

Characterization of human pituitary adenomas in cell cultures by light and electron microscopic morphology and immunolabeling

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Abstract: The morphology and hormone production of pituitary adenoma cell cultures were compared in order to highlight their characteristic *in vitro* features. Cell suspensions were prepared from 494 surgical specimens. The 319 viable monolayer cultures were analyzed in detail by light microscopy and immunocytochemistry within two weeks of cultivation. Some cultures were further characterized by scanning, transmission and immunogold electron microscopy. The viability and detailed *in vitro* morphology of adenoma cells were found to be characteristic for the various types of pituitary tumors. The sparsely granulated growth hormone, the corticotroph and the acidophil stem cell adenomas provided the highest ratio of viable cultures. Occasionally, prolonged maintenance of cells resulted in long-term cultures. Furthermore, a variety of particular distributions of different hormone-containing granules were found in several cases. Both light microscopic and ultrastructural analyses proved that the primary cultures of adenoma cells retain their physiological features during *in vitro* cultivations. Our *in vitro* findings correlated with the routine histopathological examination. These results prove that monolayer cultures of pituitary adenoma cells can contribute to the correct diagnosis and are valid model systems for various oncological and neuroendocrinological studies.

Key words: Pituitary adenoma - Hormone - Immunolabeling - Electron microscopy - Cell culture

Introduction

Correct diagnosis of the different types of human pituitary tumors is one of the most important tasks for neuroendocrine pathology. The introduction of immunocytochemistry and electron microscopy laid a new effective basis for the classification of the pituitary adenomas synthesizing and secreting different hormones [14, 18, 20]. The pathophysiological investigation of the pituitary gland is limited by its anatomical inaccessibility, the variety of cell types and hormones and its complex connections with the target organs through the circulation. Cell and tissue cultures developed into widespread model systems in neuropathological and neurooncological research [11, 23, 29]. The short-term cell cultures of pituitary adenomas proved to be an important tool for the development of

drugs and hormone therapies aimed at the control of tumor growth [1, 2, 10].

We investigated the morphology and *in vitro* behavior of human pituitary adenoma cell cultures in order to support and complement the neuropathological diagnosis and to establish a suitable model system for testing different hormones and potential drugs for the treatment of various pituitary adenomas. The cell cultures were established from tissue specimens obtained during microscope-controlled transnasal-sphenoidal operations in our institute.

In this paper we present the morphological study of 319 human pituitary adenoma cell cultures including 139 cases where both tissue sections and cell cultures have been described in detail by immunocytochemistry. Certain cell cultures were further investigated by electron microscopy and immunoelectron microscopy. The tumor-type dependent characteristic morphology of monolayer cultures and some unique morphological features are also presented.

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Materials and methods

Tissues. Human pituitary adenoma tissue was surgically removed by transsphenoidal route from 494 patients (Table 1). For light microscopy, the tissue was fixed in phosphate-buffered (0.1 M, pH 7.2) 4% formaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin and PAS. For immunocytochemistry, formalin-fixed tissue blocks embedded in paraplast were used. For the localization of different hormones, rabbit anti-human prolactin (PRL 1:800), growth hormone (GH 1:800), adrenocorticotrophic hormone (ACTH 1:500), thyroid-stimulating hormone (TSH beta 1:800), luteinizing hormone (LH beta 1:1000) and follicle stimulating hormone (FSH beta 1:1000) donated by Dr. A. F. Parlow (National Pituitary Agency, Baltimore, USA) were employed. For electron microscopy, tumor tissue was fixed in 4% glutaraldehyde in 0.2 M phosphate buffer at 4°C, washed in the same buffer, postfixed in 2% osmium tetroxide in phosphate buffer, dehydrated in graded ethanol and embedded in Durcupan ACM (Fluka, Switzerland). Ultrathin sections were stained with uranyl acetate and lead citrate and investigated with a JEM 100 B electron microscope.

Cell cultures. Monolayer cultures were prepared essentially as described in the previous report [13]. The tumor fragments were suspended in a culture medium containing 80% TC Medium 199, 20% fetal calf serum, 40 µg/ml gentamycin and explanted into Leighton tubes with glass coverslips and into 30 mm plastic Petri dishes. All media and supplements were obtained from GibcoBRL, Germany. The cultures were incubated at 37°C in 5% CO₂-containing humidified atmosphere. The culture medium was changed every other day. After the adherence and growth, cells were fixed with methanol and stained using the May-Grünwald-Giemsa technique at various time points during the cultivation in order to register the growth pattern of the monolayer. The rapidly growing cultures were subcultivated by trypsin (0.25 wt/vol% in balanced salt solution). The cells were harvested, resuspended in culture medium and seeded into plastic tissue culture flasks (Greiner). Cell growth was controlled continuously and representative samples were fixed and stained or subcultured.

For light-microscopical immunocytochemistry, we used picric acid-formaldehyde fixed monolayer cultures. The antibodies were the same that we employed in the tissue sections of tumors. The cells grown in the Leighton tube were subjected to the individual or simultaneous localization of GH and PRL. In this order, a monoclonal mouse antibody against human GH and a polyclonal rabbit antibody against PRL (BioGenex) were used in combination with the peroxidase-antiperoxidase (PAP) and the avidin-biotin-alkaline phosphatase complex (ABC-AP) techniques, respectively.

For scanning electron microscopy, the cells attached to the glass coverslips were rinsed with several changes of TC Medium 199 and fixed in 2.5% glutaraldehyde buffered to pH 7.4 with 0.1 M cacodylate for 1 h. The cultures were dehydrated in a series of graded ethanol and amyl acetate and were dried at the critical point of carbon dioxide. The coverslips supporting the cells were coated with gold (20 nm) and viewed in a Jeol Temscan-100 C electron microscope [9].

