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Growth hormone (GH) and prolactin (PRL) gene expression and immunoreactivity in GH- and PRL-producing human pituitary adenomas

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Summary. Growth hormone(GH)-producing pituitary adenomas are morphologically heterogeneous and frequently contain not only GH immunoreactivity but also variable numbers of prolactin (PRL) immunopositive cells. Paraffin sections of 59 surgically removed GH- and/or PRL-producing adenomas classified by histology, immunocytochemistry (ICC) and electron microscopy were studied using in situ hybridization (ISH) for GH and PRL mRNA and combined with ICC for the coded hormones. Somatotroph adenomas (10 densely and 10 sparsely granulated tumours) and mammosomatotroph adenomas (10 cases) contained both GH mRNA and GH immunoreactivity. In 4 densely and 4 sparsely granulated somatotroph adenomas and 4 mammosomatotroph adenomas, only GH mRNA and its product were found. In 28 cases (6 densely and 6 sparsely granulated somatotroph adenomas, 10 mixed somatotroph-lactotroph adenomas and 6 mammosomatotroph adenomas) both GH and PRL mRNA were present, although no PRL immunoreactivity was not in 2 densely granulated somatotroph adenomas. In these cases, ISH for PRL mRNA combined with GH immunostaining revealed the presence of variable numbers of mammosomatotrophs. In 9 acidophil stem cell adenomas only PRL mRNA and its product were found; one tumour expressed both GH and PRL mRNA and their products. Nine lactotroph adenomas contained only PRL mRNA and PRL immunoreactivity. The results show that GH and/or PRL mRNA content could not be correlated with ICC for coded proteins and ultrastructural features. The mammosomatotrophs were more numerous using ISH when compared with ICC. Somatotroph, mammosomatotroph and mixed adenomas are closely related and they can be considered to represent one basic tumour type originating in a cell committed to GH production. This may undergo clonal differentiation towards a mammosomatotroph and further to the lactotroph line. The results also indicate that lactotroph adenomas arise in a cell committed to PRL production. Acidophil stem cell adenomas seem to be more closely related to lactotroph cells than somatotroph.

Key words: Growth hormone – Prolactin – Immunocytochemistry – In situ hybridization – Pituitary adenomas

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Introduction

The present morphological classification of growth hormone(GH)-producing pitutary adenomas is based on histological, immunocytochemical and ultrastructural features (Horvath and Kovacs 1991; Kovacs and Horvath 1985, 1986a, b, 1987). By electron microscopy (EM), GH-producing pituitary adenomas are divided into: somatotroph adenomas; mammosomatotroph adenomas; acidophil stem cell adenomas and mixed somatotroph-lactotroph adenomas. Immunocytochemistry (ICC) revealed that both monomorphous and plurimorphous tumours can produce more than one hormone (Kovacs et al. 1989a, b; Scheithauer et al. 1986b). Thus, many somatotroph adenomas contain variable numbers of cells immunoreactive for prolactin (PRL), α-subunit (SU) and/or thyroid stimulating hormone (TSH) beside GH immunoreactivity (Beck-Peccoz et al. 1985; Kovacs and Horvath 1986a; Kovacs et al. 1982). Alpha-SU and TSH expression in GH-producing adenomas will not be dealt with here. In approximately 30–40% of patients with GH-producing adenoma serum PRL levels are elevated (Bassetti et al. 1986; Lamberts et al. 1982). The demonstration of mRNAs coding for adenohypophysial hormones by in situ hybridization (ISH) in rodent (Coghlan et al. 1985; Lewis et al. 1986; Lloyd and Landefeld 1986; Lloyd et al. 1988a, 1990; Pixley et al. 1987) and human (Kovacs et al. 1991; Lloyd et al. 1989; Nagaya et al. 1990; Stefaneanu et al. 1991) pituitaries has resulted in better understanding of the pathophysiology of pituitary adenomas. Detailed studies on GH-PRL-

producing pituitary adenomas by ISH have not been reported and the cytogenesis of these tumours is still obscure.

The aim of the present study was to obtain a deeper insight into the cellular derivation of GH-PRL-producing adenomas, using ISH combined with ICC in order to correlate the morphology of GH-PRL-producing adenomas with GH and PRL gene expression and serum hormone levels.

