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Immunohistochemical studies of human FSH producing pituitary adenomas

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Summary. Ten FSH producing pituitary adenomas were studied immunohistochemically. 9 cases were in males, and 7 showed elevated serum FSH levels. Immunohistochemically, all cases showed the presence of α-subunit and FSH-β subunits in many tumour cells. These two subunits were frequently colocalized in the same cells. However, the expression of LH-β subunit was extremely low (1 of 10 cases exhibiting occasional LH-β positive tumour cells), although it has been reported that FSH-β and LH-β subunits are colocalized in the same cells of the normal adult pituitary gland. Immunoelectron microscopically, α-subunits and FSH-β were present in the secretory granules and suggested the co-release of subunits or secretion of combined form of FSH. In 7 cases, TSH-β was positive, and in some cases, TSH-β was colocalized in the same tumour cells which contained α-subunit and FSH-β subunit. A few cases also demonstrated immunoreactivity for PRL and ACTH. Our immunohistochemical studies suggest that FSH adenomas are multihormonal and that there is abnormal gene expression in FSH cells with loss of LH-β appearance and co-expression of TSH-β.

Key words: Pituitary adenoma – Immunohistochemistry – Follicle stimulating hormone (FSH) – α-subunit – Thyroid stimulating hormone (TSH)

Introduction

Although gonadotropin producing pituitary adenoma has been considered to be a rare functioning pituitary adenoma, there is recent an increasing immunohistochemical evidence that many non-functional pituitary adenomas are associated with the production of glycoprotein subunits (Girod et al. 1986; Heitz 1979; Horvath and Kovacs 1984; Jameson et al. 1986; Klibanski et al. 1987; Koide et al. 1982; Lamberts et al. 1987; Peterson et al. 1981; Ridgway et al. 1981; Snyder et al. 1980; Snyder et al. 1984; Snyder 1985; Snyder et al. 1985; Whitaker et al. 1985; Wide and Lundberg 1981). Our previous studies on GH secreting adenoma demonstrated a frequent association with α-subunit immunohistochemical reactivity (Osamura and Watanabe; 1987). It is well known that glycoprotein hormone is composed of two subunits, i.e. α- and β-subunits, and that follicle stimulating hormone (FSH), luteinizing hormone (LH) thyroid stimulating hormone (TSH) and human chorionic gonadotropin (HCG) share a structurally similar α-subunit. Structural and functional specificity of the hormones lies in β-subunits (Klibanski et al. 1987; Ridgway et al. 1981; Snyder 1980). Our present immunohistochemical studies are aimed at the clarification of the immunohistochemical reactivities of 10 cases of FSH producing adenoma.

Our studies demonstrate that these adenomas show colocalization not only of FSH-α and FSH-β in many tumour cells but also of FSH-β and TSH-β in some of the cells.

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Materials and methods

A total of 10 cases of FSH producing adenomas and 1 case of FSH-α producing adenoma were subjected to the following immunohistochemical studies.

For light microscopic immunohistochemistry, small pieces of tissue from the adenoma were fixed in 10% formalin and embedded in paraffin. The indirect peroxidase labelled antibody method was performed on 4 μ deparaffinized sections. Final colorization was made by 0.2% 3.3′ diaminobenzidine 4 HCl (Wako pure chemicals) containing 0.005% H_2O_2. The sections were then counterstained by 1% methylgreen and mounted by

Table 1. Serum hormone levels

	Age	Sex	FSH (MIU/ml)	LH (MIU/ml)	TSH (μU/ml)	ACTH (ng/ml)	PRL (ng/ml)	GH (ng/ml)
1.	19	(M)	136.7	26.9	5.1	–	21.9	1.1
2.	58	(M)	6472	26	17.1	24	28	1.6
3.	29	(F)	700~1000	27~35	27	–	–	–
4.	60	(M)	55	25	4.2	10	29	0.7
5.	60	(M)	160	30	–	–	–	–
6.	39	(M)	101	35	–	–	–	–
7.	40	(M)	5.4	7.5		<10	35	
8.	46	(M)	25	9.4	0.8	18	31	0.8
9.	53	(M)	10.8	7.2		26	36	0.8
10.	44	(M)	10.3	12.7		<10	15.3	
11.	51	(M)	70.0	26.4		<10	10.5	

Normal levels: FSH (M4–25, F4–30 MIU/ml), LH (M7–24, F6–30 MIU/ml), TSH (10–30 μU/ml), PRL (M5–18, F6–22 ng/ml), ACTH (<50 ng/ml), GH (10–60 ng/ml)

Table 2. Results of preabsorption tests and immunohistochemical staining of adult pituitary and placenta