For electron-microscopic morphological and immunocytochemical studies, the cultures were fixed in the culture dish with 4% formaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 2 h at room temperature and washed in the buffer overnight. For structural examination only, the cultures were postfixed with 1% osmium tetroxide. After dehydration with graded ethanol and with hydroxypropyl metacrylate (HPMA) as intermediary solvent, all the cultures were embedded in Epon 812 on the bottom of the plastic Petri dishes. The ultrathin sections were stained with uranyl acetate and lead citrate.

Fine-structural immunocytochemistry was carried out on ultrathin section of non-osmicated, Epon-embedded cultures. For the simultaneous localization of GH and PRL, the sections were mounted on nickel grids free of supporting film. On one side incubations were carried out with anti-GH antibody followed by a 15 nm gold

probe coated with anti-rabbit IgG, on the other side with anti-PRL followed by a 30 nm IgG-gold probe, using the principle of Bendayan [7]. In order to check the reliability of the combination, the labels were assigned to the antibodies in the opposite order. A light uranyl acetate and lead citrate staining was applied.

Results

Over a ten year period, 494 human pituitary adenoma cell cultures were prepared of which 319 resulted in adherent cell culture suitable for microscopic analysis. The light microscopic histopathological diagnosis of tissue sections and the number of successful monolayer cultures are shown in Table 1. The neuropathological diagnosis was further enhanced by immunocytochemistry and electron microscopy in 139 cases. Table 2 presents the success rate of cell culture in these well-documented cases. The short-term cultures presented in this study were examined within two weeks after the operation.

The highest rate of cell attachment and migration was observed in the cases of sparsely granulated GH cell adenomas, corticotroph cell adenomas and acidophil stem cell adenomas. In contrast, small individual cells and cell groups of sparsely granulated PRL cell adenomas and hormonally inactive adenomas attached rather slowly or not at all and the cell migration was limited. In eleven cases, long-term cultures could be maintained from various adenoma types (Table 3). Long life span or high subcultivation number could be achieved frequently from some sparsely granulated GH adenomas and acidophil stem cell adenomas.

On the basis of our morphological, immunocytochemical and ultrastructural investigations on monolayer cultures, characteristic features had been identified by which the different types of pituitary adenomas were characterized. Further on, we summarize the *in vitro* behavior and characteristics of the various human pituitary adenomas.

Sparsely granulated PRL cell adenoma (SPRL)

The mechanical dispersion of the specimen resulted in a suspension consisting of small, round individual cells and groups. The cell attachment was slow, there was no significant attachment in about 50% of the cases. The cultures consisted of some individual small round cells and slightly spread cell groups. Occasionally, small cells with narrow processes migrated from the edge of the groups (Fig. 1, SPRL). The intensity of PRL reaction was stronger in the round cells than in the small cells with narrow processes (Fig. 2, SPRL-PRL). The surface of the round epithelial cells had numerous blebs and wrinkles. The well spread cells and their processes did not show such surface structures (Fig. 3, SPRL). Ultrastructurally, irregular nuclei with prominent nucleoli, abundant parallel arrays of the rough endoplasmic reticulum, extensively developed Golgi apparatus, small

Table 1. Viability of various pituitary adenomas in culture

Histopathology (H&E staining)	No. of cell cultures	No. of viable cultures	Ratio of viable cultures
Chromophobic adenoma	329	188	57%
Acidophilic adenoma	52	45	87%
Basophilic adenoma	36	20	56%
Mixed (chromophobic+acidophilic)	77	66	86%
Total	494	319	65 %

Table 2. Classification of pituitary adenomas based on hormone content and ultrastructure

Classification	No. of cell cultures established	No. of viable cultures	Ratio of viable cultures
Sparsely granulated PRL cell adenoma	22	11	50%
Sparsely granulated GH cell adenoma	21	21	100%
Densely granulated GH cell adenoma	17	15	88%
Mixed GH-PRL cell adenoma	10	8	80%
Acidophil stem cell adenoma	6	5	83%
Mammotroph cell adenoma	1	1	n.a.
Corticotroph cell adenoma	19	18	95%
Thyrotroph cell adenoma	5	2	40%
Null cell adenoma	32	17	53%
Oncocytoma	6	4	66%
Total	139	102	74%

spherical, pleomorphic secretory granules (125-300 nm) and misplaced exocytosis were observed (Fig. 4, SPRL). The Neberkern structure characteristic of SPRL cells is shown in Fig. 4, DGHSPRL.

Sparsely granulated GH cell adenoma (SGH)

In general, the cell suspension consisted of round cells with different size which attached individually within 24 hours. These adenomas were very active in growing, 100% of the cases resulted in cell culture. The majority of the cells had a spindle shape, only some of them remained round. Multinucleated cells with 2 to 8 nuclei were often present (Fig. 1, SGH). A strong, diffuse immunostaining was observed in the cytoplasm. Besides growth hormone (GH), a low-level prolactin production

Table 3. Lifespan of all the eleven long-term pituitary adenoma cultures

Case	Tumor type	No. of subcultivations	Life-span in days
1	Sparsely granulated GH cell adenoma	1	34
2	Sparsely granulated GH cell adenoma	2	68
3	Sparsely granulated GH cell adenoma	3	105
4	Sparsely granulated GH cell adenoma	10	216
5	Sparsely granulated GH cell adenoma	9	201
6	Densely granulated GH cell adenoma	20	347
7	Acidophil stem cell adenoma	4	128
8	Acidophil stem cell adenoma	53	656
9	Acidophil stem cell adenoma	2	74
10	Thyrotroph cell adenoma	6	142
11	Null cell adenoma	5	169

could also be detected (Fig. 2, SGH-GH, SGH-PRL). Ultrastructurally, the cell surface pattern was not uniform. There were round and spindle-shaped cells with numerous protrusions, wrinkles, blebs and microvilli but there were spindle-shaped cells with smooth surface, too (Fig. 3, SGH). The transmission electron microscopy showed pleomorphic nuclei and conspicuous nucleoli. Randomly dispersed RER profiles and prominent Golgi complexes could be identified in the well-developed cytoplasm. The secretory granules were small (100-250 nm) and sparse, they were localized along the plasma membrane (Fig. 4, SGH). Characteristic ultrastructural features were the fibrous bodies composed of type II microfilaments and smooth-walled tubules (data not shown).

