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**Short Communication**

# Expression of Steroidogenic Factor 1 and Pituitary Specific Transcription Factor 1 in Rat Pituitary Adenomas

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**Abstract:** The protein expressions of steroidogenic factor l (SF-l) and pituitary-specific transcription factor 1 (Pit-1) were investigated immunohistochemically for 53 spontaneous pituitary adenomas of the pars distalis from male Crl:CD(SD) rats. Luteinizing hormone (LH)-positive/prolactin (PRL)-negative and LH-negative/PRL-positive adenomas showed that the expression of SF-1 and Pit-1 was exclusively related to the immunoreactivity of LH and PRL, respectively. All double-positive adenomas (positive for both LH and PRL) were positive for Pit-1 and were supposed to be derived from PRL cells, although some of them also showed SF-1 immunoreactivity. In addition, all null cell adenomas (negative for all anterior pituitary hormones) were positive for SF-1 and negative for Pit-1, indicating that they originated from the gonadotroph cell lineage. This is the first report focusing on the application of transcription factors for the classification of rat pituitary adenomas. (DOI: 10.1293/tox.26.209; J Toxicol Pathol 2013; 26: 209–213)

**Key words:** pituitary adenoma, steroidogenic factor 1, pituitary-specific transcription factor 1, rat

Immunohistochemical staining for pituitary anterior hormones is useful for the classification of pituitary tumors of the pars distalis[1]. In Sprague-Dawley (SD) rats, most spontaneous pituitary tumors stained positively for prolactin (PRL) or luteinizing hormone (LH) and were classified as prolactinomas or gonadotropinomas, respectively[2]. However, there are some functionally undifferentiated adenomas that do not produce sufficient hormones to be determined. Recently, transcription factors in the pituitary gland are being researched actively, since they are known to be involved in the development and differentiation of pituitary cells and the production of pituitary hormones. Now they are being applied to diagnose human pituitary tumors as new classification markers[3, 4]. Steroidogenic factor l (SF-l) is known to be a transcriptional factor in the development of steroid hormone producing cells and to be expressed in gonadotrophs (LH/FSH cells) in the pituitary[5]. Pituitary-specific transcription factor 1 (Pit-1) is a prototypic member of the family of Pit-Oct-Unc (POU) transcription factors and plays a critical role in growth hormone (GH), PRL and thyroid-stimulating hormone β (TSHβ) expression[6, 7]. In the present study, we investigated SF-1 and Pit-1 expression in spontaneous pituitary adenomas of the pars distalis in rats to confirm the usability of these transcription factors for their classification.

Three normal pituitaries from 7-week-old male Crl:CD(SD) rats (Charles River Laboratories Japan, Inc.) and fifty three spontaneous pituitary adenomas of the pars distalis from 68- to 115-week-old male Crl:CD(SD) rats were used in the present study. These tissues were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 4 μm. For normal pituitaries, double immunohistochemical staining for LH/SF-1, PRL/Pit-1, LH/Pit-1 and PRL/SF-1 was performed, and for adenomas, single immunohistochemical staining for LH, PRL, SF-1 and Pit-1 was conducted. Table 1 shows the primary antibodies used in the present study. The experiment was conducted in accordance with the Animal Care and Use Committee of Bozo Research Center or Takeda Pharmaceutical Company Limited.

In the pars distalis from the normal rats, double immunohistochemical staining for LH/SF-1 revealed that most of the LH-positive cells were SF-1 positive, while a few cells positive for LH only (negative for SF-l) or SF-1 only (negative for LH) were also observed (Fig. 1a). Expression of Pit-1 was confirmed in all PRL cells by PRL/Pit-1 double staining (Fig. 1b). Numerous cells positive for only Pit-1 (negative for PRL) were also observed and were considered to be TSH and GH cells, which can differentiate from the Pit-1 cell lineage. No double-positive cells were observed in LH/Pit-1 (Fig. 1c) or PRL/SF-1 (Fig. 1d) double staining. From these results, it was considered that SF-1 and Pit-1 were expressed not only during the ontogenic stage but also during the adult