Antibodies	Antigens			Immunohistochemical staining	
	FSHα	FSHβ	TSHβ	adult pituitary gland	placental chorionic villi
anti HCGα(M)	–	±	+	+	+
FSHα(P)	–	±	+	+	+
FSHβ(M)	+	–	+	+	–
FSHβ(P)	±	–	+	+	–
TSHβ(P)	+	+	–	+	–

M: Monoclonal antibody; P: Polyclonal antibody; –: absorbed completely; ±: staining diminished remarkably; +: Not absorbed

Eukitt. Utilized antibodies were against FSH-α, β, LH-β, TSH-β subunits (diluted 1:1000) (supplied by NIAKDD, MA, USA), against prolactin (diluted 1:200) (kindly supplied by Prof. Paul K. Nakane, Department of Cell Biology, Tokai University School of Medicine), against GH (supplied by Biogenetics). Monoclonal antibodies against HCGα subunit (Miles Yeda 1:60) and FSHβ subunit (Immunosearch 1:100) were also used.

In order to compare two different subunits in the same tumour cells, consecutive mirror sections were used for the immunohistochemical staining (Osamura and Watanabe 1987).

For immunoelectron microscopic localization, preembedding method was used in conjunction with the indirect peroxidase labeled antibody method. Details of the procedure are described elsewhere (Osamura et al. 1986; Osamura and Watanabe 1987). In brief, the tissue was fixed in 4% paraformaldehyde overnight, washed in phosphate buffered saline with graded concentration of sucrose up to 20% and quickly frozen in alcohol dry ice. The indirect peroxidase labelled antibody method was performed, as described for light microscopic immunohistochemistry, but the incubation time for each antibody was elongated to overnight. As a second antibody, horseradish peroxidase (Sigma type VI) was conjugated to IgG Fab in order to facilitate its better penetration in the tissue sections. After the antigen-antibody reaction, the tissue sections were fixed by 2% glutaraldehyde for 10 min and reacted first in the above described DAB solution without H_2O_2 for 1 h and then in DAB solution with H_2O_2 for 2 min. After postfixation in 2% osmic acid for 30 min, the sections were dehydrated and embedded in Quetol as described previously. Ultrathin sections were examined by JEOL 1200EX electron microscopy.

The immunohistochemical cross reactivities of anti FSHα, HCGα, FSHβ, TSHβ subunits antibodies were evaluated with the available antigens (FSHα FSHβ, TSHβ supplied by NIADDK). In brief, each of the above diluted anti FSHα, FSHβ and TSHβ antibodies was preincubated with FSHα, FSHβ or TSHβ (20 μg/100 μl) for overnight at 4° C. The subsequent immunohistochemical staining was performed as described above. Adult pituitary glands and placenta were stained immunohistochemically by the above antibodies for confirmation of their specificities.

Results

Except for one case, 9 cases of FSH secretory adenomas were male. 7 cases showed elevated serum FSH levels, but in 3 cases it was within normal limits (Table 1). In haematoxylin and eosin stained material, the tumour was generally composed of ovoid or spindle cells frequently arranged around the blood vessels, exhibiting pseudorosettes. By Grimelius staining the tumour cells were frequently argyrophilic.

The results of immunohistochemical preabsorption tests and the staining of the adult pituitary gland and placental chorionic villi are summarized in Table 2. Monoclonal antibody against

Table 3. Immunohistochemical staining of FSH producing adenomas

	Age	Sex	FSHα	FSHβ	LHβ	TSHβ	ACTH	PRL	GH
1.	19	(M)	++++	++++	−	+	−	−	−
2.	58	(M)	+++	++	−	+	−	+	−
3.	29	(F)	++++	++++	−	−	++	−	−
4.	60	(M)	+++	+++	−	++	−	−	−
5.	60	(M)	++	++	−	++	−	−	−
6.	39	(M)	++	++	−	+	−	++	−
7.	40	(M)	++	++	−	−	−	−	−
8.	46	(M)	++	+	+	+	+	+	−
9.	53	(M)	+	++	−	−	−	−	−
10.	44	(M)	+	++	−	++	−	−	−
11.	51	(M)	+++	−	−	−	−	−	−
			11/11	10/11	1/11	7/11	2/11	3/11	0/11