Densely granulated GH cell adenoma (DGH)

Larger individual cells and small groups of 8 to 20 big round cells were seen in the suspension. The cell attachment occurred within 48 hours, 88% of the cases resulted in viable cell cultures. Both the polygonal and the elongated cells were closely connected with each other without cytoplasmic processes and formed epithelial colonies (Fig. 1, DGH). The GH immunostaining was generally strong in the epithelial groups, but moderate PRL immunoreactivity was also observed in some cells (Fig. 2, DGH-GH, DGH-PRL). Numerous wrinkles and blebs were present on the surface of the cells (Fig. 3, DGH). Round nucleus, well-developed RER, prominent Golgi apparatus containing immature developing granu-

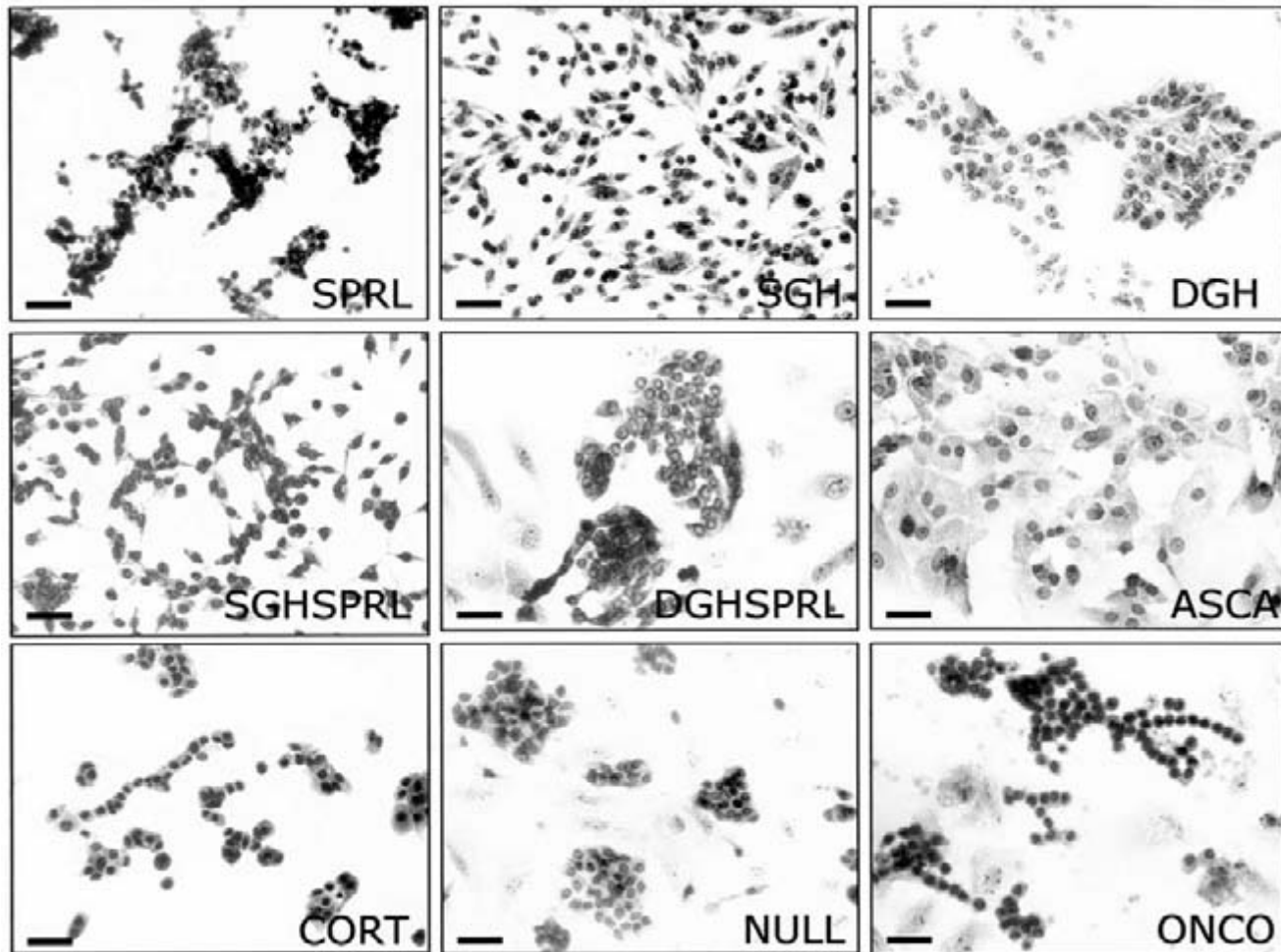


Fig. 1. May-Grünwald-Giemsa staining of pituitary adenoma cell cultures grown for 6 to 10 days shows *in vitro* morphology characteristic for the cell types. Note the slightly spread cell groups with processes in SPRL. Spindle shaped, often multinucleated cells form the SGH and SGHSPRL cultures. The DGH and DGHSPRL cultures consist of epithelial colonies of polygonal cells. Note the large polygonal cells with abundant cytoplasm in ASCA cultures. Peculiar rows of angular or round cells are present in CORT and ONCO cultures, respectively. NULL cultures contain small polygonal cells in epithelial groups. Bar = 20 μ m.

les, elongated cristate mitochondria, and numerous electron-dense secretory granules with variable diameter (600 nm or more) were observed in the cytoplasm (Fig. 4, DGH). Exocytosis was occasionally observed.

