Review

Histopathological Review of Silent Pituitary Somatotroph Adenoma

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Silent pituitary somatotroph adenoma is an adenoma that expresses growth hormone (GH) and GH mRNA and has ultrastructural features common to somatotroph adenoma, without evidence of acromegaly. Forty-one silent somatotroph adenomas were reported in the literature, and their relatively common clinical symptoms include amenorrhea and/or galactorrhea. Random GH and insulin-like growth factor-1 levels are within normal in nearly half of the patients, whereas random PRL level is elevated in about 80% of patients. Generally, the GH immunostaining of silent somatotroph adenoma is weaker than that of classical somatotroph adenoma. Tissue culture studies indicate silent

somatotroph adenoma has a very low basal secretory capacity of GH. Most of the silent somatotroph adenomas reported in the literature are sparsely granulated adenomas. The mechanism of silence may be attributed to unknown translational abnormalities of GH gene product, impaired secretion of GH, lack of biological activity of secreted GH, or it may represent an early stage of classical somatotroph adenoma. Meticulous endocrinological and histopathological examination may be helpful for the identification of silent somatotroph adenoma among patients with clinically non-functioning adenomas, especially female patients with amenorrhea and/or galactorrhea.

Key words: silent pituitary somatotroph adenoma, immunohistochemistry, *in situ* hybridization, electron microscopy

I. Introduction

Pituitary adenomas are usually assigned to functioning or clinically non-functioning adenomas. Functioning adenomas present clinical symptoms related to the excess of anterior pituitary hormones. The majority of clinically non-functioning or clinically inactive pituitary adenomas release small amounts of glycoprotein hormones or their subunits without any symptoms related to excess of these hormones.

Recent advent of sophisticated techniques, such as cell culture, electron microscopy, immunohistochemistry and *in situ* hybridization (ISH), has led to a much wider understanding of clinically non-functioning or clinically inactive pituitary adenomas. From these studies, the concept of silent pituitary adenoma was developed for pituitary adenomas that produce anterior pituitary hormone with morphological features corresponding to functioning adenomas, whereas they show no clinical symptoms related to excess of these hormones. Their representative forms include silent somatotroph adenomas that have been published in the literature [5–8, 10–16, 18–20]. In this review article, we discuss the histopathological features of silent somatotroph adenomas, with consideration of their clinical and patho-

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logical significance.

II. The Definition of Silent Somatotroph Adenoma

Silent somatotroph adenoma is now defined as an adenoma that expresses growth hormone (GH) and GH mRNA and has ultrastructural features common to somatotroph adenoma, but no evidence of acromegaly, even in association with slightly elevated or subnormal GH and insulin-like growth factor 1 (IGF-1) levels.

III. Clinical Symptoms of Silent Somatotroph Adenoma

Forty-one silent somatotroph adenomas that were reported in the literature are shown in Table 1. Most of them (80.5%) developed predominantly in younger women, and only eight male patients were reported. The symptom of amenorrhea and/or galactorrhea that is usually observed in patients with mammothroph adenomas was common in female patients with silent somatotroph adenoma. In particular, menstruation disorder was noted in 27 (81.8%) out of 33 female patients with silent somatotroph adenoma.

Serum GH, PRL and IGF-1 values of the patients reported in the literature are shown in Table 1. Random GH values were within normal range in 20 (48.8%) out of 41 patients. IGF-1 values were within normal range in 6 (42.9%) out of 14 patients studied. Random PRL values were within normal range in 7 (17.1%) out of 41 patients. The lack of GH suppression in oral glucose tolerance test (OGTT) was noted in 22 (91.7%) out of 24 patients studied. An abnormal rise of GH to thyrotropin-releasing hormone (TRH) loading was observed in 9 (36.0%) out of 25 patients studied.

