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ORIGINAL ARTICLE

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Immunohistochemical demonstration of oncocytes in nongonadotrophic pituitary adenomas

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Abstract An immunohistochemical study to demonstrate oncocytes in nongonadotrophic pituitary adenomas was performed. The adenomas were 10 prolactinomas, 2 ACTH-producing adenomas (ACTHomas), and 28 growth hormone-producing adenomas (GHomas); we also studied 5 pituitary oncocytomas. GHomas were divided into two groups: GHomas with (GHomas-1) and without (GHomas-2) fibrous bodies. A small number of solitary large cells showed intense cytoplasmic granular reactivity for mitochondrial protein and cytochrome oxidase, resembling oncocytes in oncocytomas. The proportions of the mitochondrial protein-positive cells ranged from zero to 2.1% (0.3±0.4%). They were more frequent in GHomas, GHomas-1 in particular, than other types of adenomas ($P<0.01$), and were mostly negative in prolactinomas and ACTHomas. In multivariate analysis, the proportions showed positive correlation with age ($P<0.01$) and the Ki-67 (MIB-1) labeling index ($P<0.01$) and tended to increase in number with recurrence ($P<0.05$). In GHomas, these cells were more common in cases with low basal GH level ($P<0.01$) and large tumor volume ($P<0.01$). We consider that these cells represent oncocytes existing in varying numbers in adenomas. We suggest that oncocytic change in nongonadotrophic adenomas indicates poor differentiation and/or some aggressiveness, which lead to a decrease in the endocrine activity of the tumor.

Key words Pituitary adenomas · Growth hormone-producing adenomas · Immunohistochemistry · Mitochondria · Oncocytes

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Introduction

Oncocytes (oncocytic cells) are a special type of epithelial cells characterized by an abundance of intracytoplasmic mitochondria. Oncocytomas, tumors composed predominantly or exclusively of oncocytes, are usually benign neoplasms that occur in various organs, such as the salivary glands, thyroid, parathyroid, kidney, and the adenohypophysis [4]. Pituitary oncocytomas, a variant of null cell adenomas, are clinically nonfunctioning adenomas. However, they are closely related to gonadotrophic adenomas, and a continuous spectrum may exist among them [2]. On the other hand, a varying number of oncocytes may exist in the normal adenohypophysis [10, 14, 15] as well as in functioning adenomas. Most gonadotrophic adenomas have at least focal oncocytic change [2]. Although oncocytic change can be found in any adenoma type, it is rare in nongonadotrophic, adenomas except for acidophil stem cell adenoma. In addition, the significance of oncocytic changes in gonadotrophic adenomas and in acidophil stem cell adenoma seems to be quite different. Meanwhile, it has been suggested that oncocytic change may correlate with grade of differentiation and age in adenoma [9]. Thus, many aspects of oncocytic change in functioning adenomas, particularly in nongonadotrophic adenomas, remain poorly understood.

Recently, we reported that oncocytes could be detected using immunohistochemistry with antibodies specific for mitochondrial protein and its enzymes in pituitary oncocytic adenomas and in normal adenohypophysis [13–15]. The aim of this study was to affirm the usefulness of immunohistochemistry in the detection of oncocytes in nongonadotrophic adenomas and to consider the significance of oncocytic change within these adenomas.

Materials and methods

Surgical specimens of 40 nongonadotrophic pituitary adenomas obtained from 38 patients were examined (Table 1). There were 15

Table 1 Summary of 40 nongonadotrophic adenomas (*MP* proportion of large cells positive for mitochondrial protein, *M* male; *F* female, *FB* fibrous bodies, *VD* visual disturbance(s), *AG* amenorrhea-galactorrhea, *rad* radiated case, *rec* recurrent case)

