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Detection of growth hormone, prolactin and human β-chorionic gonadotropin messenger RNA in growth-hormone-secreting pituitary adenomas by in situ hybridization*

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Summary. In a series of 39 adenomas from patients with the clinical hyperfunction syndrome of acromegaly and in one from a case of prolactinoma we studied the mRNA expression of growth hormone (GH), prolactin (PRL) and β-human chorionic gonadotropin (HCG) by using the technique of in situ hybridization (ISH). This technique allows the direct identification and localization of cells expressing mRNA and thus synthesizing the respective hormone. The aim of our study was to demonstrate the frequent co-expression of PRL mRNA and HCG mRNA in pituitary adenomas of acromegalic patients. Probes for ISH of the above-mentioned hormones were obtained by subcloning cDNA fragments into pGEM plasmids. Subsequent Sp6-polymerase catalysed in vitro transcription with ^{35}S-CTP revealed radiolabelled single-stranded antisense RNA probes [the probe for βHCG detects β-luteinizing hormone (βLH) simultaneously because of a sequence homology of 90%]. To localize the labelled hybrids, autoradiography was carried out. Light microscopical evaluation of the tissue sections demonstrated positive signals in all cases for GH, in 80% of cases for PRL and in 25% of cases for HCG [LH] mRNA. The comparison of mRNA content shown by ISH with immunocytochemical (ICC) hormone detection revaled that in all cases the detection of GH corresponded to GH mRNA content of the cells. For PRL and HCG [LH] positive mRNA detection (ISH) and negative hormone detection (ICC) occurred in some cases (PRL 17.5%; HCG [LH] 15%). In contrast, negative mRNA detection (ISH) and positive hormone content (ICC) was also demonstrated (PRL 5%; HCG [LH] 37.5%). The remaining adenomas showed both mRNA and the respective hormone, as well as negative ISH and ICC.

Key words: In situ hybridization – Pituitary adenoma – Immunocytochemistry

Introduction

In the surgical pathology of pituitary adenomas immunocytochemical demonstration of hormones is commonly carried out. However, a certain amount of immunoreactive peptide must be present for a positive reaction, which depends on peptide synthesis, secretion, intracellular transport, peptide degradation, and possibly peptide phagocytosis (Höfler 1987). Immunocytochemistry (ICC) only allows the visualization of the amount of cell product present but gives no data on current peptide synthesis. In contrast, the detection of specific hormone-coding messenger RNA (mRNA) by in situ hybridization (ISH) allows the identification and localization of individual cells, which are synthesizing the specific peptide.

In this report we used single-stranded cRNA probes specific for growth hormone (GH), prolactin (PRL) and human chorionic gonadotropin (HCG) [LH] mRNA in the tissue sections. Our aim was to demonstrate the frequent co-secretion and co-expression of PRL in pituitary adenomas of patients suffering from acromegaly as shown in recent studies (Barkan 1989; Lloyd et al. 1989). Other studies have demonstrated the expression of gonadotropins in pituitary adenomas by Northern blotting (Jameson et al. 1986), so we decided to confirm and extend these results using ISH techniques.

Materials and methods

Subcloning was performed using techniques described by Maniatis et al. (1982) and ISH was carried out as described by Fehr et al.

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Table 1. Results of in situ hybridization (ISH), immunocytochemistry (ICC), and clinical data