Mixed GH-PRL cell adenoma (DGHSPRL, SGHSPRL)

Eight out of ten adenomas provided viable cell cultures. Two different types had been distinguished in this group of bimorphous and bihormonal adenomas; the sparsely granulated PRL cells can be mixed with either sparsely or densely granulated GH cells. In DGHSPRL, the cell suspension contained individual cells with variable size and larger groups of cells. The polygonal cells adhered close to each other and formed epithelial islets. At the edge of the colonies, big pale cells with irregular cytoplasm were present (Fig. 1, DGHSPRL). Immunocytochemistry revealed the presence of both GH and PRL

in the cultured cells. The double immunostaining showed separate GH- and PRL-positive cells (brown and violet colors), however, many cells displayed the two colors simultaneously (Fig. 2, DGHSPRL-MIX). Numerous blebs and wrinkles could be seen on the surface of the epithelial cells by SEM (Fig. 3, DGHSPRL). TEM could distinguish the two distinct cell types based on the features mentioned earlier such as the Neberkern formation (Fig. 4, DGHSPRL).

The other group of the mixed adenomas with the sparsely granulated GH and sparsely granulated PRL cells provided cell suspensions of small groups of 5 to 6 cells. The attachment of the cells was rapid and a loose monolayer was formed. The majority of the cells was elongated with one or two narrow processes. Some round or spindle-shaped cells with no processes were also present (Fig. 1, SGHSPRL). Two-thirds of the cells showed GH immunoreactivity and about one-third was PRL-positive. The double immunostaining for GH and

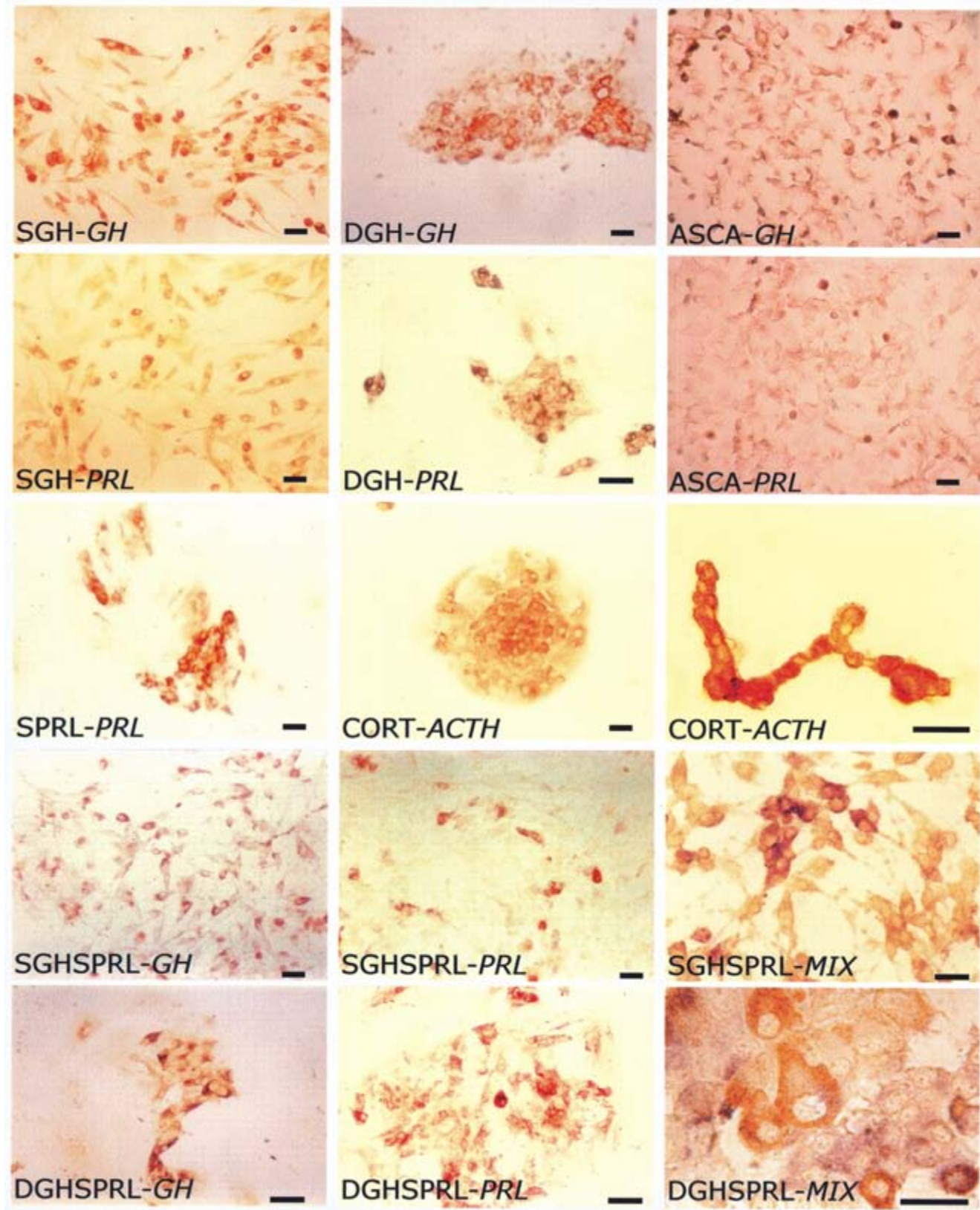


Fig. 2. Hormone content of 7-day pituitary adenoma cell cultures revealed by immunocytochemistry. Note high GH content and the presence of moderate prolactin staining in the SGH, DGH and ASCA cases. High PRL and ACTH levels were observed in SPRL and CORT cells, respectively. In the double stained cultures (MIX), brown (DAB) and red (Fast red) colors show the presence of GH and PRL, respectively. Note the bihormonal cells present in DGHSPRL-MIX. Bar = 20 μ m.