Patient	Age, gender (ng/ml)	Adenoma type	Tumor stage (Hardy)	Clinical symptom(s)	Basal GH level	Other(s)	MP (%)	Ki-67 (MIB-1) labeling index
1	26 F	Prolactinoma	III-A	VD, AG syndrome			0	0.88
2	34 F	Prolactinoma	IV-D	VD, AG syndrome			0	1.12
3	35 F	Prolactinoma	III-A	VD, hypopituitarism		rad, rec	0.4	1.43
4	24 F	Prolactinoma	I-A	VD, AG syndrome			0	0.84
5	17 F	Prolactinoma	II	AG syndrome			0	0.88
6	33 F	Prolactinoma	III-B	VD, hypopituitarism		rad	0.2	1.15
7	40 F	Prolactinoma	II-A	VD, AG syndome			0	0.70
8	28 M	Prolactinoma	III-A	VD, loss of libido			0	0.96
9	33 F	Prolactinoma	II	AG syndrome			0	0.80
10	38 M	Prolactinoma	II-A	VD			0	0.62
11	49 F	ACTH adenoma	II	Cushing syndrome			0.1	1.11
12	54 F	ACTH adenoma	I-A	Cushing syndrome			0	1.14
13	49 F	GH adenoma with FB	II	acromegaly	32		0.3	0.87
14	42 F	GH adenoma with FB	III-C	VD, acromegaly, hypopituitarism	160		0.6	0.96
15	39 F	GH adenoma with FB	III-A	acromegaly	85		0.2	0.88
16	52 F	GH adenoma with FB	III-B	VD, acromegaly	100		0.4	1.95
17	55 F	GH adenoma with FB	III-B	VD, acromegaly, hypopituitarism	40	rec	2.1	2.70
18	30 F	GH adenoma with FB	II-B	VD, acromegaly	110		0.4	1.95
19	53 F	GH adenoma with FB	III-C	VD, acromegaly, hypopituitarism	200		0.8	1.50
20	49 F	GH adenoma with FB	II-A	VD	22		0.2	0.41
21	52 M	GH adenoma with FB	II-B	VD, acromegaly	76		0.5	1.24
22	60 M	GH adenoma with FB	III-B	VD, acromegaly	48		0.9	1.05
23	61 M	GH adenoma without FB	II	acromegaly	47		0	0.88
24	52 F	GH adenoma without FB	II-A	acromegaly, hypopituitarism	140		0	0.62
25	35 M	GH adenoma without FB	III-A	VD, acromegaly	120		0.2	0.62
26	64 F	GH adenoma without FB	II-B	VD, acromegaly, hypopituitarism	47		0.4	0.64
27	28 M	GH adenoma without FB	III-A	VD, acromegaly	63		0	0.87
28	78 F	GH adenoma without FB	III-A	acromegaly	47		1.2	0.63
29	69 F	GH adenoma without FB	III-B	VD, acromegaly	62		0.6	0.90
30	50 M	GH adenoma without FB	II-A	VD, acromegaly	23		0.2	2.18
31	26 M	GH adenoma without FB	II-A	VD, acromegaly	82		0	0.70
32	27 M	GH adenoma without FB	III	acromegaly	28		0	0.61
33	59 M	GH adenoma without FB	III-B	VD, acromegaly, hypopituitarism	55	rad	0.1	1.03
34	54 M	GH adenoma without FB	III-B	VD, acromegaly, hypopituitarism	38	rec	0.6	1.33
35	44 F	GH adenoma without FB	II-A	Acromegaly	61		0.3	1.01
36	59 F	GH adenoma without FB	IV-B	VD, acromegaly, hypopituitarism	78		0.4	0.55
37	66 M	GH adenoma without FB	II-B	VD, acromegaly	59		0.5	0.53
38	34 M	GH adenoma without FB	I	Acromegaly	62		0	0.40
39	51 F	GH adenoma without FB	II-A	VD	18		0.3	0.65
40	45 M	GH adenoma without FB	III-A	Acromegaly	28		0.4	1.20