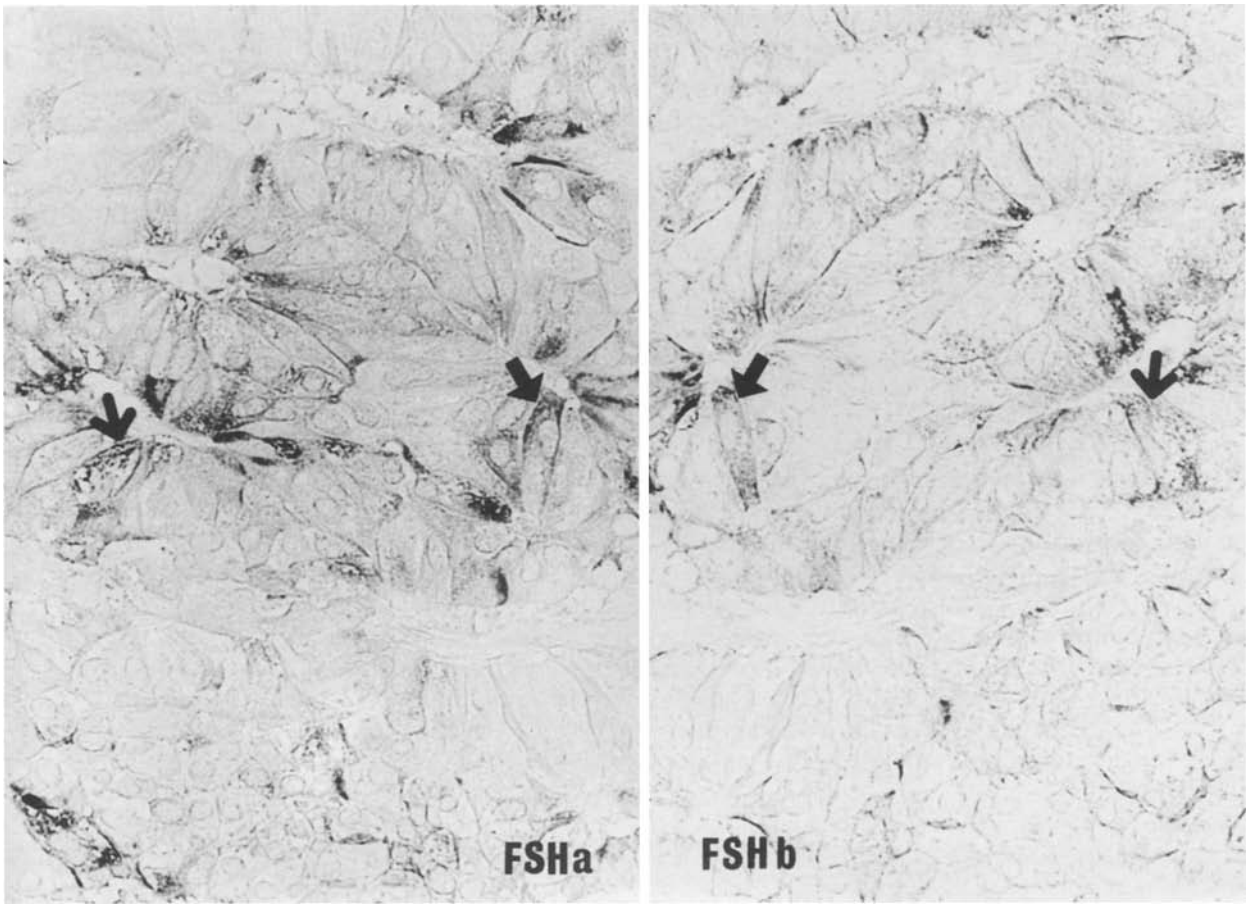

Fig. 1. Case 3. Immunohistochemical localization of α-subunit and FSH-β by mirror sections. Many tumour cells contain both α-subunit and FSH-β in their cytoplasm. (*arrows*) (× 690)

FSHβ subunit and polyclonal antibody against TSHβ subunit showed specific absorption patterns with the corresponding antigens. However, the staining of anti HCGα, FSHα antibodies was remarkably diminished by preabsorption with the antigen FSHβ and the staining of anti FSHβ antibody was also diminished by FSHα subunit. When these antibodies were applied to the placenta, anti HCGα and FSHα subunits antibodies stained chorionic villi but anti FSHβ antibody did not stain them. The staining of the pituitary adenomas by antibodies against FSHα and HCGα subunits was

