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PROP1 coexists with SOX2 and induces PIT1-commitment cells

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ABSTRACT

Prophet of PIT1 (PROP1) is a pituitary-specific factor and responsive gene for the combined pituitary hormone deficiency in *Ames* dwarf mice and human patients. Our immunohistochemical studies demonstrated that PROP1 is consistently expressed in SOX2-expressing stem/progenitor cells in the rat pituitary from embryonic (E) to postnatal periods. At E13.5, all the cells in Rathke's pouch, the primordium of the pituitary, express PROP1. Afterward, PROP1-positive cells localize along the marginal cell layer, a putative stem cell niche in the pituitary, and stratify in the parenchyma of the anterior pituitary. In the embryonic period, PROP1 coexists transiently with PIT1, which is the anterior pituitary-specific factor and is a target of PROP1, but not any hormones. Thus, the present results imply a regulatory role of PROP1 not only in pituitary organogenesis but also in conversion of PIT1-lineage cells.

Introduction

Prophet of PIT1 (PROP1), a pituitary-specific factor and heritable responsive gene for the combined pituitary hormone deficiency in *Ames* dwarf mice [1] and human patients [2–4], participates in morphogenesis, apoptosis and proliferation in the developing pituitary [5], and in pituitary organogenesis including the differentiation of cell lineages [1,6]. Several lines of evidence have indicated that PROP1 participates continuously not only in the specification of pituitary cell lineages but also in the proliferation of cells after birth [7–9]. It is reported that *Prop1* expression in mouse embryonic ontogeny remarkably diminished its level at E14.5 [1], while *Prop1* expression in human is observed in the pituitary adenoma as well as normal adult pituitary [10–12] and in porcine adult pituitary [13]. Meanwhile, cells expressing SOX2, a marker of stem/progenitor cells, are present in the anterior pituitary [14,15]. In this study, we aimed to identify the PROP1-expressing cells during pituitary organogenesis which progress in a unilateral direction from stem/progenitor to terminally differentiated cells. We first generated a specific antibody for PROP1 and conducted immunohistochemical studies on the rat pituitary from embryo to neonate.

Materials and methods

Immunohistochemistry. For immunohistochemistry, embryonic (E) and postnatal (P) pituitaries from Wistar–Imamichi rats were fixed with 4% paraformaldehyde (PFA) in 50 mM phosphate-buffered saline (PBS), pH 7.5, maintained overnight at 4 °C, followed by substitution with 30% sucrose in PBS. Cryosections of 10 μm thickness were reacted with primary antibodies at the appropriate dilution at room temperature overnight. Primary antibodies used were guinea pig antiserum against rat PROP1 (1:1000, in the present study as described in the Supplemental materials and methods), rabbit antiserum against rat PIT1 (1:250, Santa Cruz Biotechnology, Santa Cruz, CA, USA), goat antiserum against human SOX2 (1:500 dilution, Neuromics, Edina, MN, USA), rabbit antiserum against pituitary hormones (ovine LHβ (1:2000 dilution), rat TSHβ (1:2000 dilution), rat GH (1:8000 dilution) and rat PRL (1:1500 dilution), which were kindly provided by the National Institute of Diabetes and Digestive and Kidney (NIDDK) through the courtesy of Dr. A.F. Parlow, and human ACTH (1:1000 provided by Dr. S. Tanaka at Shizuoka University, Shizuoka, Japan). After washing with PBS, incubation with secondary antibodies was then carried out using FITC- or Cy3-conjugated AffiniPure donkey anti-guinea pig, rabbit and goat IgG (Jackson ImmunoResearch, West Grove, PA, USA) or Alexa Fluor 488 conjugated goat anti-rabbit IgG (Molecular Probes, Inc., Eugene, OR, USA). The sections were washed with PBS and then enclosed in VECTASHIELD