		ISH					ICC			Serum level	Sex	Age	Tumour size
		+++	++	+	(+)	−	+	++	+++				
		percentages of adenoma cells					percentages of adenoma cells						
	GH	−	30	50	10	10	10	10	10	12.5			
1	PRL	−	−	15	55	30	30	20	10	18.3	m	31	m(P)
	HCG/LH	−	−	−	−	100	−	−	−	−			
	GH	−	−	25	40	35	40	40	−	13.7			
2	PRL	1	1	3	5	90	5	−	−	9.3	m	35	M
	HCG/LH		−	−	−	−	100	−	−	−			
	GH	−	10	40	30	20	20	10	10	9.6			
3	PRL	10	10	20	10	50	−	−	−	12.0	m	45	M
	HCG/LH		−	−	−	−	100	30	−	−			
	GH	−	−	5	80	15	60	10	−	75.5			
4	PRL	−	−	−	−	100	20	10	1	46.8	f	48	M(S)
	HCG/LH		−	−	−	−	100	20	10	−			
	GH	−	−	60	40	−	10	10	−	36.0			
5	PRL	0.5	2.5	5	7	85	−	−	−	*	m	57	M(P)
	HCG/LH	−	−	−	1	99	−	−	−	−			
	GH	−	−	60	40	−	20	30	40	5.8			
6	PRL	1	−	1	−	98	1	1	−	12.4	f	62	M(P)
	HCG/LH	−	−	1	−	99	5	5	5	−			
	GH	−	−	−	15	85	20	30	20	230			
7	PRL	−	−	−	−	100	−	−	−	<2.0	f	37	M(P)
	HCG/LH	−	−	−	−	100	−	−	−	−			
	GH	−	5	80	15	5	10	10	10	21.7			
8	PRL	1	1	4	3	91	10	−	−	*	f	38	M(P)
	HCG/LH	−	−	−	−	100	30	10	−	−			
	GH	−	−	5	95	−	30	30	30	16.9			
9	PRL	1	2	2	1	94	−	1	1	5.6	m	51	M
	HCG/LH	−	−	−	−	100	8	−	−	−			
	GH	−	−	10	80	10	5	5	5	37.3			
10	PRL	1	3	4	7	85	5	5	2	14.4	f	21	M(P)
	HCG/LH	−	−	−	−	100	−	−	−	−			
	GH	−	2	60	38	−	30	30	30	10.0			
11	PRL	1	1	2	1	95	−	−	−	10.5	f	43	M
	HCG/LH	−	−	−	−	100	10	10	10	−			
	GH		−	−	20	80	−	10	20	10	8.1		
12	PRL	1	3	3	3	90	−	−	−	*	m	49	m
	HCG/LH	−	−	−	−	100	−	−	−	−			
	GH	−	−	15	80	5	20	5	−	13.8			
13	PRL	−	−	−	−	100	−	−	−	*	m	55	M(P)
	HCG/LH		−	−	−	−	100	−	−	−			
	GH	−	30	60	5	5	40	10	5	11.6			
14	PRL	1	4	30	55	10	20	10	10	*	m	32	m
	HCG/LH	−	−	−	−	100	−	−	−	−			
	GH	40	30	18	10	2	40	30	20	13.8			
15	PRL	10	20	40	15	15	10	10	10	*	m	43	M(P)
	HCG/LH	−	−	−	−	100	10	5	−	−			
	GH	15	25	50	10	−	30	30	30	7.4			
16	PRL	15	25	35	10	15	10	20	10	12.6	f	34	M
	HCG/LH	−	−	−	−	100	−	−	−	−			
	GH	−	10	10	75	5	40	30	10	96.2			
17	PRL	2	5	4	3	86	20	−	−	*	m	49	M(P)
	HCG/LH	−	−	−	−	100	−	10	−	−			
	GH	−	5	75	10	10	50	20	10	34.0			
18	PRL	30	10	20	10	30	−	−	−	*	m	27	M
	HCG/LH	−	−	1	−	99	−	−	−	−			
	GH	5	15	60	20	−	30	10	5	21.4			
19	PRL	20	40	35	5	−	20	10	10	*	f	47	M
	HCG/LH	−	−	−	−	100	10	−	−	−			
	GH	−	−	50	40	10	30	20	10	11.2			

Table 1 (continued)