Materials and methods

Fifty-nine surgically removed GH- and/or PRL-producing pituitary adenomas randomly selected were used in this study. Densely granulated somatotroph adenomas (10 cases), sparsely granulated somatotroph adenomas (10 cases), mixed somatotroph-lactotroph adenomas (10 cases), mammosomatotroph adenomas (10 cases), acidophil stem cell adenomas (10 cases) and lactotroph adenomas (9 cases) were diagnosed by histology, immunocytochemistry and EM. The tumour cell types were established at the ultrastructural level. In addition, two non-neoplastic pituitaries obtained at autopsy from patients with no endocrine disorders and two non-neoplastic fragments of surgically removed adenohypophyses were used as positive controls for ISH. Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections of 5 μm thickness were mounted on 3-aminopropylethoxylane-coated slides.

The oligonucleotide probe for human prolactin (hPRL) was d(GGC TTG CTC CTT GTC TTC GGG) and d(GGC GCG GAG CAT AGC GTT GTG) for human growth hormone (hGH). The oligonucleotides were complementary to the region of hPRL 66–72 (Cooke et al. 1981) and hGH 11–17 (Martial et al. 1979) coding for these residues. The GH and PRL probes were synthesized and purified using PAGE by Genosys Biotechnologies (The Woodlands, Tex., USA). The probes were labelled by the 3'-end method with (35S) dATP and terminal deoxynucleotidyl transferase using a kit (NEP-100, Du Pont Canada, Missisauga, Ont.) and purified with the NENSORB-TM 20 cartridge included in the kit.

ISH was performed on 5 μm paraffin sections applying a 5×10^5 cpm probe as previously described (Kovacs et al. 1989b). For combined ISH and ICC for GH and PRL the avidin-biotin-peroxidase complex (ABC) method was used after 2 × SSC (1 × SSC = 0.15 M sodium chloride 0.015 M sodium citrate) washings (Kovacs et al. 1989). The following controls for ISH were performed: predigestion of tissue sections with 100 μg/ml RNase A (Sigma, St. Louis, Mo., USA); competition studies with 100-fold excess of unlabelled probe to assure specificity; as negative controls, human liver and parathyroid adenomas were used; and combined ISH with ICC using hGH probe and anti-hGH antiserum or hPRL probe and anti-hPRL antiserum.

The ABC technique was used for demonstration of GH and PRL content. The following antisera were applied: anti-hGH (1:2000 dilution, DAKO, Santa Barbara, Calif., USA), anti-hPRL (1:1000 dilution, provided by Dr. H. Friesen, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada). The immunostainings and the appropriate controls have been described elsewhere (Hsu et al. 1981a, b).

Results

As shown in Tables 1–6, 36 patients with high levels of serum GH were diagnosed as having acromegaly, 2 patients with normal levels of serum GH and 3 patients with no available data for serum GH showed the clinical features of acromegaly. In 9 of these patients, serum PRL levels were also elevated. Nine patients with high serum PRL concentrations were diagnosed as having lactotroph adenomas. In 4 patients with galactorrhoea and amenorrhoea, the diagnosis of acidophil stem cell adenoma was made on the basis of morphology. No clinical data were available in 5 patients with acidophil stem cell adenoma.

Immunocytochemical staining revealed diffuse GH immunoreactivity in 14 out of 41 adenomas (6 densely and 4 sparsely granulated somatotroph adenomas and 4 mammosomatotroph adenomas) removed from acromegalic patients. In the remaining 27 patients with acromegaly (4 densely and 6 sparsely granulated somatotroph adenomas, 10 mixed somatotroph-lactotroph adenomas, 6 mammosomatotroph adenomas and 1 acidophil stem cell adenoma), the tumours contained both

Table 1. Clinical, immunocytochemical (ICC) and in situ hybridization (ISH) results in densely granulated somatotroph adenomas associated with acromegaly

Case no/age/sex	Serum level		Hardy's grade	GH (ICC; ISH)		PRL (ICC; ISH)	
	GH (ng/ml)	PRL (ng/ml)					
1 53, M	68	N	II	D;	D (++, +++)	−;	−
2 36, M	40	N	III	D;	D (++, +++)	−;	−
3 50, F	20	NA	II	D;	D (++)	−;	−
4 33, M	90	N	III	D;	D (++, +++)	−;	−
5 50, F	240	N	II	D;	D (++, +++)	−;	D (++)
6 57, M	26.6	24	II	D;	D (++)	−;	D (+)
7 20, F	55	N	III	D;	D (+, ++)	Few;	Few (+)
8 67, F	55	NA	III	D;	D (++, +++)	Few;	Few (+)
9 53, M	50	110	III	a) D;	D (++, +++)	Scattered;	Scattered (++)
				b) −;	D (+)	D;	D (++, +++)
10 48, M	30	46	II	D;	D (+, ++)	D;	D (++, +++)
Normal range	<5	0–20					

