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Cell type-selective expression of the zinc finger-containing gene *Nolz-1/Zfp503* in the developing mouse striatum

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HIGHLIGHTS

- Nolz-1 protein is expressed in early differentiating striatal neurons.
- Nolz-1 protein is not expressed in striatal interneurons.
- Nolz-1 is expressed in neurons of the developing ventral striatum.
- Nolz-1 is not expressed in the dorsal LGE.
- Nolz-1 may regulate development of striatal projection neurons.

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ABSTRACT

The zinc finger-containing gene *Nolz-1/Zfp503* is a developmentally regulated striatum-enriched gene. In the present study, we characterized the cell type-selective expression pattern of Nolz-1 protein in the developing mouse striatum. Nolz-1 immunoreactivity was present in Isl-1-positive ventral LGE (vLGE, striatal primordia), but absent in Pax6-positive dorsal LGE (dLGE, non-striatal primordia). In the vLGE, Nolz-1 immunoreactivity was detected in early differentiating TuJ1-positive neurons, but not in Ki67-positive proliferating progenitor cells. Moreover, many Nolz-1-immunoreactive cells co-expressed Foxp1 or Foxp2, markers for striatal projection neurons. To further characterize Nolz-1 expression with respect to D1R-containing striatonigral and D2R-containing striatopallidal projection neurons, we used the *Drd1*-EGFP and *Drd2*-EGFP transgenic mice. Nolz-1 and EGFP double labeled neurons were found in the developing striatum of *Drd1*-EGFP and *Drd2*-EGFP mice, indicating Nolz-1 expression in both populations of striatal projection neurons. Notably, Nolz-1 protein was not expressed in Nkx2.1-positive interneuron progenitors, Lhx8-positive cholinergic interneuron progenitors, nNOS and calretinin-positive interneurons in E18.5 striatum. In the developing nucleus accumbens and olfactory tubercles of ventral striatum, many Nolz-1-positive cells co-expressed Sox1, an important transcriptional regulator for ventral striatum, suggesting a role of Nolz-1 in regulating development of the ventral striatum. Finally, in contrast to postnatal down-regulation of Nolz-1 in the dorsal striatum, Nolz-1 protein was persistently expressed in the olfactory tubercle from E15.5 to adulthood. Taken together, our study suggests that Nolz-1 serves as a marker for early differentiating striatal projection neurons and that Nolz-1 may regulate development of striatal projection neurons.

1. Introduction

The zinc finger-containing gene *Nolz-1* (*Zfp503*, Mouse Genome Informatics) is a mammalian member of the *nocA/elb/tlp-1* (NET) family [11]. The NET family members share conserved motifs of Sp, buttonhead box and C_2H_2 zinc finger [11], and they function as transcription regulators, primarily as transcriptional repressors [1,6,10,12,13]. The NET family members play important roles in neural development. For instance, *Nlz1* and *Nlz2*, the zebrafish homologues of *Nolz-1*, regulate formation of rhombomeres in hindbrain and closure of the optic fissure [1,5,12,13], and the chick homologue of *Nolz-1* specifies subtypes of motor neurons in developing spinal cord [6].

We have previously identified *Nolz-1* as a mammalian homologue of the NET family by subtractive cDNA library cloning [2]. We and other groups have shown that *Nolz-1* mRNA is enriched in the developing striatum of rodent telencephalon [2,16]. Recent study has further indicated that *Nolz-1* increases neurogenesis in striatal cell culture by regulating retinoic acid signaling [16].

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To fully understand the biological function of Nolz-1 in developmental regulation of striatal neurons, it is important to know if Nolz-1 is expressed by specific subtypes of striatal neurons during development. In the present study, we aimed to characterize the cell type-selective expression pattern of Nolz-1 protein in the developing mouse striatum.

2. Materials and methods

2.1. Animals

The animal protocols were approved by the Animal Care and Use Committee of National Yang-Ming University. The ICR mice (National Experimental Animal Center, Taipei, Taiwan), *Drd1-EGFP* and *Drd2-EGFP* BAC transgenic mice (Mutant Mice Regional Resource Center, North Carolina, USA) were housed in the animal center of National Yang-Ming University.

2.2. Preparation of brain tissue

Preparation of 4% paraformaldehyde-fixed brain tissue was as previously described [17]. The fixed brains were sectioned at 14–20 μm by cryostat (Leica CM1900, CM3050S).

2.3. Production of affinity-purified anti-Nolz-1 antibodies

The 21B6 and 22B6 anti-Nolz-1 antisera were produced by immunization of the rabbits with the synthesized peptides [MAP(8)-AS] of Ac-CVEDKSSFKPYSKPGSD-amide (amino acid 174–189; NCBI NP_663434.2; Quality Controlled Biochemicals). For generation of the FLP anti-Nolz-1 antibody, GST-tagged full length recombinant Nolz-1 protein was first produced by *Escherichia coli* BL21 (DE3) transformed with pGEX-3X or pGEX-3X-Nolz-1, and the FLP anti-Nolz-1 antiserum was produced by immunization of the rabbits with GST-Nolz-1 recombinant protein (LTK BioLaboratories). The anti-Nolz-1 antisera were further purified by affinity columns.