		ISH					ICC			Serum level	Sex	Age	Tumour size
		+++	++	+	(+)	−	+	++	+++				
		percentages of adenoma cells					percentages of adenoma cells						
20	PRL	1	–	1	1	97	–	5	–	*	f	48	M
	HCG/LH	–	–	–	–	100	–	–	–	–			
	GH	–	–	5	25	70	10	10	10	11.9			
21	PRL	–	–	–	–	100	–	–	–	12.3	f	34	M
	HCG/LH	–	–	–	–	100	–	–	–	–			
	GH	–	10	90	–	–	30	10	10	8.3			
22	PRL	3	10	20	17	50	–	5	–	15.4	f	54	M
	HCG/LH	–	–	–	–	100	5	3	–	–			
	GH	5	5	15	70	5	50	20	10	150			
23	PRL	1	1	–	–	98	–	–	–	*	f	36	M
	HCG/LH	–	–	–	–	100	60	5	5	–			
	GH	–	–	–	2	98	30	10	10	36.4			
24	PRL	–	–	–	–	100	–	–	–	*	m	54	M
	HCG/LH	–	–	–	–	100	–	–	–	–			
	GH	–	–	–	30	70	50	20	20	26.4			
25	PRL	1	1	3	10	85	5	5	–	*	m	37	M
	HCG/LH	–	–	–	1	99	–	–	–	–			
	GH	–	–	2	90	8	–	10	10	70.2			
26	PRL	–	–	–	–	100	–	–	–	*	f	60	M
	HCG/LH	–	–	–	–	100	–	–	–	–			
	GH	30	20	45	5	–	30	20	10	14.8			
27	PRL	–	–	–	–	100	–	–	–	4.5	m	55	M
	HCG/LH	–	–	–	–	100	–	–	–	–			
	GH	–	–	30	70	–	30	10	10	>75			
28	PRL	–	1	–	–	99	20	10	–	1.1	f	35	M
	HCG/LH	–	–	–	1	99	10	–	–	–			
	GH	–	5	10	85	–	30	40	–	165			
29	PRL	2	2	3	3	90	–	5	1	19.8	f	50	M
	HCG/LH	–	–	–	1	99	20	20	10	–			
	GH	–	5	55	45	–	10	10	10	157.3			
30	PRL	5	20	40	30	5	20	10	5	36.3	f	42	M
	HCG/LH	–	–	–	–	100	–	–	–	–			
	GH	–	15	30	50	5	20	10	10	48.2			
31	PRL	5	40	30	15	10	5	10	5	22.3	f	58	M(P)
	HCG/LH	–	–	–	–	100	10	2	–	–			
	GH	5	15	60	20	–	10	40	40	17.4			
32	PRL	1	1	8	5	85	60	–	–	3.3	m	47	M
	HCG/LH	–	–	1	–	99	5	5	–	–			
	GH	5	10	75	10	–	30	30	20	11.7			
33	PRL	15	25	30	20	10	5	2	–	*	f	53	m
	HCG/LH	–	–	1	–	99	–	–	–	–			
	GH	–	–	80	20	–	20	60	10	37.9			
34	PRL	–	1	1	–	98	–	–	–	*	m	30	M
	HCG/LH	–	–	–	–	100	10	10	80	–			
	GH	10	10	–	80	–	30	30	30	4.1			
35	PRL	15	10	25	10	40	–	10	–	*	m	45	M
	HCG/LH	–	–	–	–	100	20	20	–	–			
	GH	5	15	60	10	10	70	10	–	8.6			
36	PRL	1	2	1	2	95	5	5	–	*	f	54	m
	HCG/LH	–	–	–	–	100	30	20	10	–			
	GH	–	–	5	90	5	20	10	5	88.9			
37	PRL	–	–	–	–	100	10	–	–	*	f	30	M
	HCG/LH	–	–	–	–	100	20	–	–	–			
	GH	–	–	2	88	10	not done			7.7			
38	PRL	–	–	1	1	98	not done			9.3	m	43	m
	HCG/LH	–	–	–	–	100	not done			–			
	GH	–	5	90	5	–	10	10	5	55.1			

Table 1 (continued)

		ISH					ICC			Serum level	Sex	Age	Tumour size
		+++	++	+	(+)	−	+	++	+++				
		percentages of adenoma cells					percentages of adenoma cells						
39	PRL	−	−	5	15	80	−	1	−	2.6	f	46	M(P)
	HCG/LH	−	−	−	−	100	−	−	−	−			
	GH	−	−	2	70	28	5	3	−	59.3			
40	PRL	20	10	50	15	5	30	10	5	292	f	41	M
	HCG/LH	−	−	1	−	99	−	−	−	−			

GH, Growth hormone; PRL, prolactin; HCG, human chroionic gonadotropin; LH, luteinizing hormone
Normal range: GH < 2.5 µl; PRL2–15 µg/l (m); PRL3–26 µg/l (f); PRL2–19 µg/l (f; post-menopause)
Tumour sizes:
m, microadenoma (< 10 mm)
M, macroadenoma (> 10 mm)
(P), patients were preoperatively treated with dopamine agonists; (S), preoperative treatment with somatostatin; *pre-operative serum range not available
+++, Very dense silver grains or high immunocytochemical reactivity; ++, dense silver grains or medium immunocytochemical reactivity; +, low density of silver grains or low immunocytochemical reactivity; (+), very low density of silver grains or very low immunocytochemical reactivity; −, No silver grains or no immunocytochemical reactivity

(1987). During the whole procedure precautions were taken to avoid RNase contamination.