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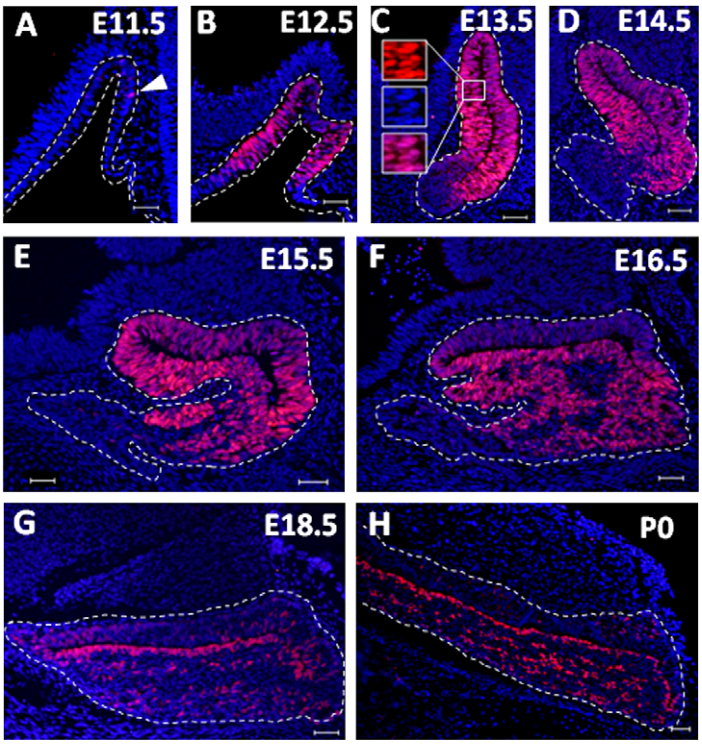

Fig. 1. Immunohistochemistry of PROP1 during rat pituitary development. Immunohistochemistry was performed with anti-PROP1 antiserum on the cryosections of embryonic (E) and postnatal (P) pituitaries. PROP1 is labeled with Cy3 (red) and is overlaid with nuclear staining by DAPI (blue). The Rathke's pouch and anterior and intermediate pituitaries are enclosed within dashed lines. Insets at E13.5 represent PROP1-positive signals (top), DAPI staining (middle) and a merged image (bottom) by enlargement the marked area. Embryonic and postnatal days are indicated. (Scale bars: 50 μm).

mounting medium with DAPI (Vector, Burlingame, CA, USA). Immunofluorescence was observed under fluorescence microscopy with a Confocal Laser Scan microscope; CLSm (Carl Zeiss, Oberkochen, Germany) and BZ-8000 (KEYENCE, Osaka, Japan).

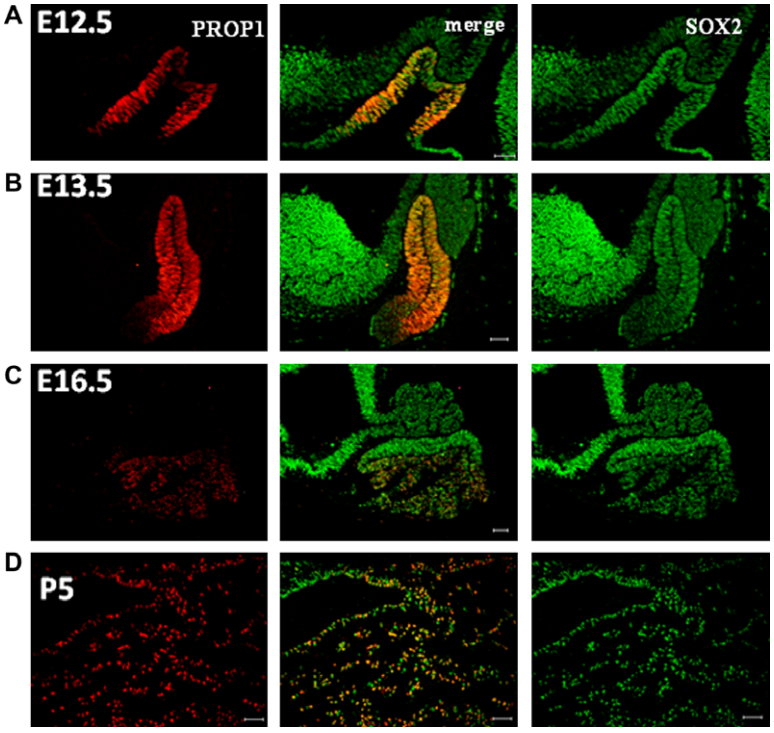

Fig. 2. PROP1-positive cells consistently coexisted with progenitor cell marker, SOX2. Immunohistochemical staining of pituitaries with PROP1 (red, left panel) and SOX2 (green, right panel) at E12.5 (A), E13.5 (B) and E16.5 (C) and P5 (D) was performed, and the merged images (middle panel) are shown. (Scale bar: 50 μm).