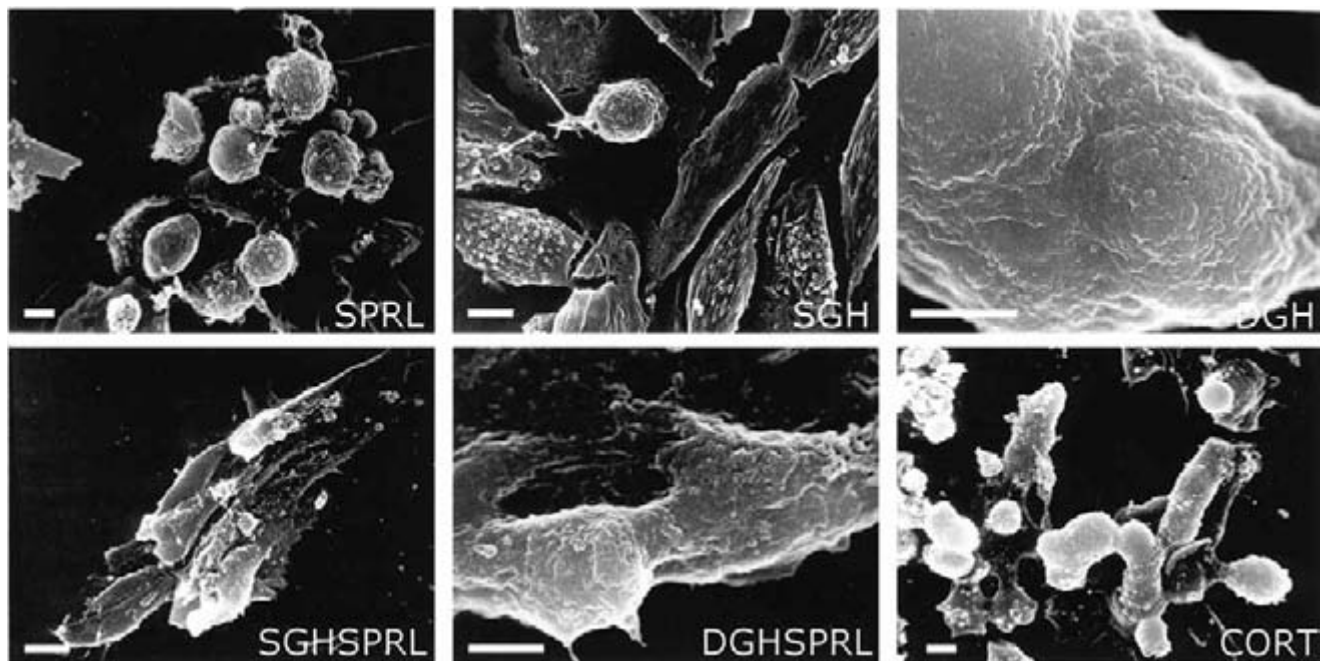


Fig. 3. Surface topography of various adenoma cells by SEM after 7 days in culture. Note the variations in the presence of blebs, wrinkles and microvilli on the cell surface. The cell rows are present in the CORT culture. Bar = 10 μ m.

PRL showed no signs of double-labeled cells (Fig. 2, SGHSPRL-MIX). The elongated cells showed by SEM smoother surface than the DGHSPRL cells (Fig. 3, SGHSPRL). The ultrastructure of the cells was similar to that of the monomorphous cell types (Fig. 4, SGHSPRL).

Acidophil stem cell adenoma (ASCA)

After the mechanical dispersion the cell suspension consisted of large round individual cells and small cell groups. The cell attachment was rather quick within 24 hours. The individual cells and islets began to spread and formed tight monolayers by the end of the first week. The polygonal cells in the colony were closely attached to each other without bearing processes (Fig. 1, ASCA). Out of the six cases, five short-term cultures could be obtained, of which three resulted in long-term cultures, one of them with a life span of over 600 days (Table 3). GH and PRL positivity were present even in the cells after several subcultivations (Fig. 2, ASCA-GH and ASCA-PRL). The TEM analysis showed monomorphous, immature adenoma cells with poorly developed cytoplasmic structures and a number of microvilli. Few small secretory granules (150-300 nm) and mitochondrial abnormalities, often changes typical of oncocyctic adenoma cells, could be observed (Fig. 4, ASCA).

Corticotroph cell adenoma (CORT)

Midsized individual cells and some small cell groups were mainly present in the cell suspension. Eighteen out

of 19 corticotroph cell adenomas gave rise to short-term cultures. A peculiar growth pattern has developed after the initial attachment and spreading: angular cells formed long rows of 10 up to 20 cells. Occasionally, small colonies of a dozen or so tightly attached cells could also be observed together with the cell rows (Fig. 1, CORT). Both the colony- and the row-forming cells displayed strong ACTH immunoreactivity (Fig. 2, CORT-ACTH). The scanning electron micrograph (Fig. 3, CORT) shows that the surface of the angular cells forming the typical cell rows contains several blebs and wrinkles, in contrast to the colony-forming counterparts. The angular cells with oval nuclei, well-developed rough endoplasmic reticulum, numerous free ribosomes, prominent Golgi complex, type 1 microfilaments and abundant spherical or irregular secretory granules (250-700 nm) were observed in the TEM images (Fig. 4, CORT). The eighteen cases of corticotroph cell adenoma could be grouped into densely and sparsely granulated variants. However, this variation did not correspond to any clinical feature.