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**Table 1.** Antibodies Used in the Study

| Antibody[a] | Host | Dilution | Antigen retrieval[b] | Source[c] |
|---|---|---|---|---|
| LH | Rabbit | 1: 2000 | Trypsin | Accurate Chemical & Scientific Corp. |
| PRL | Rabbit | 1: 8000 or 4000 | Trypsin | AbD Serotec |
| PRL | Mouse | 1: 1000 | Trypsin | AbD Serotec |
| FSH | Rabbit | 1: 4000 | Trypsin | AbD Serotec |
| GH | Mouse | 1: 200 | Trypsin | R&D Systems, Inc. |
| TSH | Rabbit | 1: 2500 | Trypsin | AbD Serotec |
| ACTH | Mouse | 1: 100 | Trypsin | Novocastra Laboratories Ltd. |
| SF-1 | Mouse | 1: 1000 | Citric acid | Perseus Proteomics Inc. |
| Pit-1 | Mouse | 1: 100 | Citric acid | BD Biosciences |

[a] LH, luteinizing hormone; PRL, prolactin; FSH, follicle-stimulating hormone; GH, growth hormone; TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic hormone. [b] Trypsin, 0.1% trypsin for 37 °C at 30 minutes; citric acid, 10 mmol/L citrate buffer for 121 °C at 15 minutes. [c] Accurate Chemical & Scientific Corporation, Westbury, NY, USA; AbD Serotec, Oxford, UK; R&D Systems, Inc., Minneapolis, MN, USA; Novocastra Laboratories Ltd., Wetzlar, Germany; Perseus Proteomics Inc., Tokyo, Japan; BD Biosciences, Franklin Lakes, NJ, USA.