D, Diffuse localization; N, within normal limits; NA, not available; GH, growth hormone; PRL, prolactin
−, Negative signal; +, weak signal; ++, moderate signal; +++, intense signal; ++++, very intense signal

Table 2. Clinical, immunocytochemical (ICC) and in situ hybridization (ISH) results in sparsely granulated somatotroph adenomas associated with acromegaly

Case no./age/ sex	Serum level		Hardy's grade	GH(ICC; ISH)		PRL(ICC; ISH)	
	GH (ng/ml)	PRL (ng/ml)					
11 39, M	12	N	II	D;	D (+)	Rare (NT?)	–
12 45, F	90	N	III	D;	D (++, +++)	–;	–
13 65, F	10.2	N	II	D;	D (+, ++, +++)	–;	–
14 NA, M	50	N	II	D;	D (+)	–;	–
15 21, M	NA	NA	NA	D;	D (++)	Scattered;	Few (+)
16 27, M	7.2	N	I	D;	D (+)	Scattered;	Few (+)
17 61, M	40	N	II	D;	D (+, ++)	Few;	Some (+, ++)
18 27, F	68	36	II	D;	D (+)	Scattered;	Few (+)
19 NA, F	NA	NA	NA	D;	D (++)	Scattered;	Many (++, +++)
20 39, F	35.6	55	II	D;	D (+)	Few;	D (+, ++)

NT, nontumourous cells

Table 3. Clinical, immunocytochemical (ICC) and in situ hybridization (ISH) results in mixed GH-PRL cell adenomas associated with acromegaly

Case no./age/ sex	Serum level		Hardy's grade	GH(ICC; ISH)		PRL(ICC; ISH)	
	GH (ng/ml)	PRL (ng/ml)					
21 76, F	40	N	II	Some;	Many (+)	Few;	Few (+)
22 45, F	16	N	NA	Most;	Many (++)	Few;	Few (++)
23 49, M	41	N	III	Many;	D (+, ++)	Few;	Some (++)
24 53, F	150	N	II	Many;	D (++)	Scattered;	Scattered and focal (++)
25 65, F	19	N	I	Most;	Most (++)	Many;	Some (++)
26 56, M	N	78	III	Many;	Many (+, ++)	Many;	Many (++, +++)
27 45, M	NA	NA	NA	Some;	Most (+++)	Scattered;	Many (++)
28 22, M	N	>926	III	Some;	D (+)	Some;	D (++, +++)
29 26, F	>200	265	II	Many;	D (+)	Scattered;	Many (+++)
30 36, M	25	N	II	Many;	D (+)	Scattered;	Most (+)

Table 4. Clinical, immunocytochemical (ICC) and in situ hybridization (ISH) results in mammosomatotroph adenomas associated with acromegaly

Case no./age/ sex	Serum level		Hardy's grade	GH(ICC; ISH)		PRL(ICC; ISH)	
	GH (ng/ml)	PRL (ng/ml)					
31 48, M	20	N	II	Most;	D (+)	–;	–
32 27, M	39	N	II	Some;	Most (++)	–;	–
33 38, M	41	N	II	Most;	D (+++)	–;	–
34 56, F	26.2	N	II	Many;	D (+)	Rare (NT?);	Rare (NT?)
35 67, F	21	N	I	Most;	D (++)	Scattered;	Scattered and focal (++)
36 55, M	13.7	N	II	Many;	D (++)	Scattered;	Scattered (++, +++)
37 62, M	15	26.9	II	Most;	D (+)	Few;	Few and focal (+)
38 31, F	37	N	II	Some;	D (++, +++)	Scattered;	Scattered (+++)
39 41, M	97	N	III	Many;	D (+++)	Many;	Most (++)
40 29, F	36.4	181.6	NA	Many;	D (+)	Many;	Many (+, ++, +++)

GH- and PRL-immunoreactive cells; the GH immunoreactivity was seen in most adenoma cells, and the PRL-immunopositive cells were either focally or diffusely distributed. Nine acidophil stem cell adenomas and 9 lactotroph adenomas contained only PRL-immunoreactive cells.

