Morphological and Functional Properties of Pituitary Adenomas in Culture

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In recent years morphological study of pituitary adenomas has remarkably proceeded by the development of electron microscopic technic. Besides, a great amount of studies on pituitary hormones have been presented in the field of endocrinology. But there are still many unsolved problems on the correlation between morphological property and hormone secretion of each pituitary adenoma cell and there is much confusion in existence of secretory granules and endocrinological activity of chromophobe adenomas. In order to make clear the relationship between cell types and the functions of pituitary adenoma cells, the author investigated the hormone producing activity of them by means of cell culture with major emphasis on the chromophobe adenomas.

Materials and Methods

Thirty pituitary adenomas removed at surgery and one non-neoplastic pituitary gland from a woman with breast cancer having metastasized to bones were submitted to this experiment. Five of the pituitary adenomas were eosinophilic with acromegaly, one was a chromophobe adenoma with Nelson's syndrome, one was also chromophobic with possible TSH-activity, and the remaining 23 tumors were so called non-functioning chromophobe adenomas.

The materials were cultured by the following methods. The tissue fragments were minced, dispersed in the cell population of $3 \times 10^6 - 5 \times 10^6$ cells/ml, and cultured on coverslip by monolayer method in Eagle's MEM containing 20% fetal calf serum. The cultures were incubated at 37°C in a CO₂ incubator (5% CO₂ and 95% air). Complete media changes were made one or two times a week. The cultures were observed under phase microscope for 50 to 150 days. For purpose of the observation by scanning electron microscope, the cells on coverslips were fixed in 1.25% glutaraldehyde for 30 minutes, dehydrated with a graded series of acetone solutions, dried in the air for more than half a day, and coated with evaporated carbon and gold. JSM-U3 was used for the observation. The media derived from the cultures were frozen and later assayed for GH, LH, and FSH by radioimmunoassay procedures. The standard used by the radioimmunoassays were Wilhelmi GH-HS 1544C for GH and LER-907 for LH and FSH. The tissues underwent routine histological diagnosis and transmission electron microscopic study. Electron microscopic specimen were fixed in 2.5% glutaraldehyde, postfixed in 1% O₂O₄, and embedded in Epon prior to thin sectioning and uranyl acetate-lead staining.

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Results

1. Cells from non-functioning chromophobe adenomas
   In 7 to 14 days of cultivation, round epithelial cells proliferated forming colonies. At least two types of cells could be recognized under the phase microscope; larger cells which were 25-30μ in diameter with granules in their cytoplasms and smaller clear cells which were 15-20μ in diameter (Fig. 1). There might be another intermediate cells which the author could not clearly characterize. Under scanning electron microscopy two types of cells were also recognized (Fig. 2a,b). One of them was large and irregular in shape with granules in the periphery of the cytoplasm, the other was small and round with smooth cell surface. These scanning electron microscopic findings were in accordance with the phase microscopic observations.

2. Eosinophilic adenoma cells from acromegalic patients
   Electron microscopy of the tissue showed densely packed secretory granules of about 280 mp and about 140 mp in diameter (Fig. 3a). In the cultures, large round cells with dark cytoplasms due to high contents of secretory granules were conspicuous (Fig. 3b).

3. Chromophobe adenoma from a patient of Nelson’s syndrome
   A pituitary adenoma developed in a patient of Cushing’s syndrome after bilateral adrenalectomies. The patient became pigmented severely and progressively. Large amounts of ACTH (>1000 pg/ml) were found in the patient’s plasma. Histological
Fig. 2a  Type (1) cell of chromophobe adenoma, 14-day-old culture. The cytoplasm has many granules. Scanning electron micrograph, ×6000.

Fig. 2b  Type (2) cell, round and with smooth cell surface. Scanning electron micrograph, ×6000.
Fig. 3a Eosinophilic adenoma, marked with densely packed secretory granules (about 280 μm and 140 μm). Electron micrograph, the scale indicates 1μm.

Fig. 3b Eosinophilic adenoma cells, 6-day-old culture. Some of them look dark due to high contents of secretory granules. Phase micrograph, ×180.
Fig. 4 Chromophobe adenoma of Nelson's syndrome. A lot of secretory granules (about 250 m\( \mu \)) are shown in the margins of the cytoplasms. Electron micrograph, the scale indicates 2\( \mu \).

