Expression of Adrenocorticotropic Hormone, Prolactin and Transcriptional Factors in Clinically Nonfunctioning Pituitary Adenoma

KAZUNORI KAGEYAMA, HIDETOSHI IKEDA*, TAKESHI NIGAWARA, SATORU SAKIHARA AND TOSHIHIRO SUDA

Abstract. We describe here a case of a clinically nonfunctioning pituitary adenoma, but with expression of ACTH and PRL. A 42-year-old woman was referred to our department for further evaluation of pituitary tumor. She had no acromegalic features, and no typical Cushingoid features. She had no galactorrhea, and had regular menses. GH, IGF-I, LH, FSH, TSH, ACTH and cortisol levels in blood were all within the normal ranges, while PRL levels were mildly elevated. Both ACTH and cortisol levels were adequately increased in response to CRH, and both were suppressed by a small dose of dexamethasone. Plasma ACTH and cortisol levels were decreased at night, suggesting the circadian rhythms for plasma ACTH levels were undisturbed. Based on these findings we did not clinically suspect ACTH-producing tumor, however immunohistochemistry revealed ACTH immunoreactivity in the pituitary adenoma. Therefore, the tumor was considered a silent corticotroph adenoma. PRL was co-expressed in a significant subpopulation of ACTH-immunoreactive tumor cells. Ptx1, Neuro D1, and T pit were densely expressed and Pit-1 was sparsely expressed in the nuclei of adenoma cells. It is therefore possible that a tumor originating in an immature or uncommitted adenohypophysial stem cell may later differentiate into different cell types due to a combination of certain specific transcriptional factors.

Key words: Corticotroph, Pituitary tumor, Pit-1, Ptx1, Neuro D1

NEARLY 30% of pituitary adenomas are considered as nonfunctioning. Nonfunctioning adenomas represent a heterogeneous group. Asa and Kovacs morphologically classified clinically nonfunctioning pituitary adenomas into two groups, those which have hormone immunoreactivity and ultrastructural features of known adenohypophysial cell types but are clinically silent, and those composed of cells that do not resemble nontumorous adenohypophysial cell types [1]. The former involves the silent somatotroph adenomas, silent corticotroph adenomas, and silent gonadotroph adenomas; the latter includes the silent type III adenomas, null cell adenomas, and oncocytomas.

Among nonfunctioning adenomas, null cell or silent gonadotroph adenomas are the most common types [2, 3], while silent corticotroph adenomas are rare pituitary tumors [4]. Silent corticotroph adenomas are defined as pituitary tumors with ACTH immunoreactivity, but without clinical evidence of Cushing’s disease. From 16 cases of nonfunctioning adenomas, PRL mRNA was detected in 3 (19%) and ACTH mRNA was detected in one (6%) [5]. These results suggest the possibility that PRL or ACTH is synthesized in some cases of clinically nonfunctioning pituitary adenomas.

The functional development of pituitary cells depends on the expression of a combination of transcription factors and co-factors. For example, pituitary-specific transcription factor-1 (Pit-1) is required for the
expression of GH, PRL, and TSH [6], since differentiation and maintenance of somatotroph, lactotroph, and thyrotroph phenotypes are dependent on expression of a functional Pit-1 gene [7]. On the other hand, pituitary adenomas with GH/PRL and ACTH production are generally considered as rare due to differential regulation in functional development. For example, a recent review of the English literature revealed only a few cases of both GH- and ACTH-producing tumor in the pituitary [8–12], and Matsuno et al. reported only one among 14 cases of nonfunctioning adenomas that was both PRL- and ACTH-positive on immunohistochemical analysis [5].

We describe here a case of a clinically nonfunctioning pituitary adenoma that showed expression of ACTH and PRL. We also present the immunohistochemistry findings of certain specific transcriptional factors.

Case report

Clinical summary

In November 2005, a 42-year-old woman was referred to our department for further evaluation of pituitary tumor. Magnetic resonance imaging (MRI) of the head revealed the presence of a microadenoma (8 × 8 × 6 mm) in the pituitary, which was not enhanced with gadolinium diethylenetriamine penta-acetic acid (Fig. 1A). One year later, in December 2006, MRI demonstrated enlargement of the pituitary adenoma to 12 × 13 × 8 mm (Fig. 1B). On admission to our hospital, her height was 148 cm, body weight 68 kg, and a body mass index of 31.0 kg/m². She was not taking any anti-hypertensive drugs and was normotensive with a blood pressure of 138/78 mmHg. She had no acromegalic features, and no typical Cushingoid features of central obesity, moon face, skin atrophy, purple striae and buffalo hump. She had no galactorrhea, and had regular menses on a 28-day cycle.