All 10 densely granulated somatotroph adenomas contained diffuse, moderate or intense hybridization signal for GH mRNA, which was correlated with GH immunostaining. In 6 of these adenomas (cases 5–10) both GH and PRL mRNAs were present (Fig. 1A, B). According to the PRL mRNA distribution, adenomas were

Table 5. Clinical, immunocytochemical (ICC) and in situ hybridization (ISH) results in acidophil stem cell adenomas

Case no./age/sex	Serum level		Hardy's grade	GH(ICC; ISH)		PRL(ICC; ISH)	
	GH (ng/ml)	RL (ng/ml)					
41 28, F	N	120	II	−;	−	Many;	D (+, ++)
42 45, F	N	125	III	−;	−	Some;	Many (+, ++)
43 60, F	N	365	III	−;	−	Many;	D (+)
44 20, F	N	1134	III	−;	−	Some;	D (+)
45 NA, M	NA	NA	NA	−;	−	Some;	D (++)
46 26, F	NA	278	NA	−;	−	Some;	D (+, ++)
47 28, M	NA	NA	NA	−;	−	Some;	D (+)
48 21, F	NA	NA	NA	−;	−	Some;	D (++)
49 63, M	NA	NA	NA	−;	−	Few;	Many (+)
50 53, M	46	N	II	Many;	Many (+)	Few;	Many (+, ++)

Table 6. Clinical, immunocytochemical (ICC) and in situ hybridization (ISH) results in lactotroph adenomas

Case no./age/sex	Serum level		Hardy's grade	GH(ICC; ISH)		PRL(ICC; ISH)	
	GH (ng/ml)	PRL (ng/ml)					
51 19, F	N	325	II	−;	−	D;	D (+++, ++++)
52 68, M	N	630	II	−;	−	D;	D (+++, ++++)
53 20, F	N	115	II	−;	−	D;	D (++)
54 31, F	N	>2500	II	−;	−	D;	D (++, +++)
55 34, M	N	10400	IV	−;	−	D;	D (+++)
56 39, F	N	450	II	−;	−	D;	D (++, +++)
57 32, F	N	70	I	−;	−	D;	D (+++, ++++)
58 37, M	N	1630	III	−;	−	D;	D (+++, ++++)
59 16, F	N	90	II	−;	−	D;	D (+++, ++++)

divided into three groups (Table 1). In group I, no PRL mRNA and no PRL immunoreactivity were detected, indicating that the adenomas synthesized only GH. Group II was characterized by diffuse signal for PRL mRNA without PRL immunoreactivity. In group III, varying numbers of cells exhibited both PRL immunoreactivity and PRL mRNA in the adenoma cells; in one case PRL mRNA and its product was diffusely distributed over all adenoma cells (case 10). One adenoma (case 9) was composed of two populations of cells; the predominant one was acidophilic, the other was mainly chromophobic. In the acidophilic area, most tumour cells displayed diffuse and intense positive signal for GH mRNA (Fig. 2A) and GH immunoreactivity. In the chromophobic area, tumour cells contained diffuse but lower signal for GH mRNA (Fig. 2A) and no GH immunoreactivity, and intense signal for PRL mRNA (Fig. 2B) and PRL immunoreactivity.

As shown in Table 1, positive correlation was found between GH mRNA expression and serum GH levels. Most adenomas were associated with normal serum PRL levels. Two cases (nos. 9, 10) in which tumour cells contained PRL mRNA and PRL immunoreactivity were associated with mild or moderate hyperprolactinaemia. The adenomas positive for PRL mRNA and immunonegative for PRL were not associated with elevated serum PRL levels.

In sparsely granulated somatotroph adenomas (Table 2), the results were similar to those found in densely granulated somatotroph adenomas. In cases 19 and 20, the signal for PRL mRNA was evenly distributed among adenoma cells despite the scattered PRL immunoreactivity.

Mammosomatotroph adenomas based on ICC and ISH resembled somatotroph adenomas (Table 4): all adenomas contained diffuse signal for GH mRNA, some (cases 31–34) contained only GH mRNA and GH immunoreactivity and some (cases 35–40) expressed PRL mRNA and PRL immunoreactivity with a focal or diffuse distribution. Combined ISH for GH mRNA and ICC for PRL or ISH for PRL mRNA and ICC for GH revealed the presence of mammosomatotrophs that in some cases represented the majority of tumour cells (Fig. 3A, B). Most adenomas (8 out of 10) were associated only with elevated serum GH levels.