Null cell adenoma (NULL)

The hormonally inactive pituitary adenomas provided viable cell cultures in about 50% of the cases. Two distinct adenoma types, the null cell adenomas and oncocyctomas had been found in this series of cultures.

In case of null cell adenoma, large multilayered clumps of cells were present in the cell suspension. The attachment was slow, floating cell groups were present in the culture medium even after 48 hours. The surface-

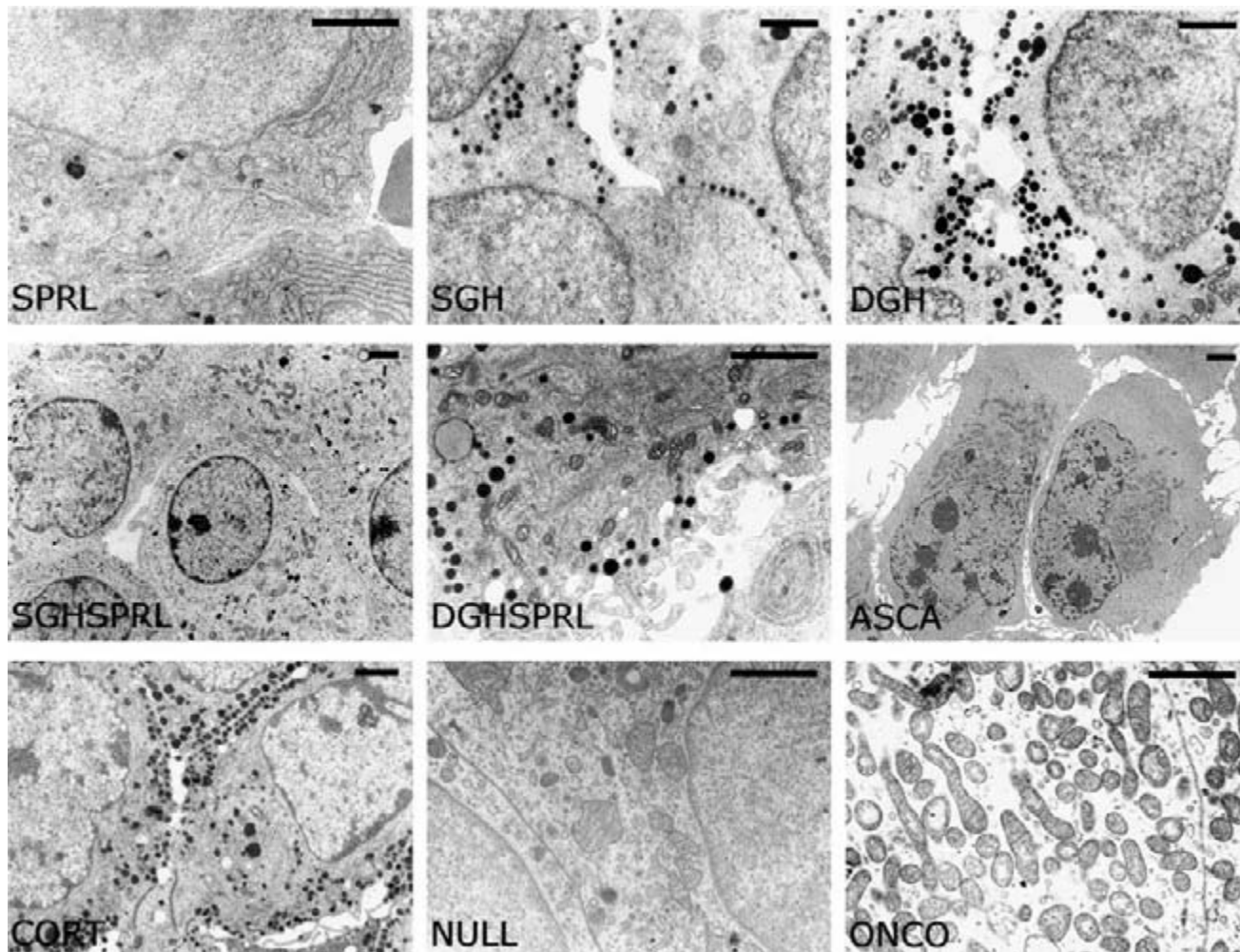


Fig. 4. Ultrastructural morphology of adenoma cells by TEM after one week of cultivation. Note abundant parallel cisternae of rough endoplasmatic reticulum in SPRL. Small uniform granules are sparsely distributed along the cell membrane in SGH. In contrast, numerous larger granules with variable diameter were found in DGH. On the DGHSPRL image a part of an SPRL cell with the Nebenkern formation of RER can be seen in the right lower corner, while the other cell presented displays a number of large granules characteristic of DGH. Immature adenoma cells with poorly developed cytoplasmic structure and microvilli are present in ASCA. Note the active, organelle- and granule-rich cytoplasm in CORT cells. NULL cells have abnormal mitochondria and a very limited number of granules. Abundant irregular mitochondria can be found in oncocytoma cells. Bar = 2 μ m.

adhered cell groups began to spread and small polygonal cells with a narrow ring of cytoplasm formed tight colonies (Fig. 1, NULL). Despite the limited attachment and spreading in general, cultures of polymorphous cells occasionally provided large monolayer colonies. One long-term culture could also be obtained from this high activity variety of null cell adenomas (Table 3). The TEM image (Fig. 4, NULL) shows cells with irregular nuclei, poorly developed cytoplasmic organelles and some small secretory granules (200-250 nm).