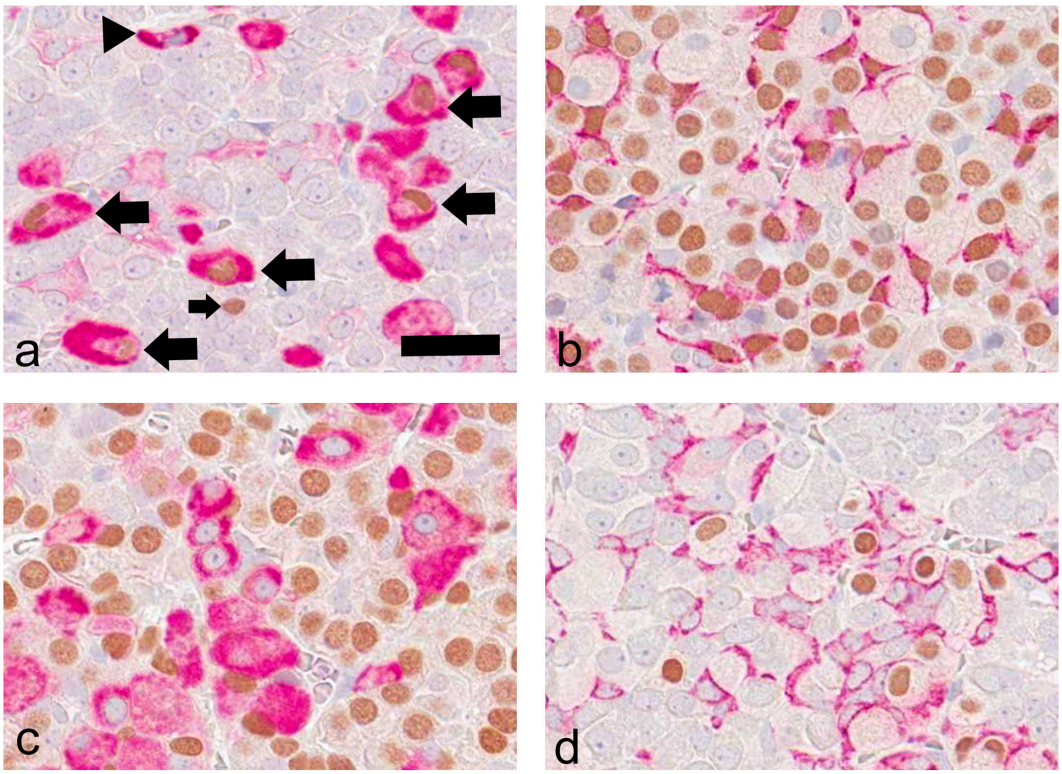


**Fig. 1.** Double immunohistochemical staining for LH/SF-1 (a), PRL/Pit-1 (b), LH/Pit-1 (c) and PRL/SF-1 (d) in the pars distalis from a normal rat. Red cytoplasm indicates positive for LH or PRL. Brown nuclei indicates positive for Pit-1 or SF-1. a) A number of LH-positive cells were SF-1 positive (arrows), while a few cells positive for LH only (negative for SF-1) (arrowhead) or SF-1 only (negative for LH) (small arrow) were also observed. b) Expression of Pit-1 was confirmed in all PRL cells. Numerous cells positive for Pit-1 only (negative for PRL) were also observed. c, d) No double-positive cells were observed in LH/Pit-1 and PRL/SF-1 double staining. Bar = 50 µm.

stage.

Adenomas of the pars distalis were divided into 4 types by immunohistochemical staining for LH and PRL as follows: PRL-positive/LH-negative, LH-positive/PRL-negative, LH/PRL double-positive and LH/PRL double-negative adenomas. Table 2 shows SF-1 and Pit-1 expression in each type of adenoma. All PRL-positive/LH-negative adenomas, the most common adenoma in this study, were positive for Pit-1 and negative for SF-1 (Fig. 2a–d) with 1 exception, which was positive for both Pit-1 and SF-1. Nine of 10 LH-positive/PRL-negative adenomas were positive for SF-1 and negative for Pit-1 (Fig. 2e–h). These results suggested that the expression of Pit-1 and SF-1 mostly corresponds to the immunoreactivity of PRL and LH, respectively. For

**Table 2.** SF-1 and Pit-1 Expression of Pituitary Adenomas with LH and/or PRL Immunoreactivity

| SF-1/Pit-1 immunoreactivity | LH/PRL immunoreactivity | | | |
|---|---|---|---|---|
| | LH(−)/PRL(+) | LH(+)/PRL(−) | LH(+)/PRL(+) | LH(−)/PRL(−) |
| SF-1(−)/Pit-1(+) | 25 | 0 | 5 | 0 |
| SF-1(+)/Pit-1(−) | 0 | 9 | 0 | 9 |
| SF-1(+)/Pit-1(+) | 1 | 0 | 3 | 0 |
| SF-1(−)/Pit-1(−) | 0 | 1 | 0 | 0 |
| Total | 26 | 10 | 8 | 9 |