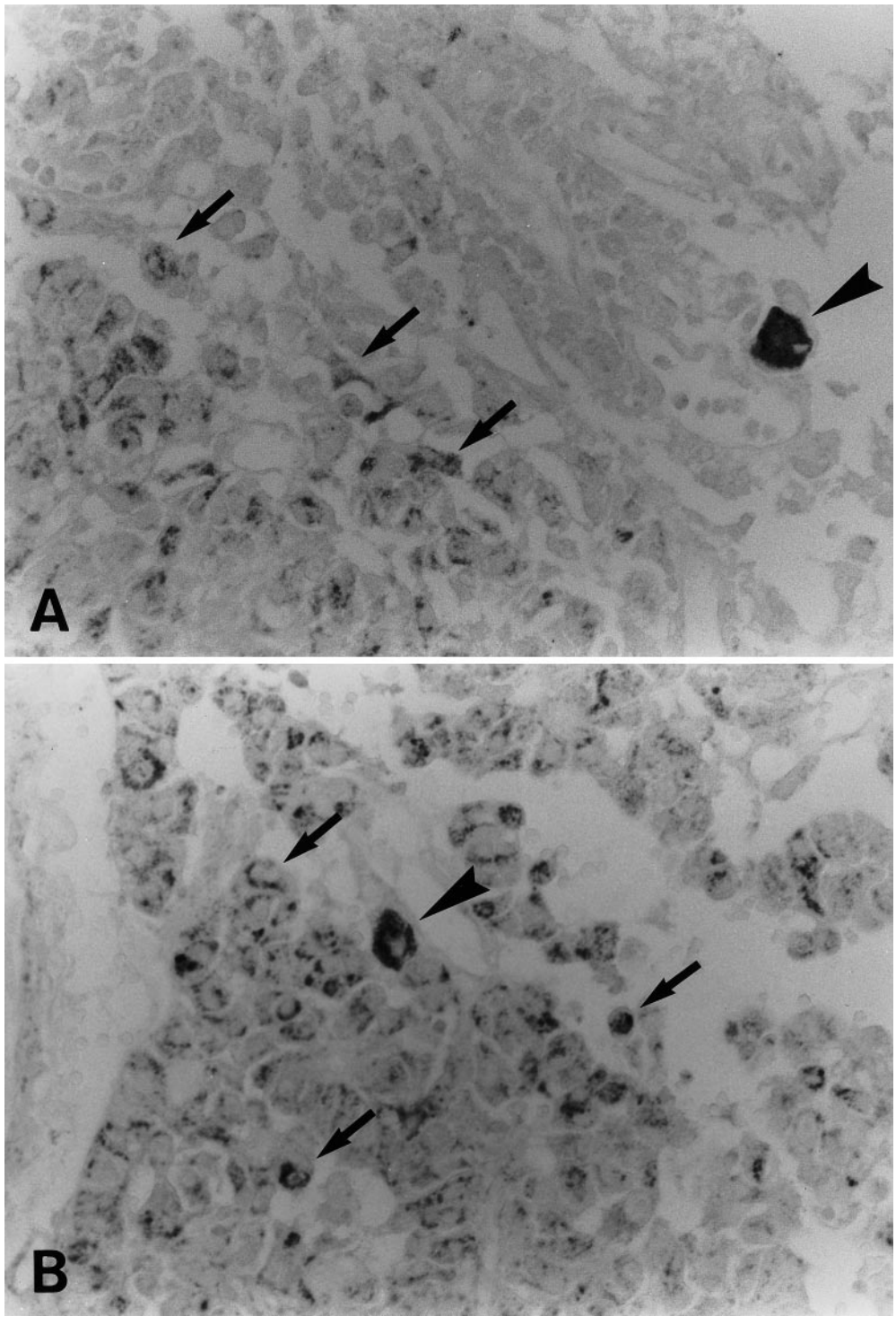

Fig. 1 Immunohistochemistry for **A** mitochondrial protein and **B** cytochrome oxidase in growth hormone (GH)-producing pituitary adenoma. A few solitary large positive cells (*arrowhead*) were observed among many small faintly positive cells (*arrows*). Methyl green counterstain, ×480

male and 23 female patients, ranging in age from 17 to 78 (mean 44.9) years. Six of them were above 60 years of age. The adenomas were 10 prolactinomas (PRLomas; 2 male and 8 females), 2 adrenocorticotropic hormone-producing adenomas (ACTHomas; 2 females), and 28 growth hormone-producing adenomas (GHomas; 13 males and 15 females). They included 3 adenomas that recurred within 3 years after the former surgery and 3 adenomas that were radiated within 1 year prior to removal. Five pituitary oncocytomas diagnosed by electron microscopy were also studied. The specimens were formalin-fixed and paraffin-embedded sections.