Results and discussion

Using the C-terminal region of rat PROP1 (amino acid residues 126–223) fused to TrxA-His-tag, anti-PROP1 polyclonal antibody was generated in guinea pig and the specificity of the antibody was confirmed by Western blotting (Supplementary Fig. 1) and immunohistochemistry (Supplementary Fig. 2). RT-PCR analysis and *in situ* hybridization confirmed the presence of *Prop1* transcripts in the postnatal rat pituitary on day 5 (P5). Signals of both mRNA and protein were found to co-exist in the same cells (Supplementary Fig. 3A and B).

Prop1 expression during rat pituitary organogenesis from E11.5 to P5 was analyzed by immunohistochemistry, and stem/progenitor cells were simultaneously surveyed using anti-SOX2 antibody. PROP1-positive signals first appeared in the invaginating oral ectoderm at E11.5 (Fig. 1A), and extended over a large area of Rathke's pouch at E12.5 (Fig. 1B). Notably, PROP1-signals were weak in the dorsal region which is in direct contact with the ventral diencephalon and were completely absent at the ventral part near the junction of the oral ectoderm; meanwhile SOX2 was present in all cells in the invaginating oral ectoderm in addition to the surrounding oral ectoderm, diencephalon and presumptive hypothalamus

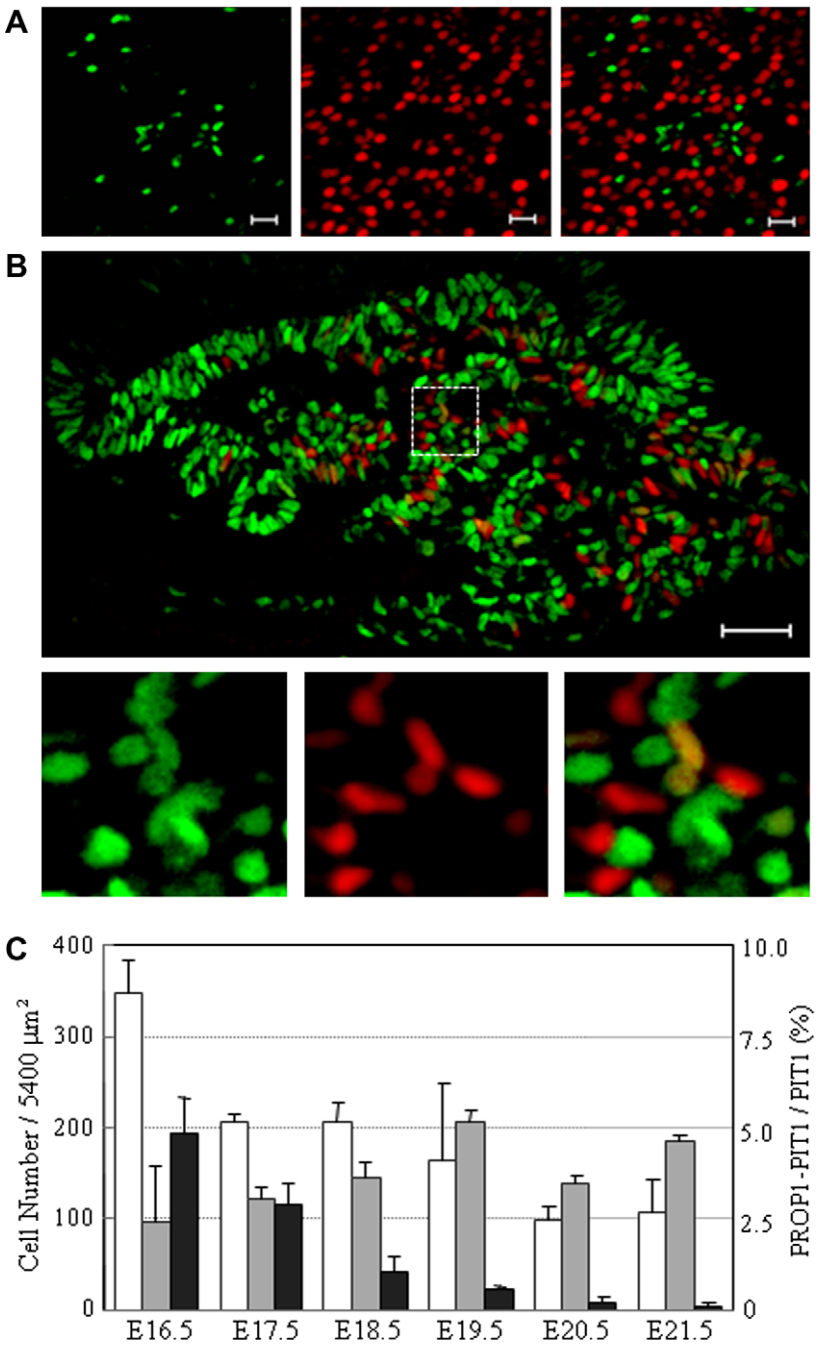

Fig. 3. Attenuation of PROP1–PIT1 coexistence. Immunohistochemistry was performed with anti-PROP1 and anti-PIT1 antisera on the cryosections of the pituitaries of P60 (A) and E16.5 (B). PROP1 and PIT1 are labeled with FITC (green) and Cy3 (red), respectively. (C) Immunohistochemistry of the pituitaries E16.5, E17.5, E18.5, E19.5, 20.5 and E21.5 was performed and numbers of PROP1-positive, PIT1-positive and PROP1-PIT1-positive cells were counted in each of the 6 separate areas (5400–9545 μm^2). The average cell number per 5400 μm^2 (open bar; PROP1-positive cells and gray bar; PIT1-positive cells) and the ratio (%) of PROP1–PIT1-positive cells against PIT1-positive cells (closed bar) are shown. (Scale bars: Fig. 3A, 20μm and Fig. 3B, 50 μm).