2.4. Western blotting

The lysates of retinoic acid-treated P19 cells and dorsal cortex, lateral ganglionic eminence, hindbrain, liver, kidney and heart of E13.5 mouse embryos were collected for Western blotting. Western blotting was performed as previously described with anti-Nolz-1 antibodies (21B6P, 1:5000; 22B6, 1:3000; FLP, 1:5000) [17]. For pre-adsorption experiment, anti-Nolz-1 antibody was pre-adsorbed with peptides (5 μg) or recombinant protein (50 μg) for overnight at 4 °C before immunoblotting. Immunoblot signals were detected using a luminescent image analyzer (FujiFilm LAS-4000).

2.5. Immunohistochemistry

For immunostaining of Nolz-1 and Nkx2.1, brain sections were processed for antigen retrieval by incubation of the sections with solution containing 10 mM citrate acid (pH 6.0) for 10 min at 95 °C. The immunostaining was performed as previously described [17]. The following primary antibodies were used: FLP rabbit anti-Nolz-1 antibody (1:1000), mouse anti-Ki67 (1:400; Novocastra/Leica), mouse βIII-tubulin antibody (TuJ1; 1:5000; Promega), mouse anti-Foxp1 antibody (1:1000; kindly provided by Dr. J. Cordell of John Radcliffe Hospital, Oxford, UK), rabbit anti-Foxp2 antibody (1:1000; abcam), 3A4 mouse anti-Isl-1 antibody (1:800, DSHB), mouse anti-Pax6 (1:500; DSHB) and rabbit anti-Sox1 antibody (1:200; Epitomics). Microscopic images of flurorescent immunostaining were acquired with fluorescence microscope (Olympus BX51, BX63) or with confocal microscope (Leica SP2).

3. Results and discussion

3.1. Generation and characterization of affinity-purified anti-Nolz-1 antibodies

To study the expression pattern and biological function of Nolz-1 protein, we generated three polyclonal rabbit anti-Nolz-1 antibodies, 21B6P, 22B6 and Nolz-1 full-length protein (FLP) antibodies.

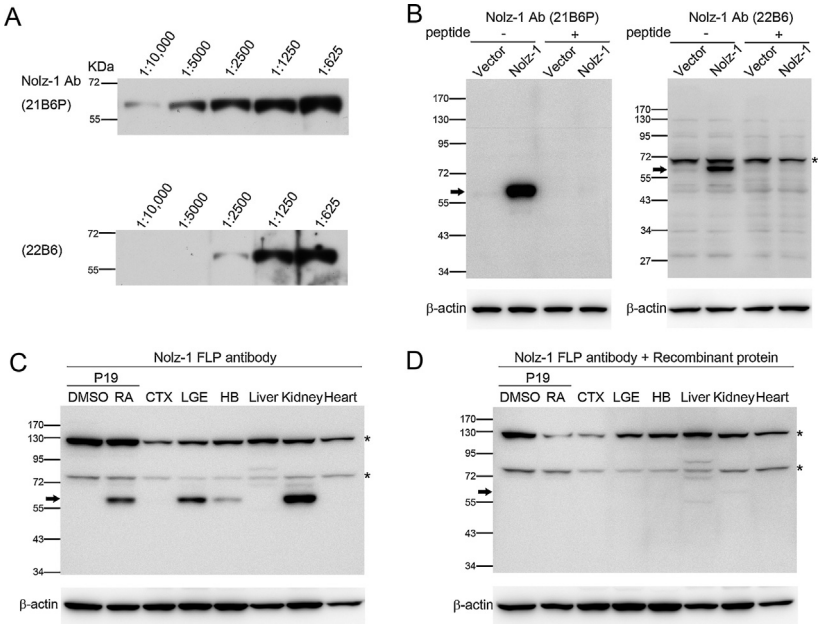

Fig. 1. Characterization of anti-Nolz-1 antibodies. (A and B) The lysates of Nolz-1 over-expressed HEK293T cells were subject to immunoblotting with 21B6P and 22B6 anti-Nolz-1 antibodies. (C) The lysates of retinoic acid (RA)-treated P19 cells, dorsal cortex (CTX), LGE, hindbrain (HB), liver, kidney and heart of E13.5 mouse embryos were subject to immunoblotting with FLP anti-Nolz-1 antibody. (B and D) The specificities of the anti-Nolz-1 antibodies are shown by loss of the signal of ~63 kDa band (arrow) in the immunoblots when the antibodies were pre-absorbed with their antigens. The asterisks indicate non-specific bands.