Immunohistochemistry was performed using the Envision labeled polymer reagent (Dako, Kyoto, Japan). The following primary antibodies were used: anti-mitochondrial protein, which recognizes a 65-kDa protein by immunoprecipitation (Chemicon International, Temecula, Calif.; 1:20), anti-cytochrome oxidase subunit II (COX, Molecular Probes, Eugene, Ore, USA; 1:100), anti-cytokeratin, which is specific for cytokeratin 7 and 8 (CAM5.2, Becton-Dickinson, San Jose, Calif.; kit), anti-GH, anti-prolactin, anti-ACTH (Dako, Carpinteria, Calif.; kit), and anti-Ki-67 (MIB-1, Immunotech, Marseilles, France; 1:100). Human autopsied liver

was used as a positive control for mitochondrial protein and COX. MIB-1 staining was performed with an antigen retrieval method; microwave heating (121°C for 10 min) with 0.01 M citrate buffer. The specificity of the immunostaining was verified by replacing the primary antibody with nonimmunized serum. The sections were stained with 3,3'-diaminobenzidine and counterstained with 1% methyl green. More than 300 cells from multiple low-power microscopic fields were counted for the proportion of positive cells and the Ki-67 (MIB-1) labeling index [1, 18].

The 28 GHomas were divided into two groups based on immunohistochemical intracytoplasmic stainings for cytokeratin: a prominent dot-like pattern (GHomas-1; 10 adenomas) and a diffuse perinuclear pattern (GHomas-2; 18 adenomas) [17, 20]. GHomas-1 were composed exclusively of cells with dot-like cytokeratin distribution, whereas a minor cell component showed a dot-like pattern in a few specimens of GHomas-2. In contrast to the weak and sparse GH immunopositivity of GHomas-1, immunohistochemistry for GH demonstrated intense and diffuse GH positivity in GHomas-2. On the other hand, some immunoreactivities for prolactin were observed in 2 of the 10 GHomas-1 and 10 of the 18 GHomas-2.

Radiological stage of the adenomas [7] was I in 1, I-A in 2, II in 5, II-A in 8, II-B in 4, III in 1, III-A in 7, III-B in 7, III-C in 2, IV-B in 1, and IV-D in 2 adenomas. Tumor volume was estimated by fine magnetic resonance imaging and/or thin-sliced computed tomography as follows: 0.5 × tumor height × width × depth (cm^3). The tumor volume ranged from 0.6 to 21.3 (mean 6.9) cm^3. In 28 GHomas, presurgical basal GH level and the tumor volume ranged from 18 to 200 (mean 69) ng/ml and from 0.6 to 18.2 (6.4) cm^3, respectively.

In addition to regression analysis, multivariate analysis (Microsoft Excel) was used for statistical analysis to rule out a reflection of the particular phenotype of the adenoma.

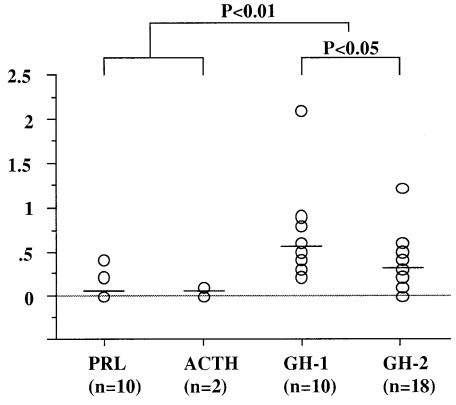

Fig. 2 Proportions of large cells positive for mitochondrial protein in different kinds of nongonadotrophic adenomas (*ACTH* ACTH-producing adenomas, *GH-1* GH-producing adenomas with fibrous bodies, *GH-2* GH-producing adenomas without fibrous bodies, *PRL* prolactinomas)

Results

Immunoreaction product deposits with antibodies to mitochondrial protein and COX appeared as intracellular granular structures. In 5 pituitary oncocytomas, not all but more than half of the adenoma cells were positive for them (mitochondrial protein: 60.0–80.5%; COX: 62.5–88.0%). The positive adenoma cells in nongonadotrophic adenomas were divided into two groups. The majority of them were faintly positive small cells that tended to accumulate in clusters or in islets. In contrast, a few solitary large cells intensely positive for mitochondrial protein (Fig. 1A) and COX (Fig. 1B), resembling the positive cells in pituitary oncocytomas, were observed in some adenomas. The proportions of the large cells positive for the former and the latter in 40 adenomas ranged from zero to 2.1% (mean ± standard deviation 0.3±0.4%) and from zero to 1.9% (0.2±0.4%), respectively. The proportions of the large mitochondrial protein-positive cells in PRLomas, ACTHomas, GHomas, GHomas-1, and GHomas-2 were from zero to 0.4% (0.1±0.1%), from zero to 0.1% (0.1±0.1%), from zero to 2.1% (0.4±0.4%), from 0.2% to 2.1% (0.6±0.6%), and from zero to 1.2% (0.3±0.3%), respectively (Table 1), whereas those of the COX-positive cells were 0–0.2% (0.0±0.1%), 0–0.1% (0.1±0.1%), 0–1.9% (0.3±0.4%), 0–1.9% (0.5±0.5%), and 0–1.0% (0.2±0.3%), respectively. These cells were more common in GHomas than other types of adenomas (mitochondrial protein, COX: $P<0.01$; Fig. 2). In addition, they tended to be more frequent in GHomas-1 than GHomas-2 (mitochondrial protein: $P<0.05$). However, there was no difference in the immunoreactivity for prolactin in GHomas.