Adenomas from 18 male and 22 female patients aged between 27 and 62 were removed by transnasal microsurgery at the Department of Neurosurgery, University of Hamburg (Dr. Lüdecke). Aliquots of tissues were shock frozen on dry ice immediately after removal and stored at −70° C until they were used for ISH. Other aliquots were fixed in formalin and embedded in paraffin for structural analysis. ICC was performed with the following antibodies: anti-GH 1:500 (Dako, Hamburg, FRG), anti-PRL 1:300 (Dako), anti-LH 1:800 (Dako) and anti-βHCG 1:100 (Dako).

Restriction enzymes, Sp6-RNA polymerase, and bacterial strain *Escherichia coli* DH5α came from BRL (Eggenstein, FRG). Plasmids pXH3C (Talmadge et al. 1984), pGH6 (Goeddel et al. 1979) and pBR322 containing the cDNA encoding PRL (Cooke et al. 1981) were obtained from ATCC (Rockville, Maryland, USA). pGEM vectors were from promega Biotec (Heidelberg, FRG), the transcription kit from Amersham (Braunschweig, FRG). Most chemical equipment was from Merck (Darmstadt, FRG), antibiotics from Hoechst (Frankfurt/M, FRG) and Sigma (Deisenhofen, FRG). X-ray films (Cronex 4 NIF100) came from Du Pont (Bad Homburg, FRG), NTB-3 emulsion from Kodak. α-[^{35}S]-CTP was obtained from Amersham.

E. coli clone pGH6 (Goeddel et al. 1979) containing 590 bp cDNA encoding GH, clone pBR322 (Cooke et al. 1981) containing 960 bp PRL-encoding cDNA and clone pXH3C (Talmadge et al. 1984) bearing 4.3 kb genomic DNA coding for βHCG-5 were grown in ×1776 medium. Plasmid isolation was performed as described previously (Maniatis et al. 1982).

The following restriction fragments were cloned into pGEM vectors: pGH6 *Eco*RI/*Rsa*I fragment of 316 bp, pBR322 *Acc*I/*Xba*I fragment of 248 bp and pXH3C *Sma*I/*Pst*I fragment of 267 bp followed by transformation into *E. coli* DH5α (Hanahan 1983). After plasmid isolation by caesium gradient centrifugation the new clones named pGEMZ-39 (GH), pGEM-38 (PRL) and pGEMZ-37 (βHCG-5) were tested by mini-preparation (Birnboim and Doly 1979) and digestion with *Eco*RI/*Hin*dIII. After sequencing the plasmid inserts (T7 sequencing Kit; Pharmacia, Uppsala, Sweden) and comparison with published sequences the clones were used for probe preparation.

After linearization of the plasmids with *Hin*dIII, labelled probes were obtained by in vitro transcription with Sp6-polymerase and α-[^{35}S]-CTP as a label. The specific activities of the resulting single stranded cRNAs were between 5.7 and 6.3 × 10^8 cpm/µg.

Tissue sections (5–6 µm) were cut on a Leitz 1720 cryostat at −20° C. Sections were mounted on 3-aminopropyltriethoxysilane-coated slides (Rentrop et al. 1986) and stored at −70° C.

ISH was performed as described previously (Fehr et al. 1987; for more details see Uhlig 1991). Briefly, slides were fixed with freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS) for 5 min, washed twice in PBS, dehydrated in 60%, 80%, 95%, 100% ethanol for 5 min each and air dried.