It is of clinical importance to identify a patient with silent somatotroph adenoma among females with a history of amenorrhea and/or galactorrhea. Random GH and IGF-1 levels are within normal in nearly half of patients, whereas random PRL level is elevated in about 80% of patients. Therefore the first step to the endocrinological identification of silent somatotroph adenoma is the repeated measurement of GH, IGF-1 and PRL values. The second step is an endocrinological confirmation of absent GH suppression in OGTT, which was regarded as the most reliable method by Sakharova *et al.* [16]. Another option is an endocrinological confirmation of paradoxical response of GH in TRH provocation test, as described in our previous reports [10–13].

IV. Histopathological Features of Silent Somatotroph Adenoma

The histopathological features of 41 silent somatotroph adenomas are summarized in Table 1. In 38 (92.7%) of 41 silent somatotroph adenomas, GH was immunohistochemically positive. The degree of immunopositivity was diverse, and weak positive staining (<10% positive cells) was ob-

served in 13 (41.9%) out of 31 silent somatotroph adenomas in which the degree of GH immunoreactivity was described. Generally, the GH immunostaining of a silent somatotroph adenoma is weaker than that of a classical somatotroph adenoma [14]. GH mRNA was detected in all (100%) of 18 silent somatotroph adenomas studied by ISH. The degree of positive signal was diverse, and weak positive signal (<10% positive cells) was observed in 9 (50.0%) out of 18 silent somatotroph adenomas in which the degree of GH mRNA signal was described. Usually, the GH mRNA signal of a silent somatotroph adenoma is weaker than that of a classical somatotroph adenoma [14].

PRL was immunohistochemically positive in 25 (61.0%) of 41 silent somatotroph adenomas. The weak positive staining (<10% positive cells) was observed in 8 (57.1%) of 14 silent somatotroph adenomas in which the degree of PRL immunoreactivity was described. PRL mRNA was detected in 13 (72.2%) of 18 silent somatotroph adenomas studied by ISH. The positive signal of PRL mRNA was generally weak, and 9 (69.2%) out of 13 silent somatotroph adenomas had weak positive signal (<10% positive cells).

In case 35, GH was immunonegative by conventional indirect method, whereas immunopositivity of GH was proven by an amplified immunohistochemical method using the catalyzed signal amplification (CSA) system. CSA system increases the sensitivity to 1,000 times higher than that of the conventional indirect peroxidase method [17]. CSA system may thus enable us to detect silent somatotroph adenomas that express only a small amount of GH.

As described in our previous report, mammothroph adenomas are related solely to PRL production [9], an adenoma causing amenorrhea and/or galactorrhea with relative hyperprolactinemia, but without acromegaly, in which immunoreactivity of GH and expression of GH mRNA are demonstrated, should be assigned to silent somatotroph adenoma with concomitant secretion of PRL.

V. Electron Microscopic Observation

Somatotroph adenomas are divided into two types on the basis of electron microscopic observations: densely granulated adenoma and sparsely granulated adenoma. The majority of silent somatotroph adenomas were sparsely granulated adenomas [6, 8, 14, 15, 18–20], as shown in the Table 1. Densely granulated type of silent somatotroph adenoma was also reported by several investigators [7, 10–14, 18]. These paradoxical responses of GH in TRH or GnRH provocation test, which were used as a hallmark of silent somatotroph adenoma in our previous reports [10–13], are observed primarily in densely granulated somatotroph adenomas. Subcellular organelles and diaminobenzidine signals of immunohistochemical staining can be observed under confocal laser scanning microscopy (CLSM) [2–4]. Therefore, when tumor specimens were not available for electron microscopy, CLSM can detect secretory granules using paraffin sections immunostained with anti-GH antibody