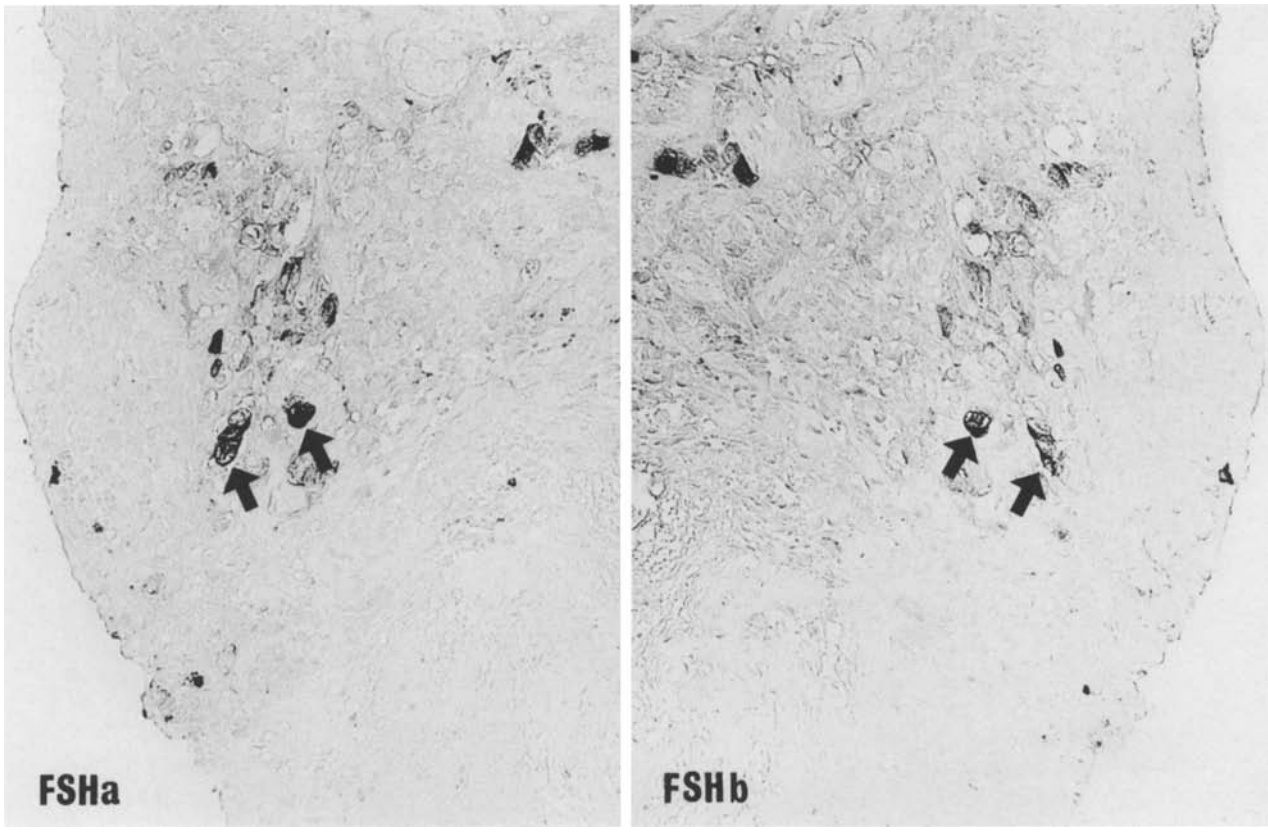

Fig. 2. Case 6. Immunohistochemical localization of α-subunit and FSH β-subunit by mirror sections. Many tumour cells have colocalized α-subunit and FSH-β in the cytoplasm. (*arrows*) (×690)

similar, as was the staining of pituitary adenomas by anti FSHβ polyclonal antibody and anti FSHβ monoclonal antibody. Therefore, we interpreted that antibodies against FSHα and HCGα were specific for the α subunit and that anti FSHβ polyclonal and monoclonal antibodies were specific for FSHβ subunit. The diminished staining of anti FSHα or anti HCGα antibodies by preapsorption with FSHβ subunit may be due to possible presence of small amount of α subunit in the supplied FSHβ subunit.

Immunohistochemically, all 10 cases showed the presence of FSH-α and FSH-β subunits in many tumour cells (Table 3). Cases 8, 9, 10 were immunohistochemically positive for FSH-α and FSH-β subunits even though serum FSH level was within normal limits. By mirror sections, in all 10 cases, FSH-α and FSH-β frequently colocalized in the same tumour cells (Figs. 1, 2). Some tumour cells possess either FSH-α or FSH-β subunits. By conventional electron microscopy, the tumour cells were spindleshaped and contained a few small secretory granules along the cell membrane (Fig. 3). Immunoelectron microscopically, FSH-α and FSH-β were mainly localized in the secretory granules of the tumour cells (Fig. 4).

As for the other immunoreactive subunits, TSHβ appeared in 7 of 10 cases. A few cases such as cases #1, showed as many TSH-β positive cells as FSH-α or FSH-β positive cells. By mirror sections, in case #6, many tumour cells contained α-subunits, FSH-β and TSH-β subunits (Figs. 5, 6).

ACTH was present in two cases. The ACTH positive cells were different from FSH-α, β-subunits containing cells (Fig. 7). It was of note that the appearance of LH-β was extremely low (in only one case) and that the immunoreactivity for GH was not observed in any cases.

One case which was immunohistochemically positive only for α-subunit showed no immunoreactivity for β-subunits, GH, PRL or ACTH.

