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SHORT COMMUNICATION

Immunohistochemical localization of anterior pituitary hormones in S-100 protein-positive cells in the rat pituitary gland

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Abstract In the anterior and intermediate lobes of the rat pituitary gland, non-hormone-producing cells that express S-100 protein coexist with various types of hormone-producing cells and are believed to function as phagocytes, supporting and paracrine-controlling cells of hormone-producing cells and stem cells, among other functions; however, their cytological characteristics are not yet fully understood. Using a transgenic rat that expresses green fluorescent protein under the promoter of the S100β protein gene, we immunohistochemically detected expression of the luteinizing hormone, thyroid-stimulating hormone, prolactin, growth hormone and proopiomelanocortin by S-100 protein-positive cells located between clusters of hormone-producing cells in the intermediate lobe. These findings lend support to the hypothesis that S-100 protein-positive cells are capable of differentiating into hormone-producing cells in the adult rat pituitary gland.

Keywords S-100 protein · Pituitary hormone · Folliculo-stellate cell · Pituitary · Intermediate lobe

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Introduction

In the anterior and intermediate lobes of the rat pituitary gland, non-hormone-producing cells that express S-100 protein, a soluble protein generally specific to glial cells (Moore 1972), coexist with various types of hormone-producing cells (Soji and Herbert 1989). S-100 protein-positive cells in the anterior lobe are usually referred to as folliculo-stellate (FS) cells. They are interconnected by cytoplasmic processes and encircle hormone-producing cells or aggregate homophilically to form pseudofollicles (Soji and Herbert 1989). In the intermediate lobe, it can be generalized that S-100 protein-positive cells have been observed as clusters wedged among masses of melanocyte-stimulating hormone-producing cells. Other populations of cells expressing S-100 protein include marginal layer cells that surround the residual lumen of Rathke's pouch. The many studies of S-100 protein-positive cells (mainly folliculo-stellate cells in the anterior lobe) have revealed that they function as phagocytes, supporting and paracrine-controlling cells of hormone-producing cells and stem cells, among other functions (Inoue et al. 1999; Allaerts and Vankelecom 2005). Here, we describe our observations that some S-100 protein-positive cells in the intermediate pituitary coexpress adenohypophyseal hormones, which supports the hypothesis that S-100 protein-positive cells can differentiate into hormone-producing cells.

Materials and methods

Animals

Transgenic S100b-GFP rats (Itakura et al. 2007) that express green fluorescent protein (GFP) under the promoter

of the S100β protein gene were donated by Prof. K. Inoue of Saitama University, Japan and were bred in our laboratory. They were housed under conditions of 12-h light, 12-h darkness and provided food and water ad libitum. All animals were treated in accordance with the Guidelines for Animal Experimentation of Jichi Medical University, which are based on the NIH Guidelines for the Care and Use of Laboratory Animals.