All mixed somatotroph-lactotroph adenomas expressed both GH and PRL mRNA and their products (Table 3). Based on ISH and ICC, four cell populations were identified in mixed somatotroph-lactotroph adenomas: adenoma cells containing only GH mRNA and GH immunoreactivity; adenoma cells containing PRL mRNA and PRL immunoreactivity only; adenoma cells positive for both GH and PRL mRNAs and their products; and adenoma cells with signals for both GH and

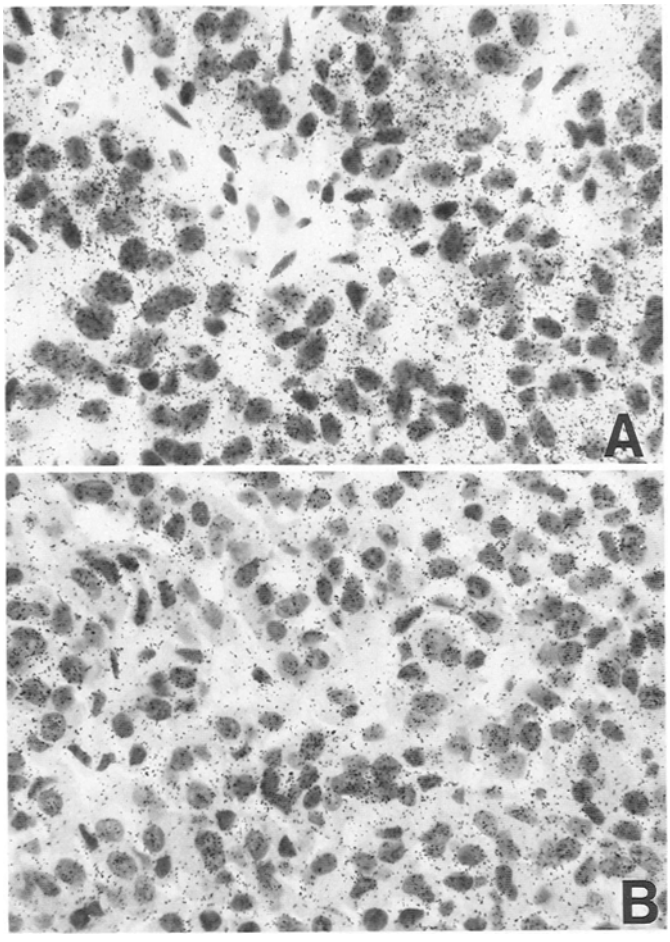

Fig. 1 A, B. Densely granulated somatotroph adenoma; in situ hybridization (ISH) revealed diffuse signal for both growth hormone (GH) (**A**) and prolactin (PRL) (**B**) mRNAs over the adenoma cells. ×400

PRL mRNAs and immunopositive for only one gene product. In 6 cases (nos. 24, 26–30), most tumour cells contained diffuse signal for GH and focal for PRL mRNAs (Fig. 4A, B). ISH for PRL mRNA combined with GH immunostaining revealed the presence of mammosomatotrophs. In the other 4 cases, varying numbers of mammosomatotrophs have been identified.

Most (9 out of 10) acidophil stem cell adenomas contained only PRL mRNA and PRL immunoreactivity (Fig. 5A, B). PRL serum level was moderately or highly elevated in these cases (Table 5). One case expressed both GH and PRL mRNA and their products, and the patient had acromegaly.

Lactotroph adenomas expressed only PRL mRNA and PRL immunoreactivity. They were negative for GH mRNA and its product. All PRL cell adenomas were associated with high serum PRL levels (Table 6).

Discussion

The investigation of GH and PRL mRNAs in different types of GH- and/or PRL-producing adenomas revealed a good correlation between ISH results and GH and PRL immunoreactivity in a majority of the cases, as previously reported by Lloyd et al. (1989), and Nagaya et al. (1990).

ISH added new insight into the understanding of the cytogenesis of GH-PRL-producing pituitary adenomas and showed that gene expression and ultrastructural features cannot be correlated in every case (Table 7). Thus, approximately half of the densely and sparsely granulated somatotroph adenomas contained a variable percentage of cells expressing PRL apart from GH mRNA and GH immunoreactivity. These bihormonal cells are called mammosomatotrophs. Some tumours classified as densely or sparsely granulated somatotroph adenomas, based on ultrastructural features, contained a diffuse signal for GH and PRL mRNAs and their product, and based on these results they fit in the mammosomatotroph adenoma group. Some adenomas with ultrastructural features of somatotroph or mammosomatotroph tumours expressed only the GH gene, or contained a variable percentage of bihormonal cells. Mammosomatotroph adenomas, which can be diagnosed only by EM, are composed of cells similar to densely granulated somatotrophs with the distinctive feature of extruded secretory granules. Extrusion of secretory granules is a reliable ultrastructural marker of normal and adenomatous lactotrophs. Mammosomatotroph adenomas share ultrastructural features of both somatotrophs and lacto-

Table 7. Growth hormone (GH) and prolactin (PRL) mRNA expression in various types of GH and/or PRL producing pituitary adenomas