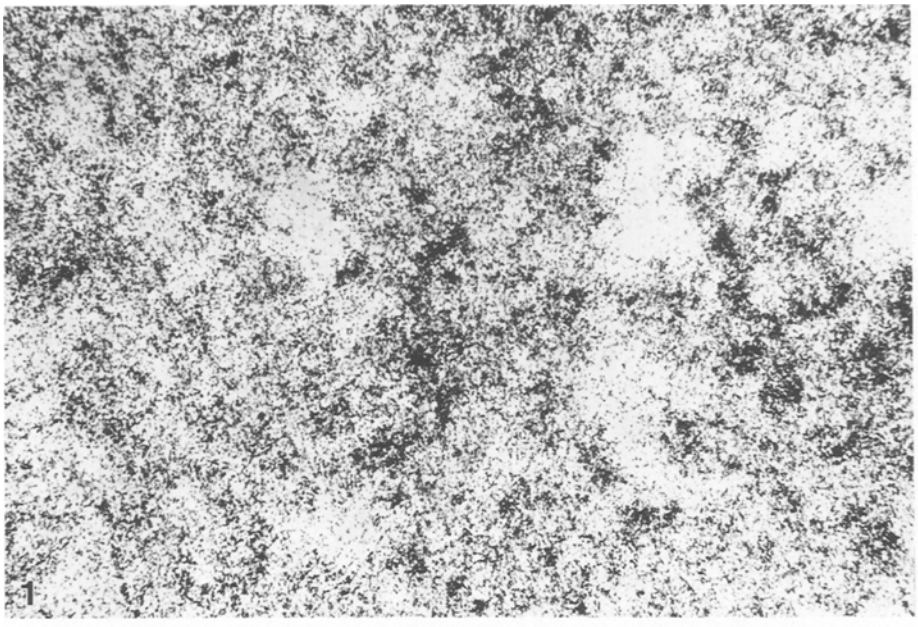
One millilitre of prehydridization buffer containing 50% deionized formamide, 0.025 M EDTA, 0.025 M PIPES pH 6.8, 0.75 M NaCl, 0.2% SDS, 5 × Denhardt's, 10 mM dithiotreitol, 250 µg/ml denatured herring sperm DNA and 250 µg/ml yeast tRNA was applied to each slide. Slides were placed flat into moist chambers lined with 50% formamide-soaked filter paper and incubated for 3 h at 50° C. Prehybridization buffer was drained off and 70 µl hybridization buffer containing 10% dextransulphate and 4 ng probe (1.7 × 10^6 cpm) was applied to the tissues in addition. Sections were covered with coverslips and ISH was carried out in the moist chambers (tape-sealed) for 16 h at 50° C.

Coverslips were removed and slides were washed twice in 4 × SSC containing 20 mM β-mercaptoethanol. Then slides were washed twice (each 5 min) in 4 × SSC and placed into buffer containing 0.5 M NaCl, 10 mM Tris-HCl pH 7.4, 1 mM EDTA and 40 mg/ml RNase A (Boehringer, Mannheim, FRG) and incubated at 37° C for 30 min. Slides were washed in the same buffer without RNase A for 30 min at 37° C and twice in 2 × SSC for 15 min at 50° C. Slides were dehydrated through 60%, 80%, 95%, 100% ethanol and air dried.

Autoradiography was carried out by dipping the slides into Kodak NTB-3 nuclear track emulsion at 42° C and developing the autoradiograms with Kodak chemicals after 5–15 days' exposure at 4° C. Some sections were counterstained with haematoxylin and eosin before they were coverslipped with Entellan.

Results

The major clinical data of the patients included in this report are summarized in Table 1. The tumours were classified as undifferentiated acidophilic pituitary adenomas (cases 1–27), highly differentiated GH-cell adenomas (cases 28–37), large-cell chromophobic pituitary



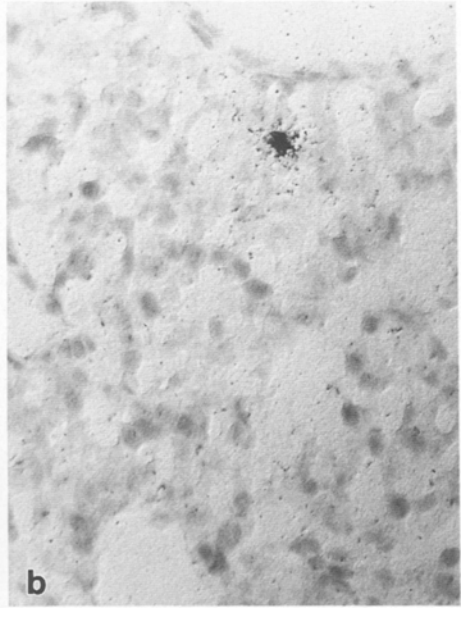


Fig. 1. Undifferentiated acidophilic pituitary adenoma (case 15). Positive cells ranging from (+) to +++. In situ hybridization for GH mRNA (autoradiography). ×225