Table 1. Summary of silent somatotroph adenomas reported in the literature

Case	Authors (Reference No.)	Age	Sex	Galac- torrhea	Menstruation disorder	GH (ng/ml)		PRL (ng/ml)		IGF-1		Tumor size (cm, or Hardy's grade)	GH sup- pression in OGTT	Paradoxi- cal rise of GH in TRH stimulation	Paradoxi- cal rise of GH in GnRH stimulation	Immunohistochemistry			In situ hybridization		Electron microscopy
						normal range		normal range		normal range						GH	PRL	Others	GH	PRL	
1	Toumaire <i>et al.</i> (18),	44	F	(+)	(+)	21		40				grade 2	(-)			(+++)	(-)	α -subunit			DG
2	Trouillas <i>et al.</i> (19)	23	F	(+)	(-), infertility	9.5		29				grade 2	(-)			(++)	(-)	α -subunit			SG (FB+)
3		23	M	(+)	(-)	6.3		220				grade 2	(-)			(+)>50%					SG
4		31	F	(+)	(+)	8.0		75				grade 2	(-)			(+)>50%					SG
5		16	F	(+)	(+)	5.0		78				grade 2	(-)			(+)>50%					SG
6		42	M	(-)	(-)	1.3		28				grade 3				(+)% N.A.					SG
7		26	F	(+)	(+)	2.6		130				grade 3				(+)% N.A.					SG
8		27	F	(+)	(+)	1.7		38				grade 3				(+)% N.A.					SG
9		36	M	(-)	(-)	<1		2080				grade 3				(-)					SG
10	Trouillas <i>et al.</i> (19)	26	F	(+)	(+)	<2		82				grade 2				(+)% N.A.					SG
11		42	F	(+)	(+)	4.4		12				grade 2				(+)% N.A.					SG
12		22	F	(+)	(+)	<5		75				grade 3				(+)% N.A.					SG
13		28	F	(+)	(+)	4.7		80				grade 2				(+++)>80%	(-)		(-)		SG
14		26	F	(+)	(+)	2.4		37				grade 2				(++)>60%	(-)		(+)		SG
15		28	F	(+)	(+)	4.2		80				grade 3				(+) 2%	(-)				SG
16		21	M	(-)	(-)	<5		180				grade 4				(+) 30%	(-)				SG
17		46	M	(-)	(-)	1.5		1490				grade 3				(+) 20%	(+++)		(+)		SG
18		25	F	(-)	(-)	36		23		7.9	0.45-2.2 U/ml	macro	(-)			(+) in scattered cells	(+) % N.A.	ACTH			SG
19	Klibanski <i>et al.</i> (6)	35	F	(+)	(-)	24	N.A.	19		2.6		macro	(-)			(+) in scattered cells	(+) % N.A.		N.A.		N.A.
20		29	F	(+)	(+)	8		43				macro	(-)			(+) in scattered cells	(+) % N.A.				
21		32	F	(+)	(+)	1.8		28.4				grade 4				(-)	(-)		(+) in a few cells		SG (FB+)
22	Kovacs <i>et al.</i> (8)	39	F	(+)	(+)	6.0-7.0		57				grade 2				(+) in many cells	(+) in a few cells		(+) in a few cells		SG (FB+)