Tumour types	Total number of cases	GH mRNA only	GH mRNA (most cells) PRL mRNA (most cells)	GH mRNA (most cells) PRL mRNA (uneven)	PRL mRNA only
GH (DG)	10	4	1	5	0
GH (SG)	10	4	2	4	0
GH-PRL	10	0	5	5	0
MS	10	4	2	4	0
ASC	10	0	1	0	9
PRL	9	0	0	0	9
Total	59	12	11	18	18

DG, Densely granulated; SG, sparsely granulated; MS, mammosomatotroph adenomas; ASC; acidophil stem cell adenomas

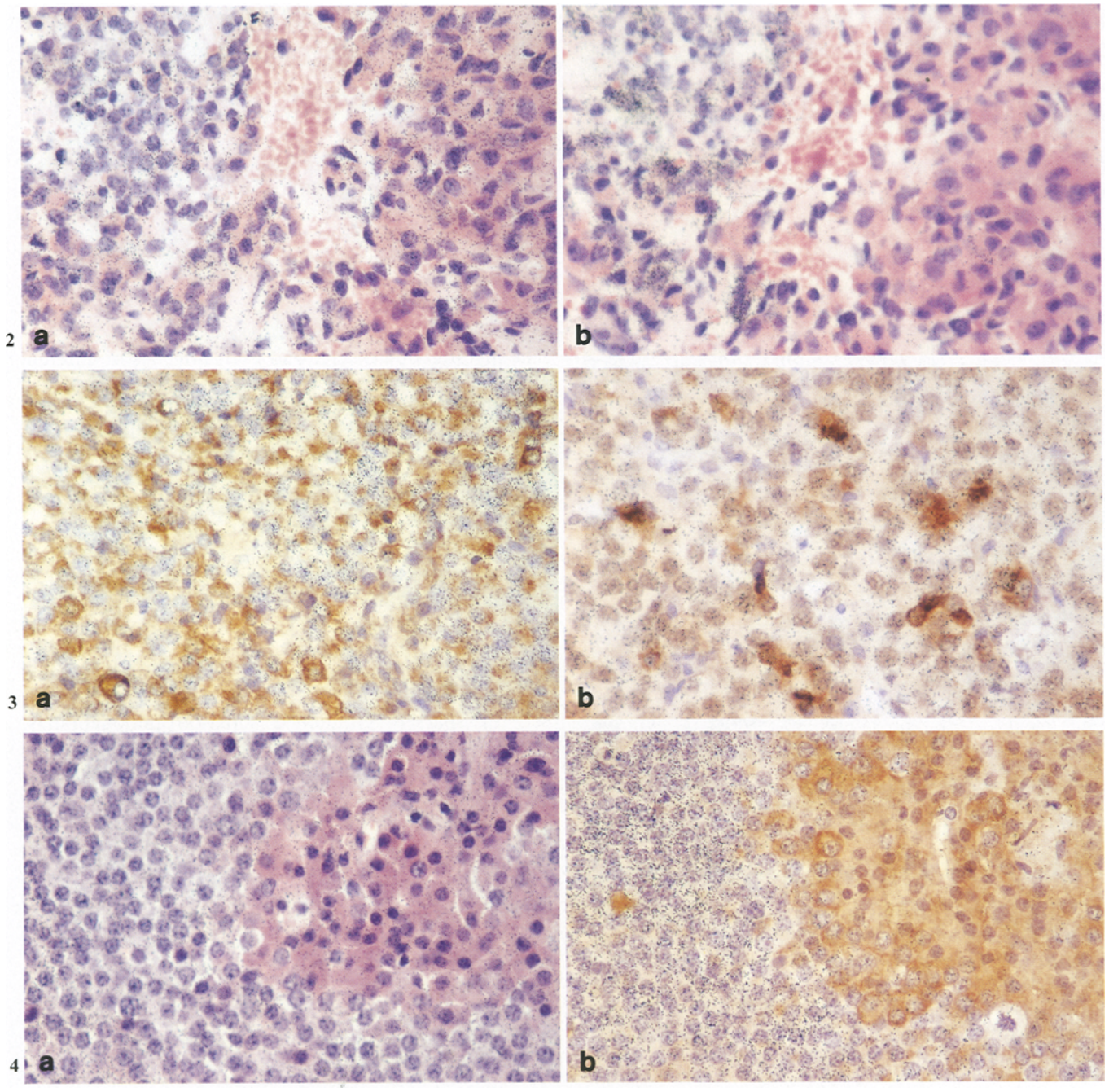

Fig. 2A, B. Densely granulated somatotroph adenoma (case 9). **A** Both acidophilic (*right*) and chromophobic (*left*) areas display diffuse signal for GH mRNA. **B** PRL mRNA is localized mainly in the chromophobic area. ×400