Immunohistochemistry

Eight- to 9-week-old male rats weighing 200–250 g were perfused with 4% paraformaldehyde in a 50 mM phosphate buffer (pH 7.4) under deep Nembutal anesthesia. Their pituitary glands were removed and soaked overnight in the same fixative at 4°C. After remaining in a 30% sucrose solution for 2 days at 4°C, the tissue was embedded in an OCT compound and frozen in liquid nitrogen. Five-μm frontal specimens were prepared using a cryostat (CM3050; Leica Microsystems, Wetzlar, Germany). Fluorescent immunostaining was performed using rabbit polyclonal antibodies against synthetic human proopiomelanocortin (POMC; residues 17–39, dilution of 1:3,200; Cymbus Biotechnology, Hampshire, UK), rat thyroid-stimulating hormone (TSH) β subunit (dilution of 1:4,000; NIDDK, Bethesda, MD, USA), rat growth hormone (GH; dilution of 1:12,800; Prof. K. Wakabayashi, Gunma University, Japan), ovine luteinizing hormone (LH) β subunit (dilution of 1:6,400; Prof. K. Wakabayashi) and rat prolactin (dilution of 1:2,500; Prof. K. Wakabayashi). After a 20-min immersion in a phosphate-buffered saline (PBS) containing 2% normal goat serum at room temperature, sections were incubated with the primary antibody in PBS overnight at room temperature. After primary immunoreactions, sections were incubated with fluorescent-labeled secondary antibodies (Alexa Fluor 568-labeled anti-rabbit IgG; Invitrogen, Carlsbad, CA, USA) in PBS for 30 min at 30°C. After the immunoreaction, specimens were coverslipped using a mounting medium with DAPI (Vector Laboratories, CA, USA), observed through an AX80TR fluorescent microscope (Olympus, Tokyo, Japan) and imaged using a DP70 system (Olympus) with the aid of Photoshop software (Adobe Systems, San Jose, CA, USA). The absence of an observable nonspecific immunoreaction was confirmed by preabsorption controls in which each antiserum was preincubated with a hormone (human POMC 1−39, rat TSH, rat LH, human GH, rat prolactin) at a molar ratio of approximately 1:20 for 2 days at 4°C and then centrifuged and the resultant supernatant was used as the preabsorbed antibody. In addition, some frozen sections were stained with hematoxylin and eosin for general observation.

Electron microscopy

Rats were perfused through the left ventricle with 2.0% cold glutaraldehyde (Merck, Darmstadt, Germany) in a 0.1 M cacodylate buffer (pH 7.4) with 2% sucrose for 5 min at 4°C. Pituitary tissues were immediately excised, cut into small pieces and immersed in the same fixatives for up to 30 min. After washing in the buffer, samples were post-fixed in 1% OsO_4 in the same buffer (pH 7.4). They were then dehydrated in an ethanol series and embedded in Quetol 812 epoxy resin (Nissin EM, Tokyo, Japan). Ultrathin sections were made and observed as previously described (Momose et al. 2006).

Results and discussion

Hematoxylin and eosin staining of a cryosection of a S100b transgenic rat is shown in Fig. 1, while Fig. 2 shows areas with representative clusters of S-100 protein-positive cells in the intermediate lobe of the six examined S100b-GFP rats. Immunoreactions for LH β subunit (a–d), prolactin (e–h), growth hormone (g, h, i–l), proopiomelanocortin (POMC; m–p) and thyroid-stimulation hormone (TSH) β subunit (q–t) were observed (shown in red) in some S-100 protein-positive cells (shown in green of GFP). In contrast, no specific signals were observed when we used primary antisera that had been preabsorbed with the antigens. In particular, LH-immunoreactive cells were more abundant than other types of hormone-producing cells in 8- to 9-week-old male rats. This is the first report to show hormone production by S-100 protein-positive cells in rat,

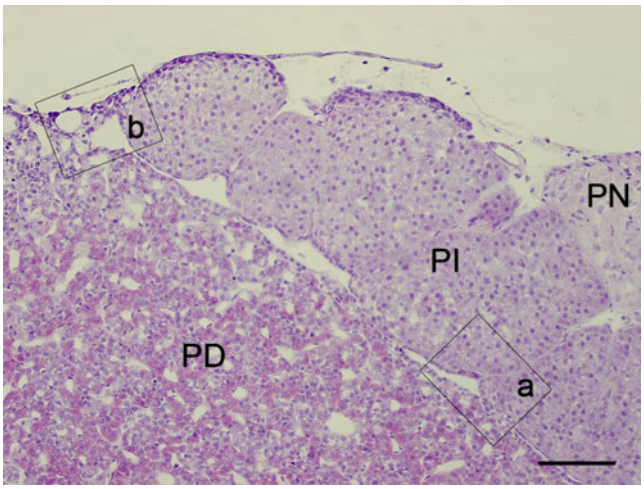

Fig. 1 Hematoxylin and eosin staining of a cryosection of S100b transgenic rat. *Boxes a* and *b* show the corresponding areas shown in Figs. 2 and 3, respectively. *PD* pars distalis, *PI* pars intermedia, *PN* pars nervosa. *Bar* 100 μm