The results of biochemical analyses carried out at admission were all within the normal ranges. The results of the hormonal data were all within the normal ranges except for PRL levels (21.3 ng/mL) (Table 1). As shown in Table 2a, plasma GH levels were mildly increased (0 min, 0.05 ng/mL; 30 min, 2.5 ng/mL) in response to intravenous TRH administration. Plasma ACTH and cortisol levels were increased in response to administration of human CRH (Table 2b), and suppressed by both 0.5 mg and 1 mg of dexamethasone on the overnight suppression test (Table 2c). Plasma ACTH (8 pg/mL) and cortisol (1.8 µg/dL) levels were decreased at night, suggesting undisturbed circadian...
rhythms. Bromocriptine (2.5 mg, per os) slightly increased GH levels (0 h, 0.32 ng/mL; 2 h, 2.11 ng/mL), while it decreased PRL concentrations (0 h, 20.2 ng/mL; 6 h, 1.4 ng/mL) (Table 2d).

On the basis of a diagnosis of nonfunctioning pituitary adenoma, total resection of the pituitary adenoma was performed through transsphenoidal neurosurgery. At 2 weeks postoperatively, blood concentrations of ACTH, cortisol, and PRL were all reduced by 0.25 mg/day replacement of dexamethasone (Table 1).

Pathological findings

Light microscopic findings

The resected pituitary tissue was fixed in 10% formalin for 8 h. Serial sections were prepared and stained with hematoxylin-eosin (HE). HE stained sections revealed chromophobic adenoma cells with oval nuclei and clear cytoplasm (Fig. 2).

Immunohistochemistry

Tissue sections were washed in phosphate-buffered saline (PBS) followed by incubation at room temperature for 1 h with primary antibodies raised in anti-GH (Dako, Glostrup, Denmark), anti-ACTH (Dako), anti-PRL (MOC, Tokyo, Japan), anti-TSH-β (MOC), anti-LH-β (MOC), anti-FSH-β (IMT, Marseilles, France), or anti-Pit-1 (Santa Cruz Biotechnology, Santa Cruz, USA). Other tissue sections were washed in PBS followed by incubation at 4°C overnight with primary antibodies raised in anti-pituitary homeobox 1 (Ptx1), anti-Neuro D1, and anti-T pit, which were gifted by Dr. Drouin, or anti-estrogen receptor (Ventana, Tucson,

Table 1. Hormonal data. Normal basal ranges are indicated in parentheses. IGF-I, insulin-like growth factor-I; UFC, urinary free cortisol.

<table>
<thead>
<tr>
<th>Hormonal data</th>
<th>Pre-operation</th>
<th>Post-operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH (7–56 pg/mL)</td>
<td>42</td>
<td>11</td>
</tr>
<tr>
<td>GH (&lt;2.1 ng/mL)</td>
<td>0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>LH (0.3–7.1 mIU/mL)</td>
<td>4.6</td>
<td>13.1</td>
</tr>
<tr>
<td>FSH (1.6–10.6 mIU/mL)</td>
<td>3.8</td>
<td>17.8</td>
</tr>
<tr>
<td>TSH (0.38–3.64 µIU/mL)</td>
<td>2.54</td>
<td>2.09</td>
</tr>
<tr>
<td>PRL (3.5–16.3 ng/mL)</td>
<td>21.3</td>
<td>15.0</td>
</tr>
<tr>
<td>Cortisol (3.8–18.4 µg/dL)</td>
<td>16.9</td>
<td>3.3</td>
</tr>
<tr>
<td>IGF-I (46–282 ng/mL)</td>
<td>233</td>
<td>248</td>
</tr>
<tr>
<td>UFC (11–80 µg/day)</td>
<td>33</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Table 2. Endocrinological examinations. (a) TRH test (TRH 500 µg, intravenous bolus). (b) CRH test (CRH 100 µg, intravenous bolus). (c) Overnight dexamethasone test (dexamethasone per os at 23:00). (d) Bromocriptine test (bromocriptine 2.5 mg, per os).

(a) TRH test

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (µIU/mL)</td>
<td>3.73</td>
<td>–</td>
<td>18.03</td>
<td>11.69</td>
<td>9.59</td>
<td>7.08</td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td>20.1</td>
<td>–</td>
<td>45.2</td>
<td>30.9</td>
<td>27.7</td>
<td>26.1</td>
</tr>
<tr>
<td>GH (ng/mL)</td>
<td>0.05</td>
<td>1.8</td>
<td>2.5</td>
<td>0.79</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

(b) CRH test

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH (pg/mL)</td>
<td>32</td>
<td>109</td>
<td>74</td>
<td>43</td>
<td>31</td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>14.6</td>
<td>20.8</td>
<td>27.8</td>
<td>15.4</td>
<td>12.1</td>
</tr>
</tbody>
</table>

(c) Dexamethasone test

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH (pg/mL)</td>
<td>38</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>23.6</td>
<td>1.3</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

(d) Bromocriptine

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>8:30;</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH (ng/mL)</td>
<td>0.32</td>
<td>0.04</td>
<td>2.11</td>
<td>0.38</td>
<td>0.90</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td>20.2</td>
<td>13.1</td>
<td>5.6</td>
<td>2.4</td>
<td>2.2</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>
They were then washed by PBS, and incubated in biotinylated IgG. The sections were immunohistochemically stained by the avidin-biotin peroxidase complex method (Vector, Burlingame, USA). The antibody-peroxidase complex was visualized with a mixture of 3,3’-diaminobenzidine (0.2 mg/ml) and 3% H2O2.