Fig. 3A, B. Mammosomatotroph adenoma (case 39). **A** Combined ISH for PRL mRNA and immunocytochemistry (ICC) for GH shows diffuse signal for PRL mRNA in GH immunoreactive adenoma cells. **B** Combined ISH for GH mRNA with ICC for PRL demonstrates the presence of GH gene expression in PRL immunoreactive cells. ×400

Fig. 4A, B. Mixed somatotroph-lactotroph adenoma (case 24). **A** ISH revealed diffuse signal for GH mRNA. **B** Combined ISH for PRL mRNA and ICC for GH shows that PRL mRNA is localized in cells immunonegative for GH. ×400

trophs and have been regarded as a distinct cell type (Kovacs and Horvath 1986a). The present results revealed that bihormonal GH-PRL-production tumours can exhibit ultrastructural features of somatotrophs or mammosomatotrophs. More studies are necessary, especially by ISH and the immunogold method at the ultrastructural level, to establish the features of neoplastic mammosomatotrophs. The mammosomatotroph adenomas represent a distinct ultrastructural group, and the significance of granule extrusion remains to be clarified.

The ISH examination of mixed somatotroph-lactotroph adenomas revealed that all adenomas displayed GH mRNA in a diffuse pattern, despite variations in GH

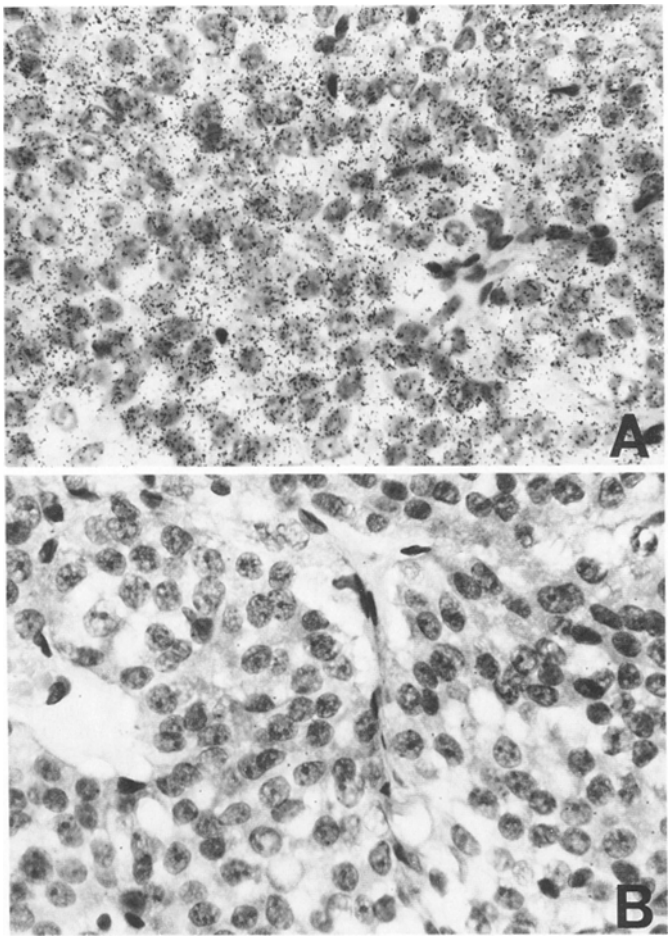

Fig. 5. Acidophil stem cell adenoma (case 41) contains a diffuse signal for PRL mRNA (**A**), while hybridization with GH probe gave negative results (**B**). ×400

immunoreactivity. PRL mRNA was present in different amounts from occasional cells up to a diffuse pattern. Unexpectedly, the islets of PRL-immunoreactive cells contained not only PRL mRNA, but also GH mRNA. Some GH-immunoreactive cells also contained mRNAs coding for GH and PRL. It appears that these mixed tumours are composed of somatotrophs and mammosomatotrophs. These data, together with ultrastructural features, suggest that mixed adenomas arise in a somatotroph that undergoes clonal differentiation toward mammosomatotrophs and subsequently to lactotrophs. An alternative origin is in a mammosomatotroph that has the potential to transform into somatotrophs and lactotrophs. The presence of GH mRNA in the islets of PRL-immunoreactive cells indicates that the clonal differentiation toward PRL lineage is not complete. This assumption is supported by the findings in a densely granulated somatotroph adenoma (case 9); this tumour was composed mainly of GH-immunoreactive cells, groups of bihormonal GH-PRL-positive cells and a smaller area with the histological features of a microprolactinoma. There were chromophobic cells with typical PRL immunoreactivity in the Golgi area. However, GH mRNA was diffusely distributed over this field. In mixed adenomas the lactotrophs did not differ from those found in the lactotroph adenomas. However, lactotroph adenomas in this and in previous studies (Lloyd et al. 1989; Nagaya et al. 1990) expressed only PRL gene and its product, indicating that lactotroph adenomas originate in a cell committed to PRL synthesis.