23		22	F	(+)	(+)	5.3-6.6		105				grade 2				(+) in a few cells	(+) in a few cells		(+) in a few cells		SG (FB+)
24	Pestell <i>et al.</i> (15)	31	F	(+)	(+)	23		40				macro	(-)			(+) in majority of cells	(+)				SG
25		19	F	(+)	(+)	38		36				micro	N.A.			no tumor cell	no tumor cell				N.A.
26		27	F	(+)	(+)	18		20		4.8	<1.9 U/ml	1.5	(-)			(+) % N.A.	(-)				N.A.
27	Yamada <i>et al.</i> (20)	46	F	(-)	(-)	7.5	<5	13.1	1.0-15.0	3.6	0.4-2.0 U/ml	macro	(-)			(+) in scattered cells	(-)		(+)		SG (FB+)
28	Naritaka <i>et al.</i> (14)	22	M	(-)	(-)	1.5		7.0		N.A.		3.5	(-)			(++)	(++)	α -subunit	(++)		DG
29	Naritaka <i>et al.</i> (14), Kobayashi <i>et al.</i> (7)	43	F	(+)	(+)	2.9		22.4		1.2		2.2	(-)			(++)	(-)	TSH β , FSH β	(-)		DG
30	Naritaka <i>et al.</i> (14)	32	F	(+)	(+)	1.8		32.9		N.A.		2.0	(-)			(+)	(+)	TSH β	(+++)		SG (FB+)
31	Naritaka <i>et al.</i> (14), Kobayashi <i>et al.</i> (7)	30	M	(-)	(-)	7.2		5.6	5.0-27.0	1.5	<2 U/ml	2.3	(-)			(++)	(+)		(+++)		SG (FB+)
32	Naritaka <i>et al.</i> (14)	24	F	(+)	(+)	4.5		52.0				3.2	(-)			(++)	(-)		(+++)		SG (FB+)
33	Naritaka <i>et al.</i> (14), Kobayashi <i>et al.</i> (7)	12	F	(-)	(-)	4.2		78.0		1.7		5.5	(-)			(+)	(+)		(++)		SG (FB-)
34	Naritaka <i>et al.</i> (14)	33	F	(-)	(-)	2.9		11.6		N.A.		3.0	(-)			(+)	(-)		(+)		SG (FB-)
35	Matsuno <i>et al.</i> (10-13)	25	F	(+)	(+)	2.1-15.1		17-59		180-220		micro	(+)			(-)(+++)>CSA	(++)		(+++)		DG (CLSM)
36	Matsuno <i>et al.</i> (11-13)	20	F	(-)	(+)	0.54-30.9	0.66-3.68	4.8-26.0		220		micro	(+)			(=)	(+)	α -subunit	(+)		DG (CLSM)
37		25	F	(-)	(+)	1.1-5.98		4.9-8.8		180-220		micro	(-)			(+)	(+)	α -subunit	(+)		DG (CLSM)
38	Kageyama <i>et al.</i> (5)	59	M	(-)	(-)	1.9	<3.1	528	3.4-16.2	600	59-215 ng/ml	macro	(-)			(++)>43%	(++)>43%		(+) in many cells		
39		44	F	(+)	(+)	4.67		35		332	100-300 ng/ml	1.5	(-)			(+) in scattered cells	(+)				N.A.
40	Sakharova <i>et al.</i> (16)	28	F	N.A.	(+)	7.15	1.18-3.77	24.5	N.A.	652	130-480 ng/ml	1.1	(-)			(+) in scant foci	(-)				
41		31	F		(+)	4.65		58		465	100-390 ng/ml	2.0	(-)			(++)>50%	(++)>35%				