In multivariate analysis, there was a positive correlation between the proportions of the large cells and the age of the patients (mitochondrial protein: $P<0.01$; Fig. 3, left). The proportions of the large cells positive for mi-

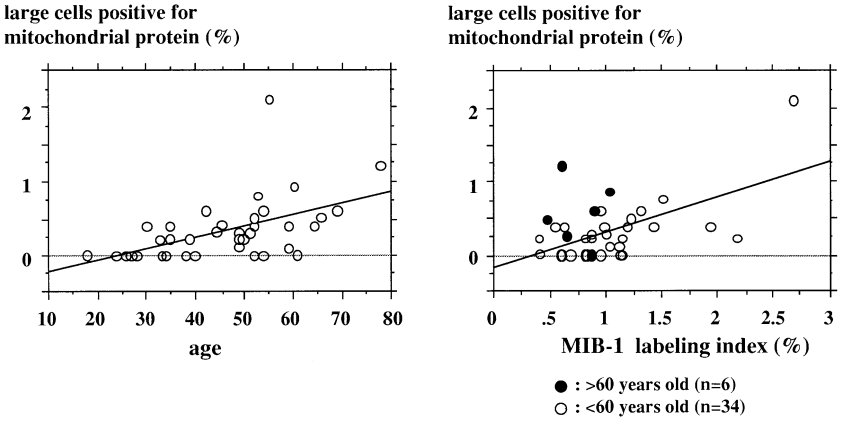

Fig. 3 Correlations between age and proportion of large cells positive for mitochondrial protein and between MIB-1 labeling index and proportion of large cells positive for mitochondrial protein

tochondrial protein and COX in 3 recurrent adenomas were 0.4%, 0.6%, 2.1% (mean: 1.0%) and 0.2%, 0.5%, 1.9% (0.9%), respectively. They were higher than those in nonrecurrent adenomas (mitochondrial protein, COX: $P<0.05$). There was no difference in terms of sex, radiological stage, tumor volume or radiation treatment.

On the other hand, the Ki-67 (MIB-1)-labeling index in 40 adenomas ranged from 0.40% to 2.70% (0.99±0.46%). There was no difference in the Ki-67 (MIB-1)-labeling index between the different types of adenomas. However, they showed a positive correlation with proportions of large cells positive for mitochondrial protein ($P<0.01$; Fig. 3, right). In multivariate analysis of 28 GHomas, the proportion of large cells positive for mitochondrial protein correlated negatively with basal GH level ($P<0.01$) and positively with the tumor volume ($P<0.01$). They also correlated positively with the patients' ages ($P<0.05$), but not with the Ki-67 (MIB-1)-labeling index.

Discussion

Oncocytes are large polygonal cells with eosinophilic cytoplasm and central small nuclei seen when stained with hematoxylin and eosin. Since it is difficult to differentiate pituitary oncocytomas from other eosinophilic adenomas by light microscopy on routine staining, it has been emphasized that their definitive diagnosis requires ultrastructural studies [9]. However, recent studies have demonstrated that immunohistochemistry with antibodies specific for mitochondrial protein and its enzymes is useful for the identification of oncocytes and oncocytomas in various organs, including the pituitary [3, 11, 13–16, 19]. Mitochondrial protein and/or COX-positive cells in oncocytomas in the present study represent oncocytes [13]. In nongonadotrophic adenomas, many adenoma cells showed cytoplasmic granular immunoreactivity for mitochondrial protein and COX. These positive cells were divided into two groups according to their cell size, immunoreactivity, and distribution. The majority of them were small and faintly positive, and tended to accumulate in clusters and in islets, whereas the minority were large solitary cells with intense reactivity, resembling oncocytes in oncocytomas. Since all cells contain some mitochondria, it was suggested that immunonegativity for these antibodies might be caused by low technical sensitivity in the present study, whereas positivity may represent an increase in either functional activity or number of mitochondria [13–15]. Although some oncocytes may manifest as small cells positive for mitochondrial protein/COX, depending on the cutting sections, we regarded the large positive cells as oncocytes.