Discussion

It should be emphasized that FSH producing adenomas possess characteristic morphological structures which distinguish them from other types of

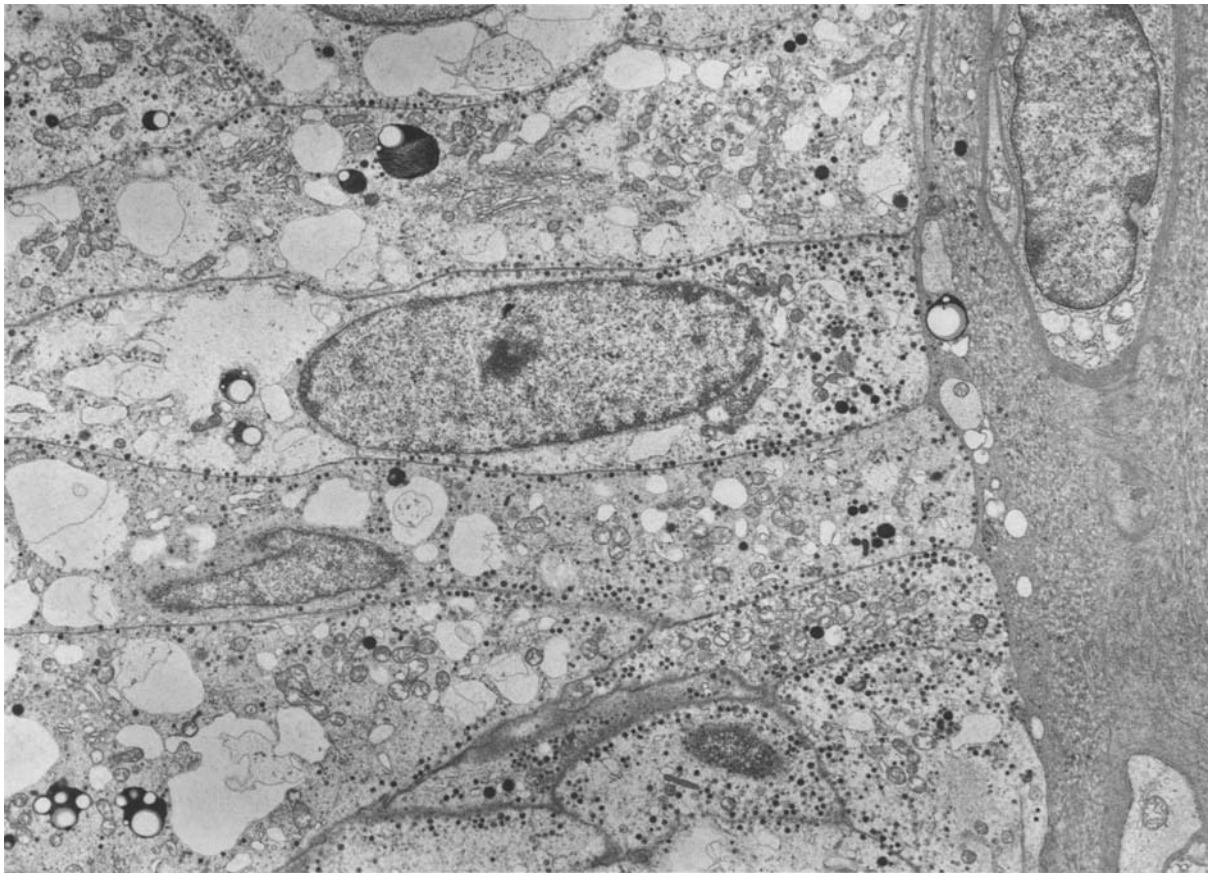

Fig. 3. General electron microscopic features of FSH producing adenoma. Spindle tumour cells are arranged around the blood vessels and contain many small secretory granules. (× 5000)

pituitary adenomas, namely spindle cells with pseudorosettes and argyrophilia.

Previous reports of cases of FSH producing pituitary adenomas showed a male preponderance (Snyder et al. 1980; Snyder 1985; Whitaker et al. 1985). In contrast, our previous study disclosed that immunohistochemical appearance of α-subunit in GH secreting adenomas was more frequent in females (Osamura and Watanabe 1988). In 7 cases here, serum FSH levels were elevated but in 3 cases they were with normal limits. Recently it has been considered that the majority of clinically nonfunctioning pituitary adenomas secrete one or more glycoprotein subunits (Girod et al. 1986; Heitz 1979; Horvath and Kovacs 1984; Jameson et al. 1986; Klibanski et al. 1978; Koide et al. 1982; Lamberts et al. 1987; Peterson et al. 1981; Ridgway et al. 1981; Snyder et al. 1980; Snyder et al. 1984; Snyder 1985; Snyder et al. 1985; Whitaker et al. 1985; Wide and Lundberg 1981). It can therefore be anticipated that the incidence of FSH- or subunit-producing adenomas may be increased when immunohistochemical staining is applied to more cases of clinically nonfunctioning adenomas.