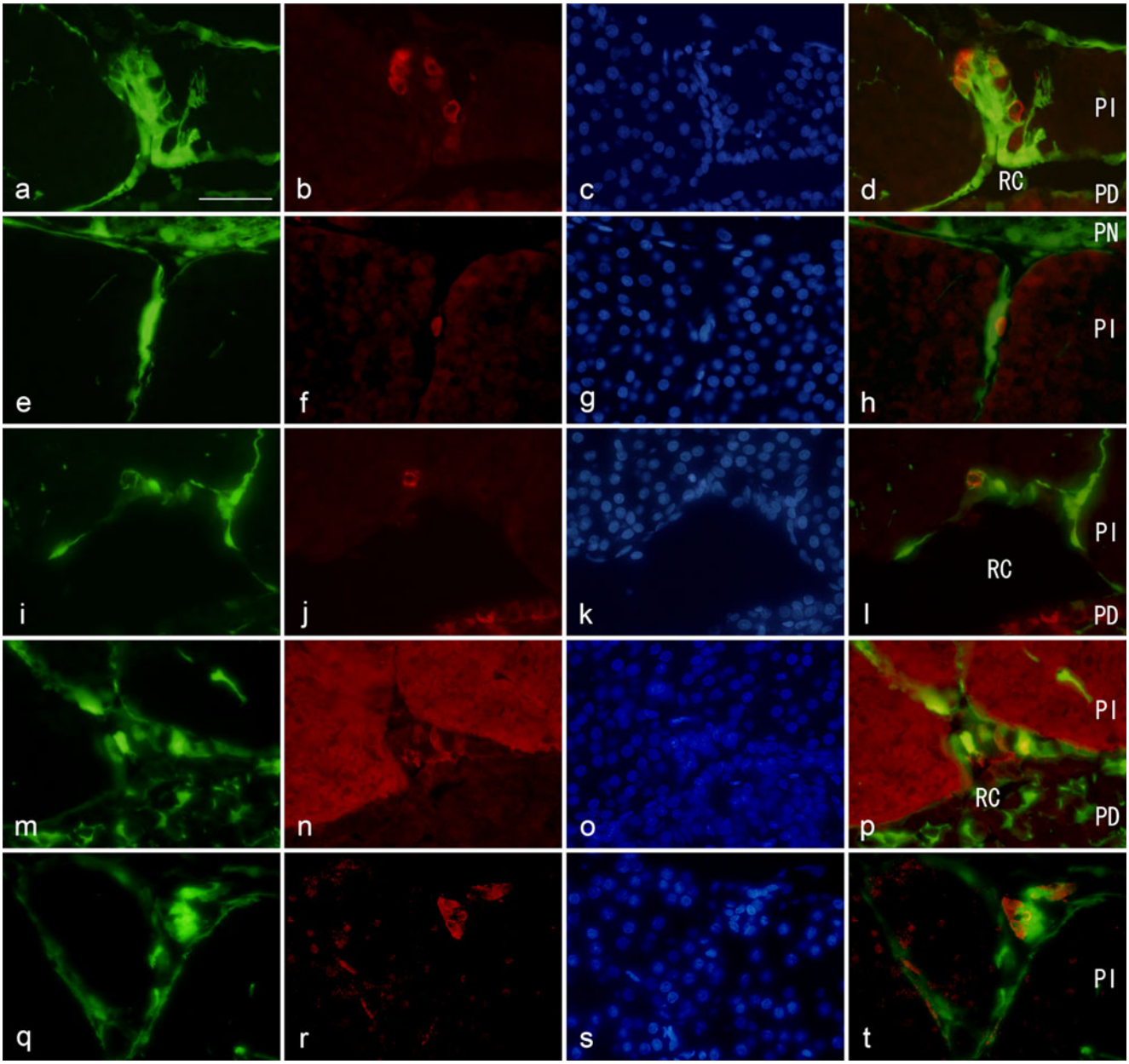

Fig. 2 Immunohistochemistry for pituitary hormones in S100b transgenic rat. **a–d** Immunoreactions for luteinizing hormone β subunit, **e–h** prolactin, **i–l** growth hormone, **m–p** proopiomelanocortin and **q–t** thyroid-stimulation hormone β subunit. *From left to right*, expression of GFP (*green*; **a, e, i, m, q**), immunoreaction (*red*; **b, f, j, n, r**), nuclear staining (*blue*; **c, g, k, o, s**) and merged images of GFP and immunoreaction (**d, h, l, p, t**). S-100 protein-positive cells were present between clusters of proopiomelanocortin-producing cells and some showed immunoreactivity for all hormones (*PD* pars distalis, *PI* pars intermedia, *PN* pars nervosa, *RC* the residual lumen of Rathke's pouch, or Rathke's cleft). *Bar* 100 μm

in either the anterior or intermediate lobe, except for that of Sands et al. (1995), who reported transient expression of S-100 by differentiated melanotropes in embryonic rat pituitary. In the present study, expression of S-100 protein was observed by using transgenic rats rather than immunohistochemical detection of S-100 protein, which is a possible explanation for this new finding. No immunoreaction against hormones was observed in S-100 protein-positive cells in the anterior lobe, although it was difficult to conclusively identify immunoreaction with optical microscopy, due to the stellate shape of the cells in the anterior lobe.

Hormone-producing cells and S-100 protein-positive cells have been regarded as totally different cell types. On the other hand, the hypothesis that S-100 protein-positive cells in the pituitary gland function as stem cells of hormone-producing cells has long been disputed (Yoshimura et al. 1977; Hosoya et al. 1997; Horvath and Kovacs 2002; Mogi et al.