+: <10%; ++: 10-25%; +++: 26-50%; ++++: >50%
 CSA, catalyzed signal amplification; N.A., not available; DG, densely granulated adenoma; CLSM, confocal laser scanning microscopy; SG, sparsely granulated adenoma; FB, fibrous body.

(Fig. 1a, b).

Naritaka *et al.* classified silent somatotroph adenomas into three groups based on the findings of immunohistochemistry, ISH, and electron microscopy [14]. Subtype 1: a moderate number of cells were immunopositive for GH, and GH mRNA was also expressed in moderate or numerous cells. Densely granulated cells were noted in this subtype. They assumed that inhibition of hormone release into circulation would be the cause of silence. Subtype 2: a small number of cells were immunopositive for GH, while GH mRNA was expressed in numerous tumor cells. The adenoma comprised sparsely granulated cells containing fibrous bodies. They suggested that posttranslational processing of the gene product might be defective. Subtype 3: only a scattered number of cells were immunopositive for GH and GH mRNA was co-localized in immunopositive cells. The adenoma cells were sparsely granulated cells containing poorly developed organelles that did not resemble those of typical sparsely granulated GH cells. They indicated that adenoma cells were largely immature cells with minimal GH lineage differentiation.

VI. Studies of Tissue Culture

Tissue culture studies were conducted in three reports [8, 19, 20]. Trouillas *et al.* studied GH release from four adenoma cells (cases 13–17), and found very low levels of GH in culture medium [19]. They stated that the tumor cells kept their ability to respond to secretagogues because their GH release was stimulated by cortisol. Kovacs *et al.* measured GH release from two adenoma cells (cases 22, 23), and found very low levels of GH in culture medium [8]. They hypothesized that GH mRNA is not translated to GH synthesis in quantities necessary to substantially raise

serum GH levels and cause acromegaly. Yamada *et al.* also found that the amount of GH released *in vitro* was small [20]. These data of tissue culture indicate that silent somatotroph adenoma has very low basal secretory capacity of GH.

VII. Cause of Silence

Several theories have been proposed to explain the absence of clinical signs of acromegaly in silent somatotroph adenoma. The first theory is the impairment of GH synthesis. One adenoma reported by Kovacs *et al.* (case 21) [8] was GH immunonegative among 18 adenomas that expressed GH mRNA. An adenoma (case 35) produced only small amount of GH because the adenoma was immunonegative by conventional indirect method [10–13], and several adenomas have low percentage of GH immunopositive cells. These adenomas would be silent because GH production was minute due to abnormalities in the synthesis or translation of GH gene product [6, 8, 10–13, 19]. The second theory is the impairment of secretion of GH. This hypothesis is supported by the data of tissue culture studies [8, 19, 20], which showed small amount of GH release from adenoma cells. Tumors reported by Trouillas *et al.* (case 14) [19] and Kovacs *et al.* (case 22) [8] contained relatively many GH immunopositive cells, but nevertheless they secreted only a small amount of GH in culture medium. The adenomas thus do not secrete GH in amounts needed to substantially raise the serum GH levels and cause a full-blown clinical symptom of acromegaly. As Yamada *et al.* stated [20], the duration of GH hypersecretion may be too short for the development of acromegaly. The third hypothesis is lack of biological activity of secreted GH [19, 20], but this theory is contradicted by tumors that have elevated

Fig. 1. **a:** Immunohistochemical study disclosed GH immunoreactive cells in the adenoma (case 36, original magnification $\times 400$). **b:** Confocal laser scanning microscopic observation of paraffin sections immunostained with anti-GH antibody revealed secretory granules that were immunostained positively for GH (case 36, N, nucleus; G, Golgi's apparatus, Bar = 2 μ m).

IGF-1 levels. The final theory is that a silent somatotroph adenoma represents an early stage of a classical somatotroph adenoma. As shown in electron microscopic studies by Naritaka *et al.* [14], subtype 3 silent somatotroph adenoma is largely immature with minimal GH lineage differentiation. Therefore, at least this subtype is not considered to represent an early stage of classical somatotroph adenoma. The two adenomas (cases 36, 37) described in our reports [11–13] do not have elevated IGF-1 level although they have GH-immunopositive cells and positive signals of GH mRNA. While the silence of these tumors can be explained by either the second or the third theory, the mechanism of silence in fact may be very complex and may not be adequately covered by these theories.

VIII. Cause of Amenorrhea and/or Galactorrhea in Silent Somatotroph Adenoma

Regarding the cause of amenorrhea and galactorrhea, some investigators have stated that amenorrhea and galactorrhea might be symptoms related to GH hypersecretion [1, 15]. Human GH and PRL bind to the same lactogenic receptors with similar affinity and produce similar biological responses [1]. Another cause of amenorrhea and galactorrhea is concomitant secretion of PRL from silent somatotroph adenomas that was often observed in previous reports, and was also observed in our patients [10–13]. The third possible cause is the so-called stalk section effect, as suggested by Kobayashi *et al.* [7], that can cause mild hyperprolactinemia.

IX. Conclusion

In this paper, the clinical and histopathological features of silent pituitary somatotroph adenoma are reviewed. Meticulous endocrinological and histopathological examination may be helpful for the identification of silent somatotroph adenoma among patients with clinically non-functioning adenomas, especially female patients with amenorrhea and/or galactorrhea.

X. References

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