In all cases, the tumour cells were immunohistochemically positive for α-subunit and FSH-β subunit which were seen by mirror sections to be frequently colocalized in the same cells. A few tumour cells contained either α-subunit or FSH-β subunit. We may speculate that the major cellular component of our cases of adenoma secretes combined FSH glycoprotein hormone or its subunits separately. Alternatively it may produce a peculiar form of FSH which reacts to antibodies for both α-subunits and β-subunit (Klibanski et al. 1987; Snyder et al. 1980; Snyder et al. 1984). In the literature, separated subunits are reported to be secreted from the adenomas (Peterson et al. 1981; Ridgway et al. 1981; Snyder et al. 1984). The secretion of free form α and β-subunits may explain the normal serum FSH levels in some FSH producing adenomas.

The extremely low incidence of LH secreting adenoma should be emphasized. In our present study, as well as in the previous study on GH sec-

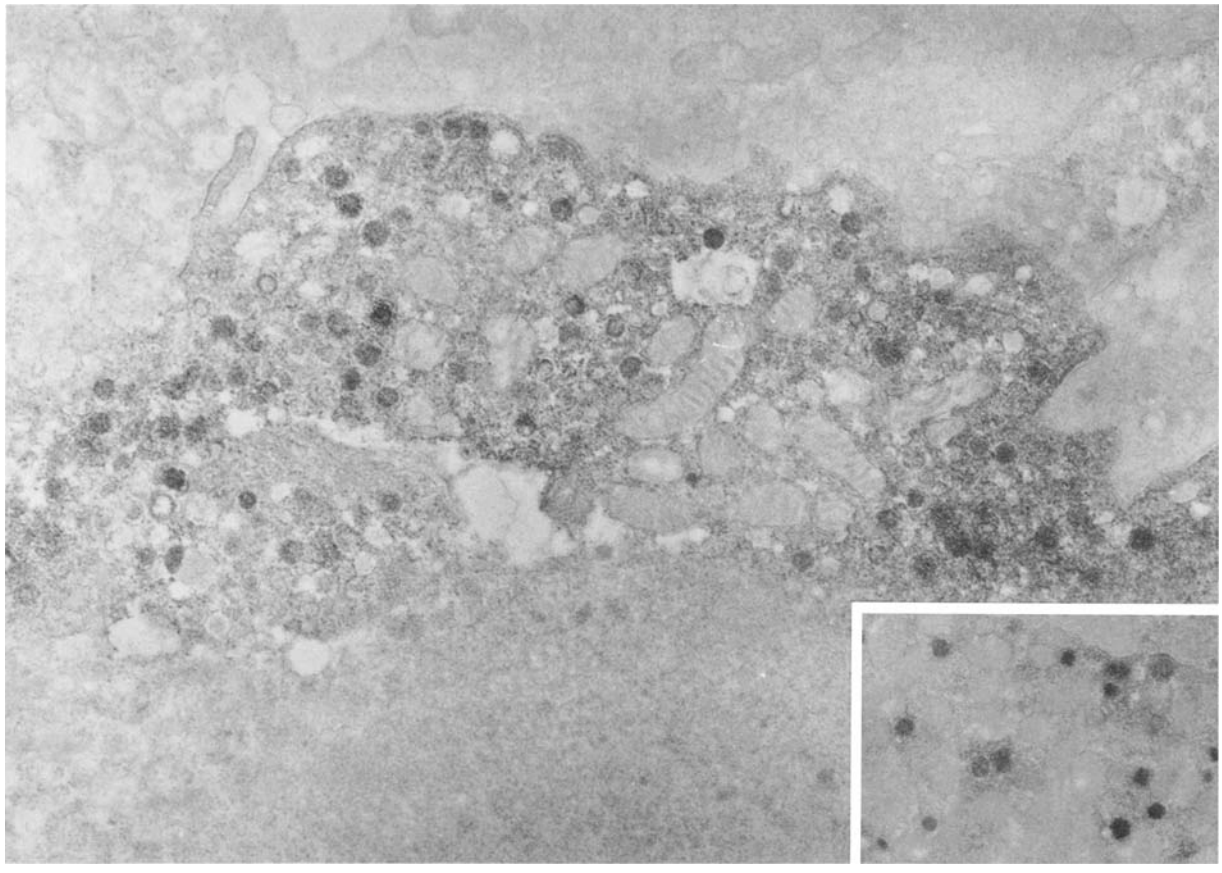

Fig. 4. Immunoelectron microscopic localization of α-subunit in the tumour cells. α-subunit was mainly localized in the small secretory granules. *Inset:* FSH β-subunit was also mainly localized in the small secretory granules. (×25000 *Inset:* ×16000)