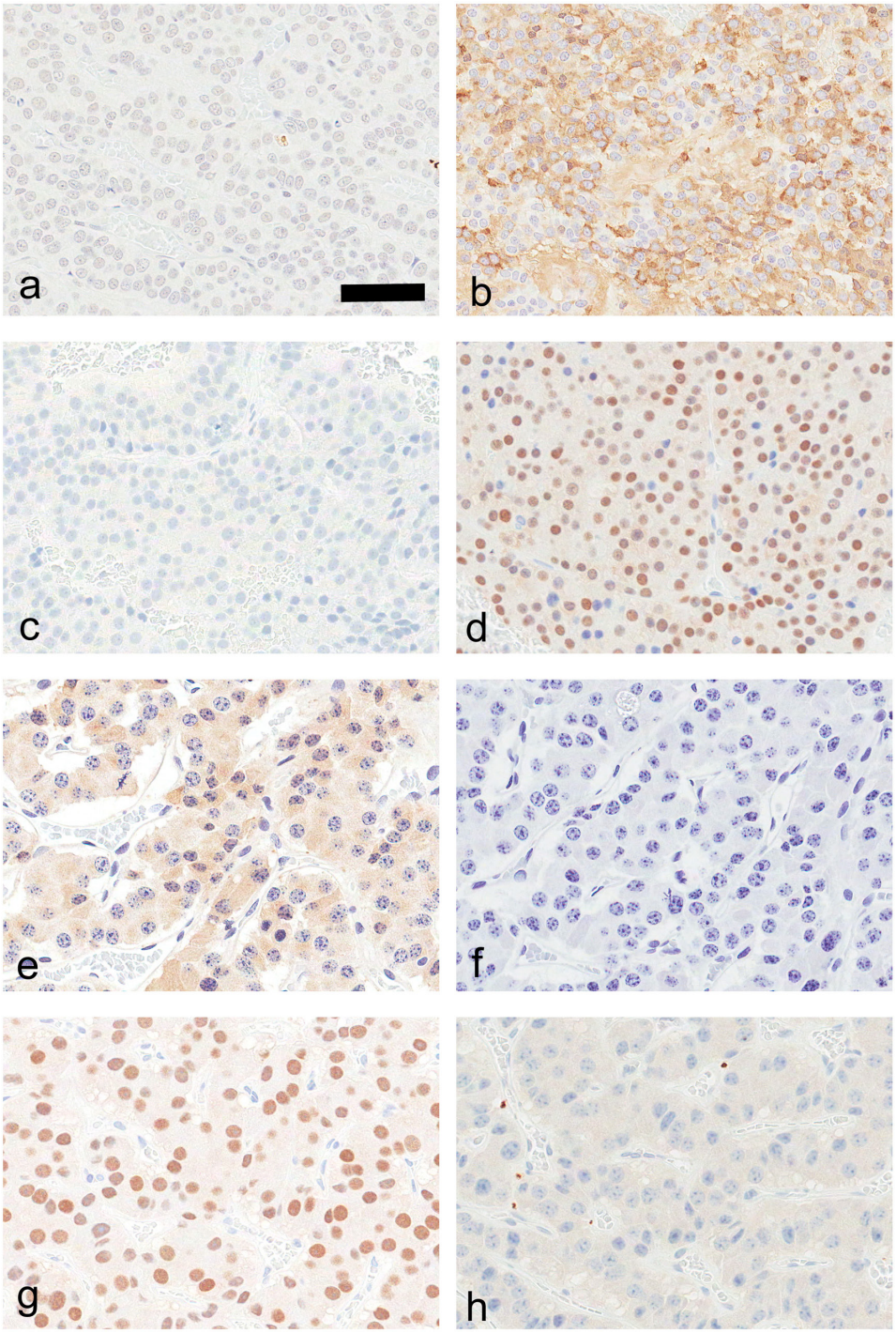


**Fig. 2.** Immunohistochemical staining for LH (a, e), PRL (b, f), SF-1 (c, g) and Pit-1 (d, h) in PRL-positive/LH-negative (a–d) and LH-positive/PRL-negative (e–h) adenomas. c, d) Most of the tumor cells that were PRL-positive/LH-negative were positive for Pit-1 and negative for SF-1. g, h) Most of the tumor cells that were LH-positive/PRL-negative were positive for SF-1 and negative for Pit-1. Bar = 50 μm.