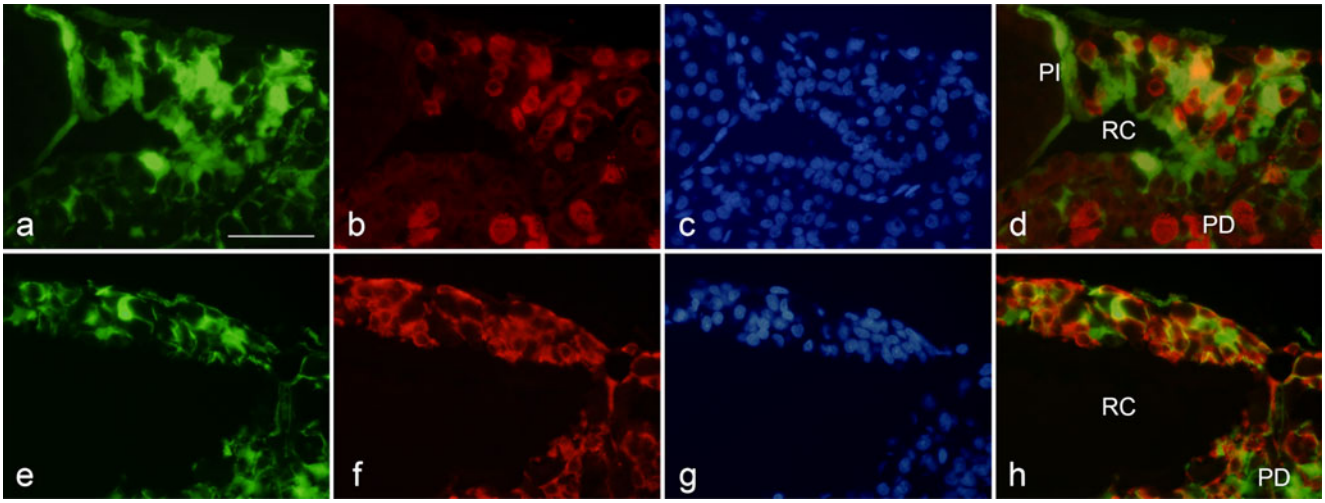

Fig. 3 Immunohistochemistry for luteinizing hormone β subunit (**a–d**) and prolactin (**e–h**) in the boundary zone between the anterior and intermediate lobes of the pituitary gland of S100b transgenic rat. *From left to right*, expression of GFP (*green*; **a, e**), immunoreaction (*red*; **b, f**), nuclear staining (*blue*; **c, g**) and merged images of GFP and immunoreaction (**d, h**). There were abundant immunoreactive cells for luteinizing hormone β subunit and prolactin specifically in these areas (*PD* pars distalis, *PI* pars intermedia, *RC* the residual lumen of Rathke's pouch, or Rathke's cleft). Luteinizing hormone-positive cells in the intermediate lobe were distinctively smaller than those in the anterior lobe. *Bar* 100 μm

2004). However, our findings suggest that nascent hormone-producing cells arise from populations of S-100 protein-positive cells, which appears to support the hypothesis. Moreover, in our observations, hormone-immunoreactive cells clearly tended to have lower GFP intensity (Fig. 2). It is possible that S-100 protein-positive cells lose the ability to express S-100 protein during the process of differentiating into hormone-producing cells. S-100 protein might negatively affect cell differentiation, although it would be premature to make this conclusion, as our results were based on the observation of GFP as a reporter of S-100 protein.

In addition, at the boundary zone between the anterior and intermediate lobes we observed large numbers of LH-positive (Fig. 3a–d) and prolactin-positive (Fig. 3e–h) cells