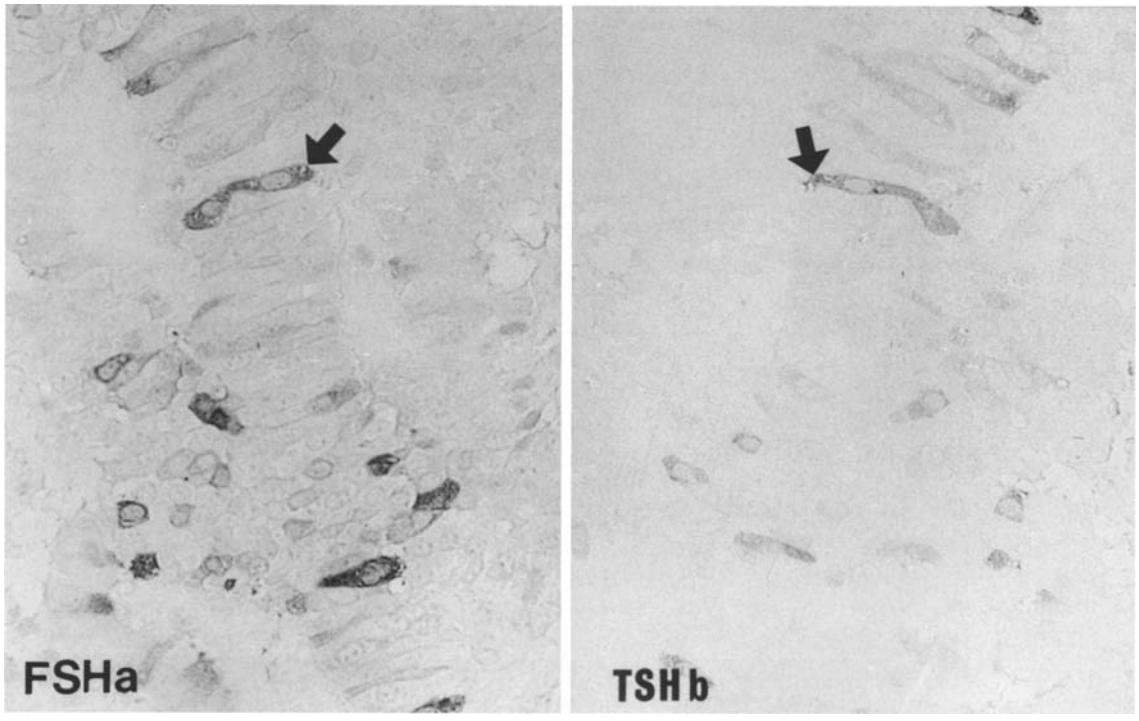

Fig. 5. Case 1. Immunohistochemical localization of α-subunit and TSH-β by mirror sections. Some tumour cells contain both α-subunit and TSH β-subunit in cytoplasm. (*arrows*) (×690)

