and hindbrain midline structures. During embryogenesis, *OAZ* is highly expressed at the dorsal neuroepithelium flanking the roof plate. *OAZ*-deficient mice are ataxic, attributed to the reduction of the cerebellar vermis and some regions of the hemispheres. Characterization of postnatal cerebellar development shows defects in Purkinje cell differentiation and granule cell proliferation. In the forebrain, dorsal telencephalic commissural neurons project axons, but these axons fail to cross the midline and midline glial cells are abnormally distributed. Moreover, there are malformations in midline structures including the septum, thalamus and hypothalamus, suggesting a pivotal role of OAZ in CNS midline patterning.
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Keywords: Midline; Corpus callosum; Cerebellum; Ataxia; OAZ; Zinc finger; Transcription factor; BMP-signaling

Introduction

In the development of the vertebrate central nervous system (CNS), the dorsal midline of the neural tube acts as an important patterning center along the entire anterior–posterior axis (Chizhikov and Millen, 2005). The mechanisms of dorsal midline-dependent patterning are best studied in the developing spinal cord, in which roof plate derived BMP and Wnt-signals control the specification, proliferation and differentiation of dorsal interneurons (Chizhikov and Millen, 2004; Helms and Johnson, 2003). Similarly, during cerebellar development, the isthmic organizer located at the dorsal mid-hindbrain junction establishes the cerebellar territory, and hindbrain roof plate contributes to cell-type specification and proliferation (Alexandre and Wassef, 2003; Chizhikov et al., 2006; Louvi et al., 2003). The molecular mechanism underlying roof plate dependent forebrain patterning is less understood. Evidence suggests that FGF and Wnt-signaling are involved in dorsal telencephalic patterning (Gunhaga et al., 2003; Storm et al., 2003), while BMP-signaling is required locally to pattern the dorsal midline itself (Hebert et al., 2002).

Our genetic disruption studies implicate the transcription factor, *Zfp423/OAZ* (*OE/EBF* associated zinc-finger protein), in both forebrain and hindbrain midline structure formation. The OAZ protein, a 30 kruppel-like C_2H_2 type zinc-finger transcription factor, is involved in multiple signaling pathways via its multiple zinc-finger domains (Hata et al., 2000; Ku et al., 2006; Tsai and Reed, 1997; Tsai and Reed, 1998; Warming et al., 2004). OAZ was originally characterized in immature olfactory receptor neurons in which it negatively regulates transcription from mature olfactory-specific promoters (Tsai and Reed, 1997). Independent studies revealed that retroviral integration events that up-regulate OAZ, or the highly homologous gene Evi3, result in B-cell lymphomas (Warming et al., 2003, 2004). In both systems, OAZ may function as an inhibitor of OE/EBF proteins, a family of atypical helix–loop–helix transcription factors involved in various developmental processes including olfactory neuron maturation and B-cell

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differentiation (Lin and Grosschedl, 1995; Wang and Reed, 1993; Wang et al., 1997).

OAZ was also identified to be a downstream mediator of the BMP-signaling pathway (Hata et al., 2000; Ku et al., 2003, 2006; Shim et al., 2002). During early *Xenopus* development, OAZ interacts with Smad proteins after BMP stimulation and functions as a coactivator with Smads at BMP-regulated promoters *Xvent2* and *Xretpos* (Hata et al., 2000; Shim et al., 2002). OAZ also recruits Parp1, a chromatin-modifying enzyme, to the OAZ-Smads complex to cooperatively modulate BMP-target gene expression (Ku et al., 2003). Interestingly, Smad6, an inhibitor of the BMP pathway, is a mammalian target gene of OAZ (Ku et al., 2006). Taken together, tissue-specific OAZ expression may contribute to the intensity and duration of the BMP-signaling pathway.

Recent studies have demonstrated the specific contributions of OAZ to development of the cerebellum (Alcaraz et al., 2006; Warming et al., 2006). OAZ-deficient mice display specific defects in proliferation and differentiation of neural and glial precursors in the developing cerebellum especially near the midline (Alcaraz et al., 2006). In the present study, we have investigated OAZ expression during neuronal development and extended the loss-of-function analysis of OAZ to the entire brain by immunostaining and micro-diffusion tensor imaging (μDTI). OAZ is highly expressed in the developing dorsal neuroepithelium flanking the roof plate and OAZ deletion in mice impairs both forebrain and hindbrain midline structures. In *OAZ*-null mice, an early fusion defect in the hindbrain results in loss of the cerebellar vermis and part of the hemispheres. During postnatal cerebellar development, Purkinje cell (PC) differentiation and granule cell (GC) proliferation are affected. In the forebrain, OAZ deletion causes anomalies in the septum, thalamus and hypothalamus. In addition, major commissural tracks including the corpus callosum and hippocampal commissure fail to cross the midline as a result of earlier midline patterning defects. These experiments provide *in vivo* evidence for the multi-functional nature of this transcription factor and underscore the importance of OAZ-mediated signaling in CNS midline patterning.