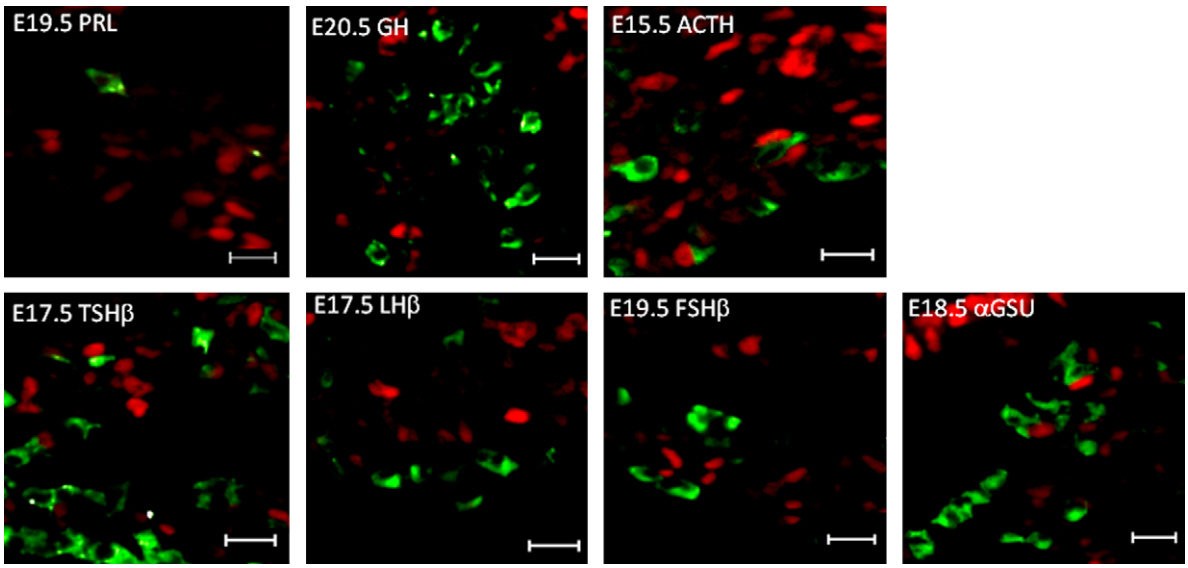

Fig. 4. Double immunohistochemical staining of PROP1 and hormones for fetal rat pituitary. Double immunohistochemical staining was performed to examine whether PROP1 localizes in hormone-producing cells in fetal rat pituitary. PROP1 is labeled with Cy3 (red), and pituitary hormones are labeled with FITC (green). PROP1 signals do not localize in any hormone-positive cells. (Scale bar: 20 μm).

(Fig. 2A). At E13.5, it is remarkable that all the cells in the Rathke's pouch expressed both PROP1 and SOX2, except for those in the rostral tip which originated from the bilateral cells bordering on the oral ectoderm (Figs. 1C and 2B). PROP1 signals at E14.5 began to decrease particularly in the dorsal region of Rathke's pouch, the prospective intermediate lobe (Fig. 1D), indicating that PROP1 is properly excluded from the development of the intermediate lobe. At E15.5 and E16.5, PROP1-positive cells started to migrate and their patchy pattern was obvious in the expanding anterior lobe. On the other hand, most of the prospective intermediate lobe was PROP1-negative at E16.5 (Figs. 1E and F, and 2C). During the fetal period examined, the PROP1-positive cells consistently coexisted with SOX2 (Fig. 2C). At E18.5 (Fig. 1G), PROP1 signals localized in the posterolateral region of the anterior lobe and lined up along the marginal cell layer, which has been postulated as a niche for pituitary stem/progenitor cells [5,16]. In addition, PROP1-positive cells at E18.5 stratified in the parenchyma of the anterior pituitary (Fig. 1G), and this distribution pattern was basically maintained during the neonatal periods (Figs. 1H and 2D). Simultaneously, the occurrence ratio of PROP1-positive cells in P60 decreased to 6% from 17% of P5. The PROP1-positive cells line the marginal cell layer and stratify the parenchyma at P5 still coexisted with SOX2 (Fig. 2D). The notion that the marginal cell layer is a niche for adult pituitary stem/progenitor cells [5,15–17] is supported by the results that the cells positive for SOX2 line the marginal cell layer of both the anterior and intermediate lobes at P0.