Fig. 5 Chromophobe adenoma of Nelson's syndrome. A markedly inflamed Golgi apparatus is conspicuous. Electron micrograph, the scale indicates 0.5\( \mu \).
Fig. 6a 21-day-old culture of chromophobe adenoma of Nelson's syndrome. Phase micrograph, ×180.

Fig. 6b Scanning electron micrograph, ×1000. The tumor cells take an acinous arrangement.
Fig. 7 21-day-old culture of the case of Nelson's syndrome. A large number of granules are demonstrated in the margins of the cells surrounding a small lumen. Scanning electron micrograph, x 6000.

diagnosis was chromophobe adenoma of papillary type. Under transmission electron microscopy many of the constituting cells were polygonal, some of them with indented nuclei and a lot of secretory granules were demonstrated in the margins of the cytoplasms (Fig. 4). The cytoplasm had abundant cisternal formations and an inflamed Golgi apparatus (Fig. 5). The size of the secretory granules was about 250 nm in diameter. In the culture the tumor cells proliferated very actively and the cell colonies had tendency to form acinous arrangements (Fig. 6a, b). Each cell was irregular in shape and had a thick cell body with granular surface. Under scanning electron microscopy a large number of granules were observed especially in the margins of the cells surrounding a small lumen (Fig. 7). The size of the granules was almost equal to that of the secretory granules recognized under transmission electron microscopy. High level of ACTH (>10^3 pg/ml) was detected in the medium by radioimmunoassay.

4. Hormone secretion by cells in culture
Among 30 cases, 18 cultures which were maintained in good conditions are
Table 1  Hormone production by pituitary adenomas in culture. GH and LH described in this table are the maximal values detected in the culture medium. The standard used by the radioimmunoassays were Wilhelmi GS-HS 1544C for GH and LER-907 for LH.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical Diagnosis</th>
<th>Histological Diagnosis</th>
<th>GH ng/ml</th>
<th>LH ng/ml</th>
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<tr>
<td>399</td>
<td>43</td>
<td>M</td>
<td>Nonfunctioning adenoma</td>
<td>Chromophobe adenoma</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>406</td>
<td>31</td>
<td>F</td>
<td>Acromegaly</td>
<td>Eosinophilic adenoma</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>407</td>
<td>47</td>
<td>F</td>
<td>TSH producing adenoma?</td>
<td>Chromophobe adenoma</td>
<td>7.6</td>
<td></td>
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<tr>
<td>416</td>
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<td>Chromophobe adenoma</td>
<td>28.8</td>
<td>13.5</td>
</tr>
<tr>
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<td>2.8</td>
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</tr>
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<td>26.0</td>
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<td>Chromophobe adenoma</td>
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<td></td>
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<tr>
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<td></td>
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<td>Chromophobe adenoma</td>
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<td>Eosinophilic adenoma</td>
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<tr>
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<td>38.5</td>
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<tr>
<td>474</td>
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<tr>
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</tr>
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<td>53.0</td>
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<td>$70.0 \times 10^3$</td>
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<td>Breast cancer</td>
<td>Normal pituitary gland</td>
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<tr>
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<td>Cerebellar Hemangioblastoma</td>
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<td>N.D.</td>
</tr>
</tbody>
</table>
listed up in the Table 1, to which one case of normal pituitary gland and one case of non-pituitary brain tumor are added for comparison. The table includes the maximal values of GH and LH, detected in the culture medium. The results of FSH measurement were omitted, because they were almost parallel with values for LH. Changes of hormone secretion in relation to the duration of culture are shown in Fig. 8 and 9. In culture media of adenomas from acromegalic patients, very high levels of GH were secreted. In all acromegalic cases which underwent radioimmunoassays for LH and FSH (3 cases), these two hormones were also detected in the media, at especially high level in one of them. Nine cases among 13 non-functioning chromophobe adenomas revealed the evidence of GH production in the culture medium, though the level was lower than normal control. Concerning LH and FSH, in many cases of non-functioning chromophobe adenomas (7/10) the hormone assays were also positive, particularly in two of them at very high levels.