Fig. 2. Hematoxylin-eosin stained sections show chromophobic adenoma cells with oval nuclei and clear cytoplasm (Original magnification ×100 (A) and ×400 (B)).

Fig. 3. (A) Immunological staining for ACTH. ACTH-positive cells are visualized by the brown precipitates. The tissue section showed sparse but distinct ACTH-stained cells in the tumor (Original magnification ×100 (upper panel) and ×400 (lower panel)). (B) Immunological staining for PRL. PRL-positive cells are visualized by the brown precipitates. PRL was co-expressed in a significant subpopulation of ACTH-immunoreactive tumor cells (Original magnification ×100 (upper panel) and ×400 (lower panel)).
H$_2$O$_2$ (0.83 µl/ml) in 0.05 M Tris buffer-saline solution. Neither ACTH nor PRL was detected in other nonfunctioning adenoma cells using this method. Examination of serial tissue sections showed sparse but distinct ACTH-stained cells (Figs. 3A and B). PRL was co-expressed in a significant subpopulation of ACTH-immunoreactive tumor cells, as a mirror image on serial tissue sections. GH-, LH-, FSH-, and TSH-positive cells were not detected in the tumor (not shown). Ptx1, Neuro D1, and T pit were densely expressed and Pit-1 sparsely expressed in the nuclei of adenoma cells (Fig. 4), while estrogen receptor was not detected in the tumor (not shown).

**Discussion**

We did not clinically suspect our patient of having an ACTH-producing tumor, but ACTH immunoreactivity in the pituitary tumor was revealed by immunohistochemistry. Therefore, the tumor was considered as a silent corticotroph adenoma. The patient had no typical Cushingoid features, hypertension, or impaired glucose tolerance, suggesting that the tumor had no autonomic ACTH secretion sufficient for causing the clinical symptoms of Cushing’s disease. Although the diagnostic criteria for preclinical Cushing’s disease have yet to be established, the following endocrinological findings may be considered as suggestive of the disease [13]: 1) the absence of Cushingoid appearance, 2) evidence of autonomic or abnormal secretion of...
ACTH such as (a) normal or high plasma ACTH and normal cortisol levels in the morning, but high cortisol levels (>2.5 μg/dL) while sleeping at night, (b) incomplete suppression of cortisol level (>3 μg/dL) in the morning by a low-dose of (0.5 mg) dexamethasone, (c) a response of plasma ACTH levels to desmopressin [14], and 3) presence of pituitary adenoma. However, our case did not sufficiently meet criterion 2).

In the different diagnosis of clinically silent corticotroph adenoma, other causes should be ruled out. Even though the discrepancy between immunoreactivity and lack of endocrine activity of silent adenomas is unclear as yet, in the case of both GH and ACTH production for example, the anabolic effects of potent GH may conceal the effects of cortisol on the metabolism, because each other are known to have antagonizing effects on protein and fat metabolism [15]. There are also rare possibilities such as the bioactivity of ACTH or sensitivity to ACTH in the adrenal glands to consider. Corticotroph adenomas are often deficient in prohormone convertase 1/3, the pacemaker enzyme of POMC processing. In these cases, bio-inactive ACTH may be rare among the multihormonal pituitary adenomas. Ptx1 acts synergistically with Neuro D1, steroidogenic factor-1 (SF-1), and Pit-1 to activate POMC/ACTH, FSH/LH, and GH-PRL-TSH transcription, respectively [24]. In this regard, PRL and ACTH are hormones of different lineages. In our case, Neuro D1, T pit, and Pit-1 were expressed in the adenoma cells. These data suggest that one cell lineage may be due to aberrant patterns of expression of transcription factors in pituitary adenomas.

Pit-1 is frequently co-expressed with GH, PRL, and TSH-producing tumors [22], and would contribute to the functional differentiation toward these tumor cells from the common precursor. Neuro D1 is a transcriptional factor containing a helix-loop-helix heterodimer for ACTH differentiation. In the pituitary, synergistic action between Neuro D1 and Ptx1 has been reported to activate POMC/ACTH, FSH/LH, and GH-PRL-TSH transcription, respectively [24]. In this regard, PRL and ACTH are hormones of different lineages. In our case, Neuro D1, T pit, and Pit-1 were expressed in the adenoma cells. These data suggest that one cell lineage may be due to aberrant patterns of expression of transcription factors in pituitary adenomas.
physial stem cell may later differentiate into different cell types with a combination of certain specific transcriptional factors.

Acknowledgments

We thank Dr. Drouin (Institut de Recherches Cliniques de Montreal) for generously providing anti-Ptx, anti-Neuro D1, and anti-T pit antibodies. We also thank the laboratories of Dr. Wakabayashi (Institute of Brain Science) and Dr. Itoh (Center for Advanced Medical Research) for technical assistance.

Kazunori Kageyama is supported by a grant from the Funds for the Promotion of Aomori Medical Research. This work was also supported in part by Health and Labour Science Research Grants (Research on Measures for Intractable Diseases) from the Ministry of Health, Labour, and Welfare of Japan, and by a grant to Toshihiro Suda from the Ministry of Education, Science and Culture of Japan (No. 18591014).

References