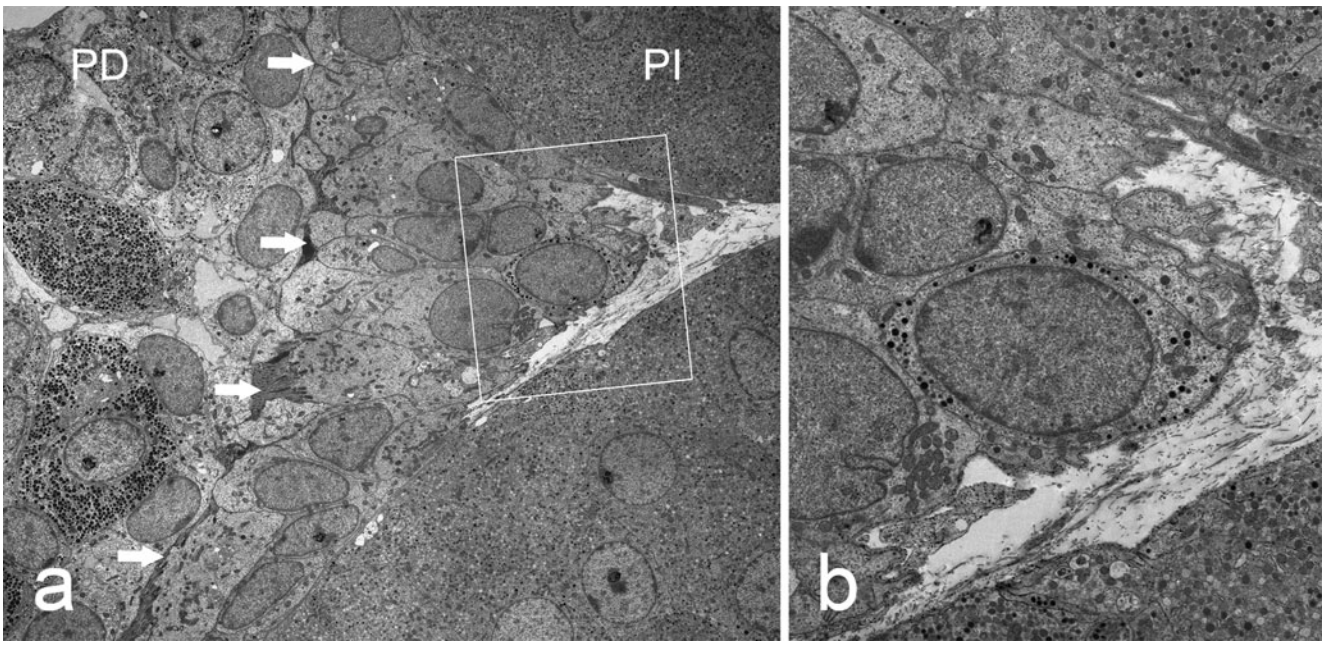

Fig. 4 Electron micrographs of the intermediate lobe of the rat pituitary gland. **a** Lower magnification (×1,400). **b** higher magnification (×4,000) of the area indicated by the box in (**a**). *PD* pars distalis, *PI* pars intermedia. Arrows show the residual lumen of Rathke's pouch (Rathke's cleft). The granulated cell in (**b**) has the immature fine structural features of a hormone-producing cell

with GFP. All LH cells in this zone were clearly smaller than those in the anterior lobe. S-100 protein-positive cells in this boundary zone may play a role in the development of nascent LH and prolactin cells.

Hove and Maxwell (1968) reported their detailed observations of non-hormone-producing cells in the intermediate lobe of the pituitary gland. According to Kurosumi et al. (1961), some of these cells were derived from cells in the wall of the hypophyseal lumen, i.e., the marginal layer. Gary and Chronwall (1995) distinguished glial-like cells in S-100 immunoreactive cells. Non-hormone-producing cells in the intermediate lobe are apparently heterologous, at least with respect to phenotype. In our observations, hormone-immunoreactive cells were preferentially located at the peripheries of S-100 protein-positive cell clusters between melanocyte-stimulating hormone-producing cells (Fig. 2). Indeed, no hormone immunoreactivity was observed in S-100 protein-positive cells that surrounded clusters of melanocyte-stimulating hormone-producing cells. We conclude that it is likely that cells capable of differentiating into hormone-producing cells are a subpopulation of S-100 protein-positive cells.