Fig. 2a. Highly differentiated GH-cell adenoma (case 30).Negative cells, and positive cells ranging from (+) to ++. In situ hybridization for prolactin mRNA (autoradiography). Interference microscopy, ×700. **b** Undifferentiated acidophilic pituitary adenoma (case 23). One positive cell, slight background signals. In situ hybridization for βHCG[LH] mRNA (autoradiography). Interference microscopy, ×450

adenomas (cases 38, 39) and 1 acidophilic PRL-cell adenoma (case 40). All of the 40 patients had the clinical diagnosis of acromegaly with the major symptoms of gigantism whereas only some of them showed hyperprolactinaemia with symptoms like galactorrhoea and amenorrhoea.

ISH of the RNA probes complementary to hGH, hPRL and βHCG [LH] showed positive hybridization signals with the specific mRNA in frozen tissue sections. The specificity of the hybridization probes was established by sequencing the cRNA probes [Sanger et al. 1977; modified by Pharmacia with the T7 sequencing kit (data not shown)] and compared with the published sequences of Goeddel et al. (1979), Cooke et al. (1981), and Talmadge et al. (1984). For microscopical evaluation of the ISH we applied a scale from − to +++. Homogeneous distribution of silver grains over the whole tissue section without any concentration in single cells was designated as −. Positive hybridization exhibited an accumulation of silver grains in single, many, or sometime in all cells. (+) corresponded to very low density of distribution of silver grains in the whole section or parts of it, + to low-, ++ to dense and +++ to very dense distribution of grains.

In all cases we demonstrated positive hybridization signals (silver grains) for GH mRNA. Thirty-two pituitary adenomas (80%) expressed PRL mRNA but only 10 tumours (25%) could be shown to contain gonadotropin mRNA. Table 2 shows the varying densities of the 40 studied tumours: 97.5% of the tumours contained cells with positive hybridization signals for GH with intensity (+), 90% +, 52.5% ++ and 22.5% +++.

Table 2. mRNA content of 40 pituitary adenomas

Intensity of hybridization signals	GH	PRL	βHCG/LH
+++	22.5%	67.5%	0%
++	52.5%	67.5%	0%
+	90%	75%	12.5%
(+)	97.5%	70%	12.5%
−	57.5%	97.5%	100%
Positive cases	40	32	10

Table 3. Correspondence of hormone mRNA (ISH) and hormone content (ICC) in 40 pituitary adenomas

Hormone	mRNA without hormone detection	Hormone detection without mRNA	mRNA and hormone detection
GH	0	0	40 (100%)
PRL	7 (17.5%)	2 (5%)	24 (60%)
HCG/LH	6 (15%)	15 (37.5%)	4 (10%)

Table 4. Combinations of hormone mRNA synthesis in 40 pituitary adenomas

Hormone combination	Number of cases
GH	8 (20%)
GH+PRL	22 (55%)
GH+PRL+HCG/LH	10 (25%)

Thirty-two pituitary adenomas contained PRL mRNA while 70% of these adenomas included cells with intensity (+), 75% +, 67% ++ and 67% +++. Only in 10 tumours (25%) could we detect gonadotropin mRNA. The intensity of these signals was weaker than the others whereas 12.5% of the adenomas contained cells with intensity (+), 12.5% of the positive gonadotropin cases included cells with intensity +. Adenoma cells with intensity ++ and +++ were not observed (Table 2).

The ICC hormone detections revealed that all tumours contained GH, whereas 19 adenomas contained gonadotropin peptides (LH, HCG) and 26 tumours expressed PRL.

The comparison of the ISH and ICC results indicates that all of the 40 pituitary adenomas contain both the GH mRNA and ICC-detectable hormone. For PRL and the gonadotropins we found different results for the two methods: negative ICC and positive ISH [PRL 17.5%, HCG [LH] 15%) and positive ICC and negative ISH (PRL 5%, HCG [LH] 37.5% (summarized in Table 3)].

Analysis of mRNA content revealed that in 8 adenomas GH mRNA alone was detectable. Twenty-two adenomas contained two hormone mRNAs while the remaining 10 tumours showed positive hybridization signals for all three mRNAs (Table 4).