Materials and methods

Mouse genetics

The OAZ^{lacZ} mouse line was generated from ES cell line XB409 (Bay Genomics—baygenomics.ucsf.edu) by blastocyst injection. The exact insertion site was mapped by Southern blot analysis and specific PCR primers were designed for genotyping (wild-type allele: forward primer, 5′-CTGGATCAGCCCTTCTTGAGTCCTA-3′; reverse primer, 5′-CAATGGCGAGGAAGAAACTCTGATG-3′; tagged allele: the forward primer paired with a third primer in the lacZ gene 5′-CCACAACGGGTTCTTCTGTTAGTCC-3′). The *OAZ* knock-out targeting vector was constructed from restriction fragments of 6 kb and 5 kb flanking exon 4 of the SVJ129 *OAZ* gene. An *IRES-YFP-pA* cassette followed by the *LoxP-TK(Δ)-Neo-LoxP (LTNL)* cassette was inserted at amino acid 112 and replaced the downstream sequence in exon 4. Homologous integrants were identified by Southern blot and correctly targeted ES cells were injected into C57BL/6 blastocysts. The F1 heterozygous mice were mated with Cre-expressing transgenic mice to remove the *LTNL* cassette. Mice were genotyped using a 3-primer PCR analysis of genomic DNA, with forward and reverse primers amplifying the wild-type allele (forward primer, 5′-GTGCCTCAAAGAGTTCCGTAGCAAG-3′; reverse primer, 5′-AGTGCTGCCTCTCTGGGTTGCGATA-3′), and a third primer in the YFP sequence (forward primer, 5′-CTTCTTCAAGGACGACGGCAACTA-3′). The *OAZ* wild-type allele is detected as a ~410 bp product and the targeted allele as a ~820 bp product. All mice were maintained on a mixed 129/BL6 background.

X-gal staining, immunolocalization and in situ hybridization

X-gal whole-mount and section staining were performed as described (Monuki et al., 2001). Immunohistochemistry was performed on paraffin or PFA sections. The following primary antibodies were used: BrdU (rat, 1:100; Abcam, Cambridge MA), GFAP (rabbit, 1:1000, Sigma, St. Louis MO), parvalbumin (mouse, 1: 2000; Sigma), neurofilament M (rabbit, 1:1000, Chemicon, Temecula, CA) and Zic1 (rabbit, 1:400; Rockland, Philadelphia, PA). Alexa 546-labeled secondary antibodies were used at 1:1000 dilutions. Biotinylated secondary antibodies were used at 1:200 dilutions and detected with streptavidin-conjugated peroxidase (Vector Labs, Burlingame, CA). DAPI was used as a nuclear counterstain in fluorescent images. *In situ* hybridization with the *OAZ* probe (NM_033327, nucleotide 102-1715) was performed on PFA-fixed sections following a standard protocol (Wang et al., 2004). Images were obtained with either a Leica stereomicroscope or a Zeiss Axioplan microscope except Zic1 staining was obtained with a Zeiss LSM 510 confocal laser-scanning microscope.

BrdU labeling and counting

Pregnant females or pups were injected intraperitoneally with BrdU (Sigma) 50 μg/g body weight 30 min before they were sacrificed. Sections were incubated with 3N HCl for 30 min before immunostaining with anti-BrdU antibody. Counting of BrdU+ cells was performed on images taken at 10× magnification.

MRI

P0 $OAZ^{-/-}$ and $OAZ^{+/-}$ heads were immersion-fixed in 4%PFA. P20 mice were perfused with 4% PFA and the brains were removed from skull and post-fixed in 4% PFA until imaged. Imaging was performed as previously described (Wang et al., 2006).

Results

OAZ expression assayed with a β-geo gene-trap reporter

To define OAZ expression, we generated genetically modified mice from an ES cell line (XB409) harboring a *β-geo* gene-trap reporter into the last intron of *OAZ* gene. This insertion resulted in the deletion of nine amino acids from the C-terminus of OAZ and expression of an OAZ-β-geo fusion protein (Fig. 1a). At embryonic (E) 8.5, $OAZ^{lacZ/+}$ mice display bilateral expression of the OAZ-β-geo reporter along the dorsal neural tube (Fig. 1b) and this expression continued through later embryonic stages. At E9.5, OAZ was expressed as two narrow continuous bands along the dorsal midline extending from the telencephalon caudally along the entire body axis (Figs. 1c, d). In transverse sections, OAZ expression was mainly detected in the neuroepithelum, with the most intense staining at the dorsal region flanking the roof plate (Figs. 1e–g). Later in embryonic development, X-gal staining was detected in limbs, lung, and the cranial sensory placodes, including the olfactory, lens, otic placodes and branchial arches (data not shown). In sagittal sections of E14.5 $OAZ^{lacZ/+}$ embryo, strong X-gal staining was detected in the olfactory epithelium, thalamus, septum, midbrain

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