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Research article

Involvement of SF-1 in neurogenesis and neuronal migration in the developing neocortex

Munekazu Komada, Mifumi Takahashi, Yayoi Ikeda*

Department of Anatomy, School of Dentistry, Aichi Gakuin University, Nagoya, Japan

HIGHLIGHTS

- SF-1 was expressed in the dorsal telencephalon at E15.5–E18.5, but not in adulthood.
- In SF-1 KO embryos, neurons in the IZ/SVZ were increased, in the CP decreased.
- The APCs increased and radial fibers showed abnormal morphology in SF-1 KO embryos.
- Cell cycle duration was shortened and exit inhibited in SF-1 KO stem/progenitor cells.
- Expression of *ESRα* was up- and of *Cyp19a1* down-regulated in SF-1 KO embryos.

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ABSTRACT

The nuclear receptor steroidogenic factor-1 (SF-1) plays essential roles in the development and function of the endocrine and reproductive systems. During embryogenesis, SF-1 is expressed in the ventromedial hypothalamic nucleus (VMH) and regulates the migration and terminal differentiation of the VMH neurons. Additionally, *in situ* hybridization data indicated *SF-1* expression in the dorsal telencephalon at embryonic day (E) 13.5. In this study, we investigated the neocortical development in SF-1 knockout (KO) mouse embryos. The number of neurons was increased in the intermediate/subventricular zones and decreased in the cortical plate in the SF-1 KO embryos. SF-1 KO embryos produced more neural stem/progenitor cells, especially apical progenitor cells, and showed abnormal radial glial fiber morphology. The increase in neural stem/progenitor cells was caused by an increased S-phase fraction in the proliferative cells and the inhibition of cell cycle exit in these cells. The mRNA expression of the estrogen receptor *ESRα* was up-regulated and that of the estrogen synthetase *Cyp19a1* was down-regulated in the dorsal telencephalon of SF-1 KO embryos. We showed that *SF-1* is expressed in the dorsal telencephalon at E15.5 and E18.5, but not in adult animals. Our data demonstrated that SF-1 is involved in cell cycle regulation, neurogenesis, and neuronal migration via controlling the estrogen signaling for proper neocortical development.

1. Introduction

Steroidogenic factor 1 (SF-1), which is encoded by the *NR5A1* gene, is a member of the nuclear hormone receptor family [1]. SF-1 is required for adrenal and gonadal steroidogenesis, controlling the expression of a number of key target genes including those encoding cytochrome P450 steroid hydroxylase and aromatase in adulthood [2]. It is expressed in the gonads, adrenal cortex, pituitary, and hypothalamus during embryogenesis [3,4], and SF-1 knockout (KO) mice exhibit agenesis of the adrenal gland and gonads, deficiency of pituitary gonadotropin secretion, and abnormal structure of the ventromedial hypothalamic nucleus (VMH), suggesting that SF-1 plays critical roles in the development of the tissues at all the levels of the hypothalamus-pituitary-gonad/adrenal axis [5,6]. The disorganization of the VMH was shown to be due to the accumulation of immature neurons in the proliferative zone of the diencephalon, which was caused by migration defects in these neurons [7]. In addition, misexpression of VMH markers and loss of neuronal projections to the bed nucleus of

Abbreviations: APC, apical progenitor cell; CP, cortical plate; DCX, doublecortin; E, embryonic day; ESR, estrogen receptor; GXD, gene expression database; IdU, iododeoxyuridine; IPC, intermediate progenitor cell; IZ, intermediate zone; KO, knockout; SF-1, steroidogenic factor-1; SVZ, subventricular zone; VMH, ventromedial hypothalamic nucleus; VZ, ventricular zone.

* Corresponding author at: 1–100 Kusumoto-cho, Chikusa-ku, Nagoya, Aichi 464–8650, Japan. Fax: +81 52 757 6755.
E-mail address: yayoi@dpc.agu.ac.jp (Y. Ikeda).

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the stria terminalis and the amygdala in SF-1 KO mice have been reported [8]. These data suggest that SF-1 is involved in the regulation of cell fate determination, neuronal migration, and terminal differentiation during the development of the VMH.

During neocortical development, apical progenitor cells (APCs, neural stem cells) in the ventricular zone (VZ) and intermediate progenitor cells (IPCs, neural progenitor cells) in the subventricular zone (SVZ) self-renew and generate the neurons [9]. APCs and IPCs are distinguished by their distribution, self-renewal ability, capacity for neurogenesis, and expression of transcription factors [10]. The division patterns of APCs and IPCs affect the number of neurons and determine the neocortical size [11]. Their proliferation and neurogenesis are orchestrated by a signaling network of factors including morphogens, transcription factors, and growth factors. For example, estrogen receptors and aromatase that are expressed in the developing dorsal telencephalon control the proliferation, migration of neural stem/progenitor cells, and neurogenesis [12,13].

In situ hybridization data available in the Gene Expression Database (GXD), a publicly available resource for gene expression information from laboratory mice, indicated that SF-1 is expressed in the dorsal telencephalon at E13.5 [14]. However, the role of SF-1 in this region remains unknown. In this study, we investigated the roles of SF-1 in the morphogenesis of the neocortex using SF-1 KO embryos. First, we analyzed the histological changes and the changes in expression of markers for neurogenesis, proliferation, and migration of neural stem/progenitor cells using immunostaining. Next, the cell cycle kinetics of neural stem/progenitor cells were examined by iododeoxyuridine (IdU)-labeling and double immunostaining for Ki67 and IdU. Finally, we compared the expression of genes involved in estrogen signaling and neurogenesis during the development of the neocortex in wild-type (WT) and SF-1 KO embryos. The results suggested that SF-1 is involved in the regulation of neurogenesis, proliferation, and neuronal migration in the developing neocortex, as well as in the hypothalamus.