A dot-like intracytoplasmic distribution of cytokeratin in GHomas has often been documented and considered to be identical with fibrous body [17, 20]. The fibrous bodies consist of spherically arranged type-2 filaments and are the most conspicuous morphologic marker of sparsely granulated GHomas [2, 9, 12, 20]. This structure has also been described in acidophil stem cell adenoma and sparsely mixed GH/PRLoma. Thus, the distribution pattern of cytokeratin and immunoreactivity for GH indicate that GHoma-1 and -2 in the present study may correspond mainly to sparsely granulated and densely granulated GHoma, respectively [17, 20]. GHoma-1 and -2 with some immunoreactivities for prolactin may also include acidophil stem cell adenoma and sparsely mixed GH/PRLoma, and mammosomatotroph adenoma and mixed densely GH/PRLoma, respectively.

It has been suggested that the grade of differentiation correlates with oncocytic change in adenoma [9]. Acidophil stem cell adenoma is a poorly differentiated adenoma assumed to be derived from common progenitor cells capable of differentiating to either GH-or PRL cells (or both). Oncocytic change is one of the characteristic ultrastructural findings of acidophil stem cell adenoma [2, 9]. A prolactin-secreting oncocytoma reported by Kalyanaraman [8] may, in fact, represent acidophil stem cell adenoma. Although there is no evidence that fibrous bodies are either a sign or an agent of cellular degeneration [12], sparsely granulated GHomas are also less highly differentiated than are densely granulated GHomas or other functioning adenomas [9]. Indeed, oncocytic change was more frequent in GHomas with fibrous bodies than in other nongonadotrophic adenomas in the present study.

Pituitary oncocytomas occur predominantly in the older age group, and the number of oncocytes in the normal pituitary increases with advancing age [9, 15]. It has been suggested that oncocytic changes also correlate with age in functioning adenomas [9]. There was a positive correlation between age and the proportion of oncocytes in the present study. The age-related increase of oncocytes in normal and adenomatous pituitaries is similar to that found in other organs, such as the thyroid, parathyroid, and salivary glands [4, 9]. It can be considered that oncocytic change represents a common appearance of age-related degeneration of adenohypophysial cells in normal pituitary, oncocytomas [4, 5, 15], and in not all, but some, nongonadotrophic adenomas.

On the other hand, oncocytic change may lead to a loss of the capability for hormone production in both normal and adenomatous pituitaries [10, 15]. In a PRL-producing oncocytoma reported by Chowdhury et al. [5] not oncocytes but about 10–15% of nononcocytic cells were likely to be secretorily active and responsible for the functional activity of the tumor. A lower GH production in sparsely granulated GHomas than in densely granulated GHomas has been reported [20, 21]. In the present study of GHomas with multivariate analysis, the proportion of oncocytes correlated negatively with basal GH level and positively with the tumor volume. This may indicate decreased GH production associated with oncocytic change. Consequently, nongonadotrophic adenomas with oncocytic change may be hormonally less active than those without them.

Sparsely granulated GHomas exhibit a faster pace of growth and are more likely to be invasive tumors with

macroadenomas; their recurrence rate appears to be somewhat higher than that of densely granulated GHomas [2, 9, 17, 20, 21]. Acidophil stem cell adenomas are characteristically aggressive, invasive, and rapidly growing tumors [2, 9]. Furthermore, 2 of 3 reported cases of functioning pituitary oncocytomas were invasive tumors [5, 6, 8]. In the present study a positive correlation was noted between the Ki-67 (MIB-1) labeling index and the proportions of oncocytes. This correlation was more prominent in patients aged under 60 years. Although it is still a controversial matter to estimate the proliferative potential of pituitary adenomas by way of the MIB-1 labeling index [1, 18], the proportion of oncocytes tends to increase in association with recurrence. In conclusion, we suggest that nongonadotrophic adenomas with oncocytic change are less highly differentiated tumors with decreased endocrine activity that may be associated with some aggressiveness.

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