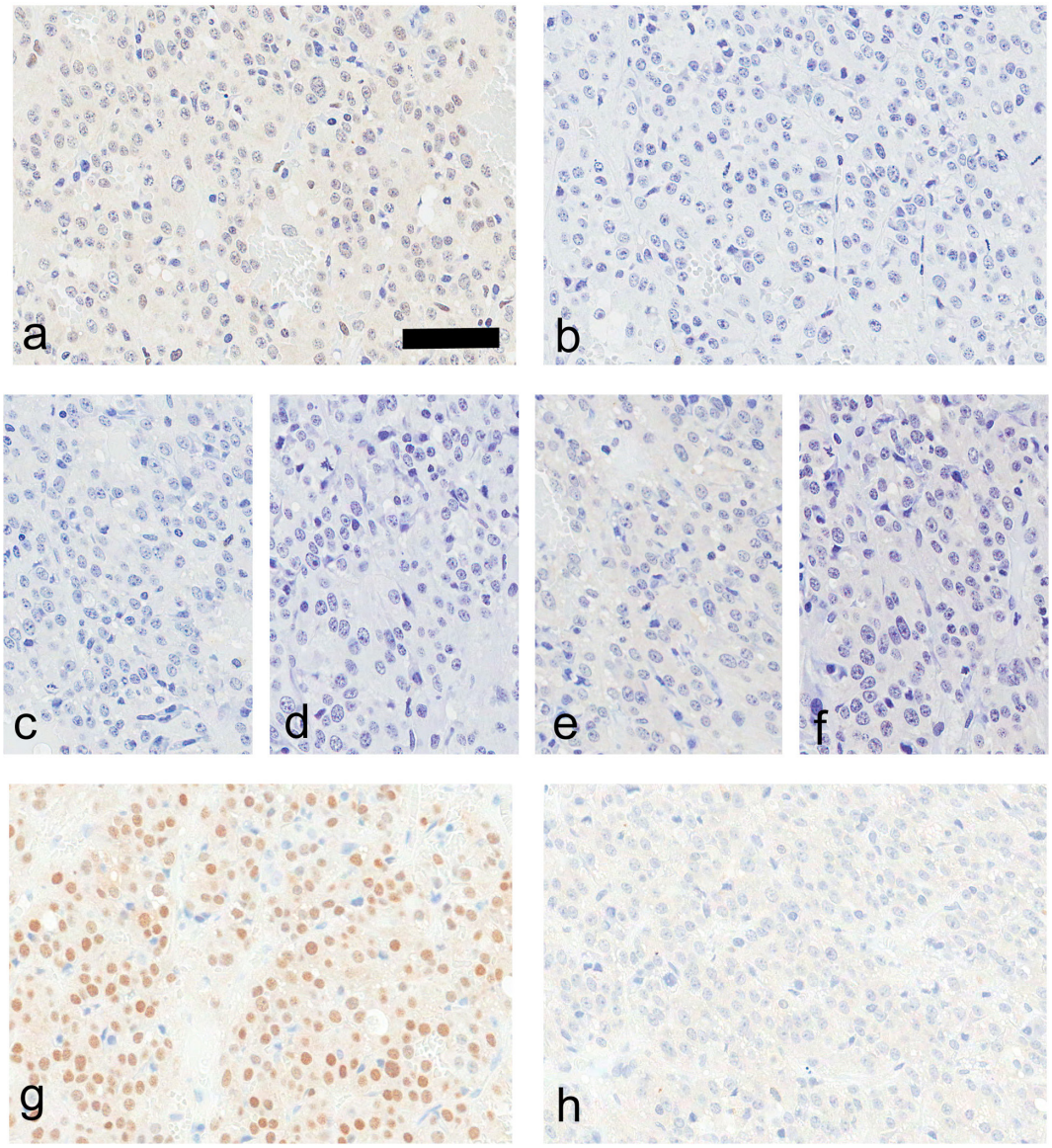


**Fig. 3.** Immunohistochemical staining for LH (a), PRL (b), FSH (c), GH (d), TSH (e), ACTH (f), SF-1 (g) and Pit-1 (h) in null cell adenomas. Null cell adenomas, which were negative for all anterior hormones, were positive for SF-1 and negative for Pit-1. Bar = 50 μm.

one LH-positive/PRL-negative (SF-1/Pit-1 double negative) adenoma, further examination for other LH-inducible transcription factors such as early growth response protein 1, pituitary homeobox 1, gonadotropin-releasing hormone and DAX-1 might be necessary[8, 9]. Eight of 53 adenomas consisted of both LH-positive regions and PRL-positive regions and were classified as LH/PRL double-positive adenomas. Additional double immunohistochemical staining for LH/PRL revealed that 7 of them had LH/PRL double-positive cells (data not shown). All these adenomas were positive for Pit-1, indicating that they were derived from PRL cells, although 3 of these adenomas also showed SF-1 expression. It was reported that LH/FSH cells could transdifferentiate into PRL cells with Pit-1 expression under pregnancy or estrogen treatment[10, 11]. Therefore, some LH/PRL double-positive adenomas might be plurihormonal adenomas originating from LH/FSH cells. Among the 53 adenomas, nine were negative for both LH and PRL (Fig 3a–b) and were diagnosed as null cell adenomas because additional immunostaining for other pituitary hormones, FSH, GH, TSH and ACTH, also gave negative results (Fig 3c–f). Interestingly, all null cell adenomas were positive for SF-1 and negative for Pit-1 (Fig. 3g–h). In humans, a large number of null cell adenomas are recognized as being of gonadotroph origin because they frequently express SF-1 and gonadotropin subunit genes, and βLH and βFSH production were confirmed *in vitro*[4, 12, 13, 14]. It is quite likely that null cell adenomas in rats originate from the gonadotroph cell lineage as well.

From the above, we conclude that the expressions of SF-1 and Pit-1 were exclusively related to the immunoreac-

tivity of LH and PRL in rat pituitary adenomas, respectively. In addition, the null cell tumors examined in this study were considered to have originated from the gonadotroph cell lineage. This is the first report focusing on the usability of transcription factors in the classification of rat pituitary adenomas.

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