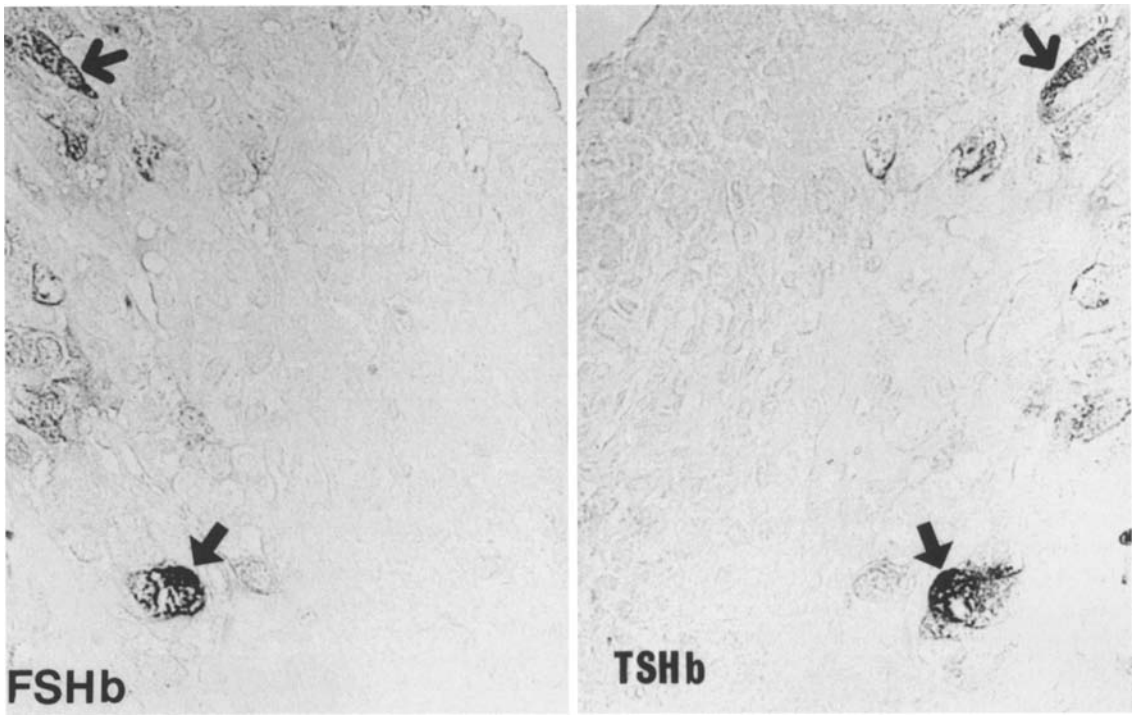

Fig. 6. Case 6. Immunohistochemical localization of FSH β-subunit and TSH β-subunit by mirror sections. Some tumour cells colocalize FSH β and TSH β subunits in the cytoplasm. (*arrows*) ($\times$ 690)

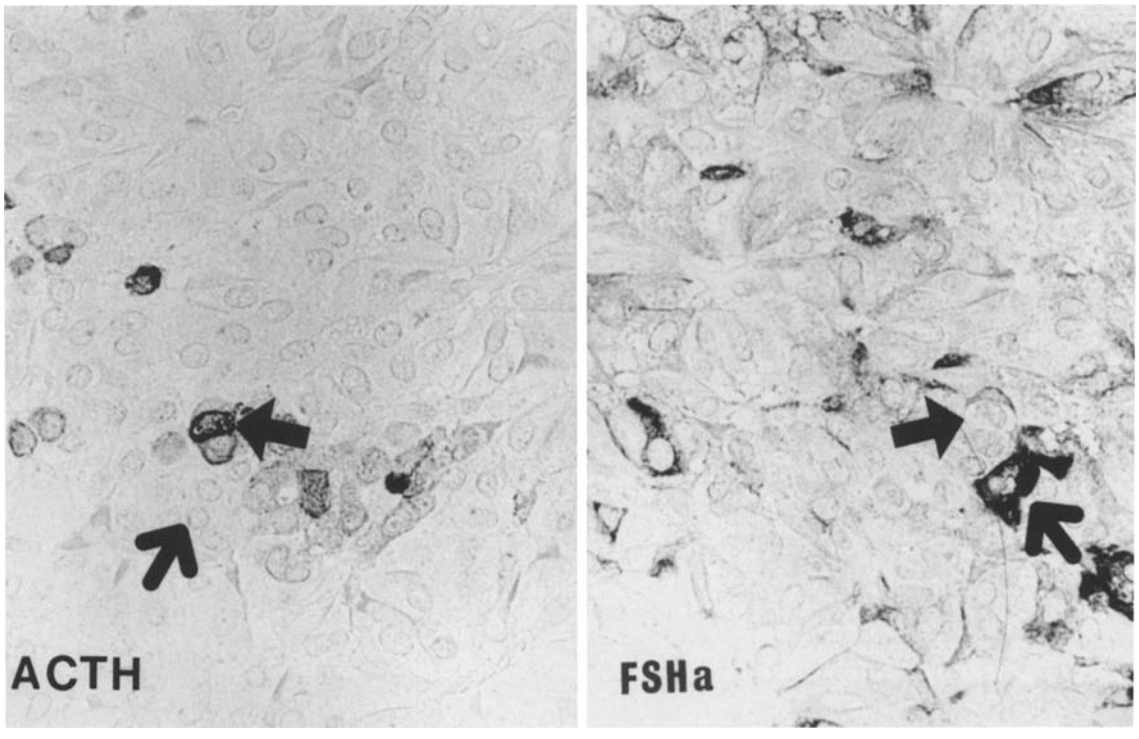

Fig. 7. Case 10. Immunohistochemical localization of α-subunit and ACTH by mirror sections. α-subunit and ACTH were apparently localized in different tumour cells. (*arrow*) ($\times$ 690)

reting pituitary adenomas, the immunohistochemical appearance of LH-β was extremely rare. For some undetermined reasons, neoplastic transformation is not accompanied by gene expression of LHβ, although, in normal pituitary gland, LH-β is colocalized in most FSH-β producing cells (Osamura and Watanabe 1985).

The immunohistochemical appearance of TSH-β was rather high in incidence and, in some tumour cells, TSH-β was colocalized with FSH-α and FSH-β. Simultaneous secretion of FSH and TSH has been reported in the literature (Koide et al. 1982). This combination of colocalization is not usually seen in the normal pituitary gland (personal observations). Therefore, the appearance of TSH-β can be considered as abnormal gene expression of FSH cells following neoplastic transformation.

A few cases showed immunohistochemically positive cells for ACTH and PRL which were noted in the different cells. It may be considered that FSH producing adenomas also possess a potentiality toward multihormonal production. This has been well documented in GH secreting adenomas (Heitz 1979; Osamura and Watanabe 1987).

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