2. Materials and methods

2.1. Immunofluorescence

Immunofluorescence staining of the sections was performed as described previously [15,16]. The antibodies used in the study and detailed methods are described in the Supplemental methods. Primary and secondary antibody incubations were conducted overnight and for 3 h, respectively, at room temperature. Nuclei were counterstained with DAPI (D9542, Sigma–Aldrich, St Louis, MO, USA; 1:1000) for 3 h at RT. Six sections from three embryos per genotype were used for immunofluorescence analysis of each marker.

2.2. IdU incorporation and cell cycle kinetics

For *in vivo* labeling of the S-phase cells and cell cycle exiting cells, IdU (I7125, Sigma–Aldrich, 50 mg/kg) was intraperitoneally injected into the pregnant mice at 1 h and 24 h prior to sacrificing embryos at E15.5, respectively. To estimate the cell cycle duration, we counted the number of Ki67+, proliferative cells that were labeled by a 1 h pulse of IdU. The fraction of IdU+/Ki67+ cells among all Ki67+ cells provides a rough estimate of the neural stem/progenitor cell cycle duration; a smaller population of IdU+ cells among the Ki67+ cells is indicative of an increased cell cycle length [17,16]. The percentage of cells in the dorsal telencephalon exiting cell cycle was estimated from the ratio of IdU+/Ki67- (postmitotic) and IdU+/Ki67+ (cell cycle non-exiting) cells to all IdU+ cells labeled by a 24 h pulse of IdU.

2.3. Quantitative immunofluorescence analysis

Immunofluorescence staining was quantified in selected areas of the dorsal telencephalon by manually counting the number of cells and measuring the areas of expression within the selected area or by measuring the positively stained area in six anatomically matched sections from three embryos using the Adobe Photoshop CS4 software (Adobe, San Jose, CA, USA) as previously described [15,16,18]. NeuN+ and DAPI+ cells were manually counted in the CP and intermediate zone (IZ). Ki67+, Tbr2+, IdU+, and DAPI+ cells were manually counted and Pax6+ and DAPI+ areas were measured in the VZ, SVZ, and IZ. DCX+ and DAPI+ areas were manually measured in sections in a frame with a 100 μm width covering a region from the ventricle to the pial surface of the dorsal telencephalon. The frames used for the counting and measuring are indicated in Fig. 1B–F and A–C. The ratios of positive cells (number of marker+ cells per number of DAPI + cells × 100) and positive areas (DCX and Pax6: marker + area per DAPI + area × 100) were calculated.

2.4. Semi-quantitative RT-PCR

The primer sequences are listed in Supplementary Table S1. Semi-quantification of mRNA expression was performed using Adobe Photoshop CS4 and ImageJ (NIH, Bethesda, MD, USA). Gene expression of the target genes was normalized to β-*actin* expression. Three animals per genotype and per developmental stage were analyzed.

3. Results

3.1. SF-1 KO embryos have a reduced number of neurons in the cortical plate of the dorsal telencephalon

We performed a histological analysis at E15.5 to evaluate whether the dorsal telencephalon of SF-1 KO embryos had an anomalous structure. H&E staining showed no structural differences between WT and SF-1 KO embryos (Fig. 1A, Fig. S1). The effects on neurogenesis and migration of neural stem/progenitor cells in the dorsal telencephalon in the SF-1 KO embryos were investigated by immunostaining of the neuronal markers DCX and NeuN. The DCX+ area in the CP was decreased (WT: 23.7 ± 0.97%, KO: 19.5 ± 0.91%, $p = 0.005$), while the DCX+ area in the SVZ/IZ was increased in the SF-1 KO compared to the WT embryos (WT: 44.8 ± 1.12%, KO: 49.5 ± 0.56%, $p = 0.003$) (Fig. 1B and G). The number of NeuN+ neurons in the SVZ/IZ was increased in the SF-1 KO compared to the WT embryos (WT: 40.0 ± 0.87%, KO: 42.4 ± 0.51%, $p = 0.012$) (Fig. 1C and H).

3.2. Apical progenitor cells, but not intermediate progenitor cells, are increased in the SF-1 KO mice

Next, we analyzed the proliferation of neural stem/progenitor cells by immunostaining at E15.5. The number of cells that were stained positively for Ki67, a marker of proliferative cells, was decreased in the SF-1 KO embryos (WT: 52.8 ± 0.86%, KO: 58.5 ± 1.78%, $p = 0.007$) (Fig. 1D and I). To estimate which neural stem/progenitor cell population (APCs or IPCs) was affected in the SF-1 KO embryos, the sections were immunostained for Pax6 and Tbr2, which are markers for APCs and IPCs, respectively. Since we could distinguish individual Pax6+ cells to count, we measured the Pax6+ area and performed the statistical analysis. Pax6+ area and Tbr2+ cells were detected in the VZ and SVZ, respectively (Fig. 1E and F). The Pax6+ area was significantly expanded in the SF-1 KO compared to the WT embryos (WT: 28.5 ± 2.13%, KO: 40.0 ± 1.49%, $p = 0.003$) (Fig. 1E and J). There was no significant difference in the