One of the functions of PROP1 is to regulate the expression of another pituitary-specific transcription factor, PIT1 [1], which functions mainly in the differentiation of the PIT1-lineage cells, TSH-, GH- and PRL-producing cells, during pituitary organogenesis [1,8,18,19]. PIT1 also regulates the hormone genes by its persistent expression in terminally differentiated PIT1-lineage cells [20]. In this sense, PIT1-expressing cells without any hormones are committed cells destined to differentiate into TSH-, GH- and PRL-producing cells. The PIT1-expressing committed cells first appeared at E16.5 (Fig. 3A) and then, terminally differentiated TSH-, GH- and PRL-producing cells appearing first at E16.5, E19.5 and E19.5 (data not shown), respectively. At E16.5, 9.7% of the PIT1-positive cells were PROP1-positive (Fig. 3B and C). This rate of coexistence of PROP1 and PIT1 declined with the progression of pituitary organogenesis (Fig. 3C). Furthermore, none of the PROP1-positive cells overlapped any endocrine cells in the embryonic period (Fig. 4). This coexistence of PROP1 and PIT1 might be an intermediate condition of the transition from progenitor cells to committed cells, supporting the argument that PROP1 functions as an early activator for the expression of the *Pit1* gene [18]. In the neonatal pituitary, no coexistence of PROP1 and PIT1 could be found (data not shown). These data suggested that PROP1 exists in the SOX2-expressing progenitor cells in the expanding anterior lobe and disappears at the first stage of the transition of progenitor cells to committed cells in the embryonic period.

Our immunohistochemical studies demonstrated for the first time that PROP1 is expressed in the SOX2-positive pituitary progenitor and plays a role in the conversion of PIT1-commitment cells from progenitor cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.05.027.

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