Acidophil stem cell adenomas with ultrastructural features of immaturity share characteristics with both adenomatous sparsely granulated somatotrophs and lactotrophs, including fibrous bodies and secretory granule extrusions (Horvath and Kovacs 1991; Kovacs and Horvath 1985, 1986a, b, 1987). These tumours are accompanied by hyperprolactinaemia, and rarely by elevated GH in the serum (Kovacs and Horvath 1986a). The finding that nine out of ten adenomas examined contained only PRL mRNA and PRL immunoreactivity suggests that this tumour type originates in an immature cell closer to the lactotroph line. These adenomas, however, show only incomplete commitment to PRL production since they may also produce GH, as seen in one of our cases and some of those previously reported (Kovacs and Horvath 1986a, b; McNicol et al. 1991; Scheithauer et al. 1986a).

Our study reveals that the mammosomatotroph component of GH-producing adenomas is higher when sought by ISH when compared with immunocytochemistry, indicating the higher sensitivity and specificity of ISH. Discrepancy between ICC and ISH results was found in two densely granulated somatotroph adenomas that contained PRL mRNA with a diffuse pattern despite negative PRL immunoreactivity. Since serum PRL levels were within the normal range in these patients, it can be speculated that the PRL mRNA was not translated or the amount of synthesized PRL was not enough to be detected by ICC. Nagaya et al. (1990) reported four adenomas in which the presence of PRL mRNA was not associated with PRL immunoreactivity and hyperprolactinaemia. In contrast to these findings, McNicol et al. (1991) described two tumours associated with elevated serum PRL levels which were positive for PRL mRNA and immunonegative for PRL, suggesting that PRL was not stored in sufficient amounts for immunocytochemical detection. The same explanation is provided by Lloyd et al. (1989) for one tumour which contained GH mRNA and no GH immunoreactivity. In this patient serum GH concentration returned to normal after resection.

The question arises as to when a cell should be called a mammosomatotroph. This cell type was defined by ICC colocalizing GH and PRL to the same cell (Kovacs et al. 1988; Losinski et al. 1989; Zurschmiede and Landolt 1987). By the reverse haemolytic plaque assay, mammosomatotrophs were identified based on the release of both hormones (Frawley et al. 1985; Lloyd et al. 1988b). ISH revealed adenoma cells containing PRL and GH mRNAs and only one of the coded peptides. Should such an adenoma be classified in the mammosomatotroph group?

The present results indicate that somatotroph, mammosomatotroph and mixed adenomas are closely related. The presence of somatotrophs, mammosomato-

trophs and lactotrophs in these tumours can be a reflection of a process occurring in the normal adenohypophysis. A developmental relationship between somatotrophs and lactotrophs was suggested by the ontogenic appearance of these cells (Horvath et al. 1981; Lloyd et al. 1988; Mulchahey and Jaffe 1988). In a recent review, Frawley and Boockfor (1991) discussed the significance of mammosomatotrophs during ontogeny and different physiological and pathological conditions. The authors envisage the acidophilic cell suspended as a pendulum swinging toward the somatotroph or lactotroph line by the intermediate bihormonal phase – the mammosomatotroph. Behringer et al. (1988) created transgenic mice for the enhancer/promoter sequence of rat GH fused to diphtheria toxin. This is a valuable experimental model for the study of the relationship between lactotrophs and somatotrophs. The authors propose a model involving two pathways for lactotrophs development: a major one from somatotrophs via mammosomatotrophs, and a minor one in which lactotroph arises directly from an acidophilic stem cell. Our findings in GH-PRL-producing adenomas support this model. Thus, it can be suggested that an adenoma arising in a cell committed to GH production can undergo clonal differentiation toward mammosomatotroph and further to a lactotroph line. The finding that lactotroph adenomas express only PRL gene indicates their origin in a cell committed to PRL production. It can be speculated that there may also be a cell line committed only to GH production, from which somatotroph adenomas producing only GH arise.

In conclusion, our study indicates that GH-PRL-producing pituitary adenomas may be considered to represent one basic tumour type. It also suggests that the terminology and distinction between the various entities depend on the methods used for their definition.

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