Oncocytoma (ONCO)

Small individual round cells were present in the cell suspension. The cells attached strongly to the surface and predominantly formed rows, often with a branching tendency (Fig. 1, ONCO). The ultrastructural analysis

showed poorly developed cytoplasmic organelles and the abundance of characteristic irregular mitochondria (Fig. 4, ONCO).

Electron microscopic double immunolabeling of GH and PRL

The pathologically monomorphous and monohormonal somatotroph adenomas are known to contain a certain amount of prolactin. In order to describe the distribution of PRL and GH within the adenoma cells and their individual secretory granules, double gold immunolabeling was performed in eight cases (Table 4).

Three cases of acromegaly including two densely and one sparsely granulated GH adenomas were also studied. The double gold labeling in these three monomorphous cell cultures revealed the presence of PRL,

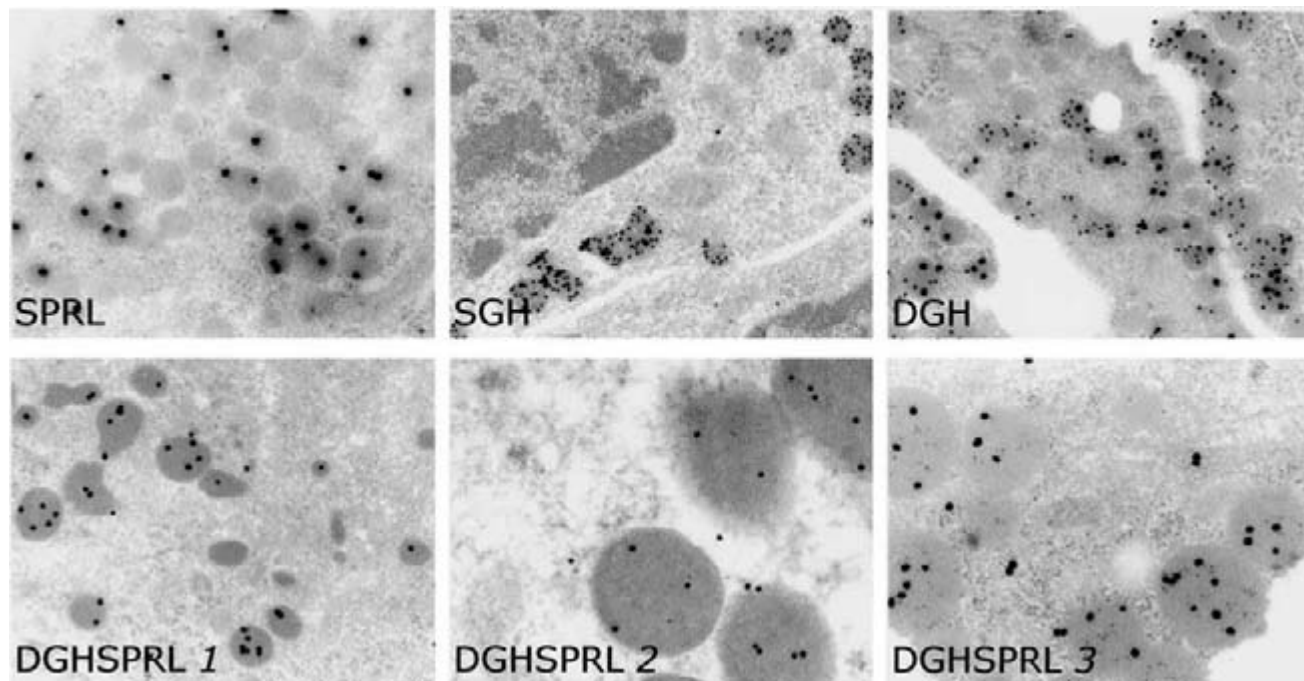


Fig. 5. Immunogold labeling of hormone-containing granules. Note the abundance of prolactin in the SPRL cells and a moderate occurrence of prolactin in the granules of SGH and DGH cells (PRL: 30 nm, GH: 15 nm). However, in mixed adenoma cultures both monohormonal (DGHSPRL 1 (PRL: 30 nm) and 2 (GH: 15 nm)) and bihormonal (DGHSPRL 3, PRL: 30 nm, GH: 5 nm) cells can be present. $\times 50\,000$.

besides GH, within the same secretory granules (Fig 5, SGH, DGH). These three somatotrophic adenomas diagnosed as monomorphous and monohormonal proved to be bihormonal by this ultrastructural analysis.

The cells of the three bimorphous and bihormonal mixed adenomas included in this experiment displayed a variety of GH/PRL colocalization patterns (Table 4). In the case of gigantism (Case 4), both monohormonal and bihormonal PRL and GH cells were observed. Granules from monohormonal PRL and GH cells can be

seen in Fig. 5 (DGHSPRL 1 and DGHSPRL 2, respectively). A PRL cell where the two hormone colocalized is also shown (DGHSPRL 3). In Case 5, only monohormonal cells were found expressing only GH or PRL, in accordance to their morphological appearance. In Case 6, only GH cells contained bihormonal granules, while the PRL cells contained only prolactin.