5. TSH producing adenoma

The pituitary adenoma of No. 407 was a very huge one and the patient showed panhypopituitarism. High levels of TSH were found in the patient's plasma. There was no evidence of primary hypothyroidism. Histologically the tumor was chromo-
phobe adenoma. Radioimmunoassay of TSH in the culture medium was negative in contrast to high levels in the plasma. The cells in the culture showed polygonal shape with a few cell processes.

Discussion

Several reports on tissue cultures of anterior pituitary gland and/or pituitary adenomas have been published\(^\text{13-4}\). According to Kohler et al\(^\text{3}\), cultured pituitaries produced large amounts of GH, LH, and TSH, cultured adenomas from acromegalic patients produced GH at higher levels, and clinically non-functioning chromophobe adenomas produced hormones such as LH, GH, and TSH in culture. Batzdorf et al\(^\text{4}\) demonstrated that adenomas from acromegalic patients produced high levels of GH in the culture and that in 2 cases of 8 non-functioning chromophobe adenomas GH was detected.

The results of author’s investigation coincided with those of the previous reports. Moreover, the present study demonstrated almost all adenomas from patients with no clinical evidence of hormone hypersecretion had capacity to secrete hormones (GH, LH, FSH) in culture. Morphologically two types of cells were recognized under
phase microscope and scanning electron microscope; type (1), larger cells with granules in the cytoplasm and type (2), smaller clear cells without granules. Type (1) cells are probably hormone-secreting cells or have capacity to secrete hormones. Bergland & Torak5] and Paiz & Henniger6 demonstrated electron microscopically that true chromophobic cells were rare or did not exist in man. Schelin7 reported that chromophobic neoplasms contained secretory granules under electron microscopy and McCormick & Halmi$> demonstrated lack of chromophobe adenoma in large series of pituitary tumors by light microscopic study. These previous reports were confirmed by the author’s correlative study on chromophobe adenoma which has multiplicity of constituting cells from view point of morphology and endocrinology by means of tissue culture.

In well maintained cultures, hormone production increased corresponding to cell proliferation (No. 523,502,490, P-K-S in Fig. 8, 9) and high levels of hormones were detected after passage (No. 523-S, P-K-S in Fig. 8,9). These results confirm that the cells retain their hormone secreting capacities in cultures.

Since Nelson’s report in 19581), there have been a number of reports10)-12) of ACTH-producing pituitary tumors following adrenalectomy for Cushing’s syndrome. The majority of these tumors were chromophobic. The tumor of Nelson’s syndrome in this report was also a chromophobe adenoma. The cultivation of this tumor showed acinous cell arrangements and active cell proliferation. The scanning electron microscopic observation of the cultured cells and the transmission electron microscopic study of the tumor revealed that the tumor cells were actively functioning secretory cells. These morphological observations were in accordance with high contents of ACTH in the culture medium and the patient’s blood plasma. The granules demonstrated in the cultured cells by scanning electron microscope were possibly the ACTH-secreting granules but there is still much debate in differentiating the ACTH-granules from the β-MSH-granules.

TSH-producing pituitary adenoma is uncommon13, and the author cannot interpret the result of the present case.

Summary

The correlation of morphological properties and hormone production of the pituitary adenoma cells were investigated using cultured cells by means of scanning electron microscopic observation and radioimmunoassay of the hormones in the culture.

1. Pituitary adenoma cells retained hormone-secreting abilities in the culture.
2. Two types of cells (25μ-30μ with granules, 15μ-20μ without granules) were recognized in the tissue cultures of non-functioning chromophobe adenomas.
3. Almost all non-functioning chromophobe adenomas had abilities to secrete hormones (GH: 1.0–310 ng/ml, LH: 13.5–2.9 × 10^2 ng/ml) in the culture for 50–150 days.
4. Concluding from 2. and 3., the non-functioning adenoma is composed of multiple types of cells which are different from each other in morphological and functional properties.

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5. Secretory granules were demonstrated in the culture cells from a patient of Nelson’s syndrome by scanning electron microscopy.

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References