It is interesting that expression of several types of pituitary hormones were observed within clusters of S-100 protein-positive cells in the intermediate lobe. Chen et al. (2005) examined "side population cells" that had been isolated from the mouse pituitary gland by fluorescence-activated cell sorting based on the exclusion of the DNA dye Hoechst 33342. They noted that these cells developed spheres to produce various hormones in vitro. S-100 protein-positive cells in the intermediate lobe may thus have multipotent characteristics similar to those of side population cells. It is expected that future studies will elucidate the cytological properties of S-100 protein-positive cells in comparison with side population cells and examine whether S-100 protein-positive cells in the anterior lobe possess differentiation potency.

In electron microscopic observation of the area with S-100 protein-positive cells in the intermediate lobe of the rat (Fig. 4), we observed a few granulated cells in addition to non-granulated cells and marginal layer cells. These granulated cells were often surrounded by an extracellular matrix including collagen fibers and were small and usually round or oval. A few secretory granules approximately 200–300 nm in diameter were distributed in the cytoplasm. The Golgi apparatus and rough endoplasmic reticulum were poorly developed. These fine structural characteristics clearly differed from those of all types of hormone-producing cells in the anterior lobe and the hormone-producing cell (i.e., melanocyte-stimulating hormone-producing cell) in the intermediate lobe. The granulated cells—but not the non-granulated cells—in this area are likely S-100 protein-positive cells expressing several types of pituitary hormones.

In conclusion, this is the first report to show that S-100 protein-positive cells in the rat intermediate pituitary gland express various anterior pituitary hormones. This finding strengthens the hypothesis that S-100 protein-positive cells function as stem cells in the adult rat pituitary gland.

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