Discussion

In our study we used single stranded antisense RNA probes in contrast to many other investigators who have used oligonucleotides or DNA probes (Penschow et al. 1987; Pixley et al. 1987; Levy and Lightman 1988a, b; Lloyd et al. 1989). The major advantages and disadvantages of oligonucleotide and cRNA probes have been discussed (Grody et al. 1987; Höfler 1987; Lloyd 1987; Wilson and Higgins 1989). Advantages of the radioactive probes obtained by Sp6-polymerase catalysed in vitro transcription are the high specific activity (up to 10^9 cpm/μg) and the possibility of removing all unspecifically bound single-stranded probes by RNase digestion.

We found GH mRNA in all 40 adenomas from patients with acromegaly and in 80% PRL mRNA. The immunocytochemical GH findings showed 100% correspondence with the respective mRNA detection. However, for PRL we observed discrepancies between hormone and mRNA detection. Seven tumours did contain mRNA but no ICC-detectable hormone. An explanation for this observation could be that the hormone was secreted immediately after synthesis and not stored, or there were only small amounts of the hormone synthesized because of possible disturbances on the translational level (Lloyd et al. 1989). Technical artefacts like alteration or loss of antigenicity of the hormones during paraffin embedding could not be excluded.

In contrast, where there was positive ICC and negative mRNA detection it is reasonable to assume that the mRNA synthesis in these cells was suppressed by intracellular hormone storage. Destruction of mRNA by endogenous ribonucleases during surgery could also explain the lack of detectable mRNA in some tumours.

Elevated PRL serum levels are found in 30–40% of the patients with acromegaly (Kanie et al. 1983; Scheithauer et al. 1986; Barkan 1989; Lloyd et al. 1989; Melmed 1990). All patients in our study had elevated GH serum levels. Our findings strongly support the hypothesis that these serum levels result from hormone synthesis in the tumours, but we could not prove a direct correlation between mRNA content in the tissue sections and serum levels. A correlation between serum level and mRNA content for PRL could not be shown because in many cases pre-operative PRL serum analyses have not been done.

Lloyd and his colleagues (1989) examined 10 prolactinomas, 16 GH-secreting adenomas and 4 normal pituitaries with ISH and ICC. They demonstrated PRL mRNA in more than 80% of the cases with GH overproduction and also found some discrepancies between hormone and mRNA detection. The recent detection of bipotential secretory cells in fetal human pituitary tissues which produce GH and PRL (Mulcahy and Jaffe 1988), and the frequent co-secretion of PRL in GH-secreting adenomas, allowed Lloyd and colleagues (1989) to pro-

pose a common phylogenetic and ontogenetic development for these cells.

For some years gonadotropin-secreting adenomas have been thought to be rare variants of pituitary tumours (Miura et al. 1985). However, increasingly pituitary tumours which secrete glycoprotein hormones (luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone) and uncombined α- and/or β-subunits have been observed (Snyder et al. 1984; Klibanski et al. 1987, 1989). Jameson et al. (1986) described the βHCG mRNA expression in an α-subunit secreting pituitary adenoma. Because of the high degree of nucleic acid homology shared by βHCG and βLH [90% (Fiddes and Talmadge 1984)], βHCG probes hybridize to both hormone mRNAs. In our study we used a βHCG cRNA probe with a sequence homology of 90% to βLH. This strong molecular similarity made it impossible to distinguish between positive βLH and βHCG signals during microscopical evaluation of the autoradiography. For this reason we discuss the gonadotropins LH and HCG together.

In our study we found only 10 tumours showing a weak hybridization to βHCG [LH] mRNA, but in 19 the hormone could be detected by ICC. Perhaps in these cases the hormone is defective or the subunits are incorrectly combined, resulting in blocked secretion. Thus the accumulated hormone may suppress mRNA synthesis in these cells.

Simultaneous occurrence of glycopeptide hormones besides uncombined α- and β-subunits has been described (Snyder et al. 1984). The observation of frequent gonadotropin production in clinically silent pituitary adenomas (Snyder et al. 1984; Miura et al. 1985; Jameson et al. 1987; Klibanski et al. 1989) could result from the synthesis of these free subunits which evoke no clinical symptoms.

Our findings indicate that ISH techniques may be powerful tools for the pathologist to examine pituitary adenomas. The advantage over other forms of mRNA detection like Northern blots is the fact that interesting cells can be directly identified and characterized morphologically on the light mciroscopical level. Clinical and immunopathological observations in ISH may be helpful in establishing the diagnosis of tumour types.

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