The two monohormonal and monomorphous prolactinomas involved did not show the presence of GH (Fig 5, SPRL), in agreement with the immunocytochemical

Table 4. Characterization of cellular composition and secretory granule content by double immunolabeling of pituitary adenomas

Case	Age / Gender	Clinical manifestation	Morphology of culture	Cellular composition by EM	Hormone content in secretory granules
1	59 / m	acromegaly	polygonal cells in groups	densely gran. GH cells	GH+PRL
2	25 / f	acromegaly	polygonal cells in groups	densely gran. GH cells	GH+PRL
3	33 / f	acromegaly	loose monolayer of round and elongated cells	sparsely gran. GH cells	GH+PRL
4	10 / f	gigantism	polygonal cells in groups	densely gran. GH cells sparsely gran. PRL cells	GH; GH+PRL PRL; PRL+GH
5	35 / f	acromegaly	polygonal cells in groups	densely gran. GH cells sparsely gran. PRL cells	GH PRL
6	35 / m	acromegaly	loose monolayer of elongated cells with processes	sparsely. gran. GH cells sparsely gran. PRL cells	GH; GH+PRL PRL
7	18 / f	amenorrhoea	loose monolayer of elongated cells with processes	sparsely gran. PRL cells	PRL
8	38 / f	amenorrhoea galactorrhoea	loose monolayer of elongated cells with processes	sparsely gran. PRL cells	PRL

analysis of 11 monohormonal prolactinoma cell cultures.

Discussion

The routine cell culture of 494 human pituitary adenomas provided the opportunity to observe the cellular composition and attachment dynamics of the cell suspension obtained by mechanical dispersion of the tumor specimens. Sixty five percent of them resulted in viable adherent cell cultures. Previous studies showed that the cell cultures initiated from a cell suspension proved to be more viable than the ones obtained from tissue culture of one cubic mm pieces of the tumor specimen [13]. The *in vitro* behavior and morphology of these cell cultures was documented by following the spreading and migration of the cells as well as by MGG staining of methanol-fixed cultures.

Comparative morphology of pituitary adenoma cell cultures

Since previous comparative morphological analysis of pituitary adenoma cell cultures is rather limited [27], we compared our light microscopic observations of 319 monolayer cultures with the histopathological analysis of the various pituitary adenomas in order to describe their unique *in vitro* features. However, the tinctorial typing does not provide clinically valuable information in many cases. In general, acidophilic adenomas form rather epithelial groups of cells (Fig. 1, DGH), whereas the peculiar rows of cuboidal cells were characteristic of basophilic adenomas (Fig. 1, CORT). In contrast, chromophobic and mixed adenomas provided cell cultures showing a wide variety of different morphologies. The detailed analysis of that diverse group of chromophobic adenomas showed that their unique *in vitro* features could provide a basis for distinguishing the various types of those tumors.

The detailed immunocytochemical, scanning and transmission electron microscopic analyses of 139 cell cultures provided more insight into the *in vitro* behavior, hormone production and morphological features of various pituitary adenomas. In our experience, the hormonally active adenomas - especially the sparsely granulated GH, the acidophil stem cell and the corticotroph types - resulted in viable cell cultures more often than the hormonally non-active varieties. According to our observations, the morphology of mixed adenomas mainly reflects the features originating from the densely or sparsely granulated GH-producing elements, namely the formation of closed epithelial groups or of loose network of individual cells, respectively. Further investigation is necessary to confirm whether the blebbing and wrinkled surface of the spindle-shaped adenoma cells reflects a higher hormone secretory activity com-

pared to the cells with smooth surface [9]. Clinically, the sparsely granulated GH adenomas seem to have a higher growth dynamics and higher recurrence rate [19]. *In vitro* these cells were less differentiated than the densely granulated variety and also had a higher growth rate in cell culture. The corticotroph adenomas showed the peculiar morphology of chains of cuboidal cells. Similar pattern with even more branching was observed in some oncocyctic adenoma cell cultures. The detailed morphological investigation of pituitary adenoma monolayer cultures revealed that the *in vitro* observations can identify various adenoma types and can complement and support the classical histopathological diagnoses based on tissue sections. Further investigations are necessary to discuss whether some of these morphological features observed in short-term monolayer cultures might have some prognostic value.

Distribution of various hormone-containing secretory granules in cell cultures

The question of clonality in the pathogenesis of the pituitary adenomas is still begging to be answered [24, 28]. The hormone secretion had been considered as an important marker of the origin of cells. Previous studies on tissue sections indicated that in several cases the morphological and hormonal heterogeneity do not overlap [22]: in about half of the monomorphous prolactinomas expression of other hormones (GH, ACTH, FSH) was also detected [24]. It has also been proved that one secretory granule might contain both GH and PRL in certain cases [5, 6, 15, 30]. Accordingly, we performed double immunogold labeling in eight short-term cultures (Table 4). Both the sparsely and densely granulated GH adenoma cells contained prolactin as well. The cultures of mixed adenomas displayed a variety of different granules and cells: in a case of gigantism, both morphologically GH- and PRL-producing cells contained monohormonal and bihormonal granules [12]. These results further validate adenoma cell cultures as *in vitro* experimental model systems for endocrinological and molecular studies, since the ultrastructural and functional features of the original tumors are well preserved during short-term cultivation.

Pituitary adenoma cell cultures as experimental models

The short-term cultures of normal pituitary cells and adenomas proved to be valuable tools for experimental neuroendocrinology. Investigations involving both tissue sections [25, 26] and *in vitro* cell cultures [4, 8, 10, 16] clarified the autocrine and paracrine mechanisms regulating hormone production in pituitary cells. The inhibition of hormone production by various compounds was also quantified [3, 5]. The importance of producing

short-term cultures from the adenoma specimens is underlined by the fact that occasionally long-term cultures can be obtained from cases with higher proliferative potential. A great variety in proliferative activity was found in tissue sections and cytological smears by Ki-67 staining [17, 21]. In our series, the sparsely granulated GH and the acidophil stem cell adenomas provided the most viable cultures, occasionally reaching high passage numbers (Table 3). However, the biochemical and molecular investigations often require larger amount of cellular material that could be readily produced from these samples.

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