Pathology of Ectopic ACTH Syndrome with Emphasis on Pituitary Crooke Cells and Adrenocortical Hyperplasia Related to ACTH Activities in Tumor Tissues

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Histopathological characteristics of ectopic ACTH-secreting tumors were confirmed by detailed examinations on 8 undoubted cases with bio- and/or radiimmunoassay for ACTH in the tumor tissue. They were unexceptionally composed of carcinoma cells of poor differentiation, and basically adenocarcinoma took more advantage of ACTH-elaboration by the tumor tissue than epidermoid carcinoma. There was no relation between carcinoma cell patterns and degrees of ACTH activities. Electron microscopic studies revealed serotonin-like granules in cells of colon carcinoma but failed to demonstrate them in thymic carcinoma.

Additional 4 cases with atypical Cushing's syndrome without assay for ACTH activities by the tumor tissue included a case of bronchial carcinoma with clinical signs of gonadotrophic activities and an exceptional pattern of histology as colloid carcinoma.

Appearance of Crooke cells in the pituitary gland was remarkable. The ratio of Crooke cells to beta 1 cell (R cell) in number had a close relation to adrenocortical hyperplasia rather than to the value of ACTH activities in the tumor tissue.

Ectopic ACTH syndrome, a variant of Cushing's syndrome associated with tumors of non-hypophyseal and non-adrenal origin, is the most popular disease among paraneoplastic syndromes, and actual case reports have been recently increased year by year in the literature. A histopathological study usually requires a considerable amount of specimens and precise examinations by a pathologist with his own eyes. Even such fundamental procedures have not been carried out in this syndrome because of rare occurrence and insufficient knowledge of the disease.

The present paper concerns histopathological findings on tumors and endocrine glands from 12 autopsy cases of ectopic ACTH syndrome diagnosed by clinical and endocrinological findings in Japan.

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MATERIALS AND METHODS

Materials examined were obtained from 12 autopsy cases covering a majority of actual cases available in Japan. They were composed of 3 cases of our own and 9 cases which were generously offered by several pathological laboratories to us for the present investigation. These cases were employed (Tables 1 and 2) after a strict criticism following the criteria of ectopic ACTH syndrome proposed by Azzopardi et al.  

<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical course</th>
<th>Primary cancer</th>
<th>Cancer (Histology)</th>
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<tr>
<td>1</td>
<td>68</td>
<td>F</td>
<td>2 y</td>
<td>Lung</td>
<td>Undif. squamous cell carc.</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>M</td>
<td>6 m</td>
<td>Lung</td>
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<td>3</td>
<td>66</td>
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<td>1.5 m</td>
<td>Lung</td>
<td>Oat cell carc.</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>M</td>
<td>3 m</td>
<td>Lung</td>
<td>Small spindle cell carc.</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>M</td>
<td>6 y</td>
<td>Lung</td>
<td>Papillary adenocarc.</td>
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<tr>
<td>6</td>
<td>32</td>
<td>F</td>
<td>1 y</td>
<td>Thymus</td>
<td>Small cell carc.</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>M</td>
<td>6 y</td>
<td>Thymus</td>
<td>Undif. cell carc.</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>M</td>
<td>5 m</td>
<td>Thymus</td>
<td>Small spindle cell carc.</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>F</td>
<td>8 m</td>
<td>Pancreas</td>
<td>Islet-cell carc.</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
<td>F</td>
<td>5 m</td>
<td>Pancreas</td>
<td>Aciar cell carc.</td>
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<tr>
<td>11</td>
<td>26</td>
<td>M</td>
<td>6 m</td>
<td>Pancreas</td>
<td>Islet-cell carc.</td>
</tr>
<tr>
<td>12</td>
<td>58</td>
<td>M</td>
<td>3 m</td>
<td>Colon</td>
<td>Undif. adenoarc.</td>
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TABLE 1. Cases examined: (2) endocrinological findings.

<table>
<thead>
<tr>
<th>No.</th>
<th>ACTH-activity in tumors</th>
<th>Adrenal weight (g)</th>
<th>Urinary 17-KS</th>
<th>Urinary 17-OHCS</th>
<th>Serum K (mEq/l)</th>
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<tr>
<td></td>
<td>Bioassay (mU/g)</td>
<td>Radio-immunoassay (mg/g)</td>
<td>L</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.06</td>
<td>40</td>
<td>6.1</td>
<td>7.1</td>
<td>8.6</td>
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<tr>
<td>2</td>
<td>0.33</td>
<td>12</td>
<td>15</td>
<td>7</td>
<td>21.6</td>
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<tr>
<td>3</td>
<td>0.18</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>23.3</td>
</tr>
<tr>
<td>4</td>
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<td>14.0</td>
<td>13</td>
<td>15</td>
<td>12.1</td>
</tr>
<tr>
<td>5</td>
<td>0.2(Thy), 3.0(Rec), 7.9(Heart)</td>
<td>Excised</td>
<td>12.8</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>0.57-2.84</td>
<td>13.8</td>
<td>13.8</td>
<td>14.2</td>
<td>10.6-12.3</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>20</td>
<td>21</td>
<td>15</td>
<td>16.5</td>
</tr>
<tr>
<td>8</td>
<td>3.6(Liver)</td>
<td>20</td>
<td>21</td>
<td>15</td>
<td>14.1</td>
</tr>
<tr>
<td>9</td>
<td>195(Liver)</td>
<td>20.3</td>
<td>19.1</td>
<td>28.4</td>
<td>27.0</td>
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Above all, biochemical demonstration of an ACTH-like substance by bioassay and/or radioimmunoassay in carcinoma tissue was particularly useful for histological analysis and carried out in 8 cases (in Nos. 1–3, 6 and 9 the concentration was determined by Dr. Imura, Kyoto University Hospital, and in Nos. 7, 10 and 12 the bioassay was performed by Dr. Shimizu, Tokyo University Hospital and the radioimmunoassay by Dr. Miura, Tohoku University Hospital). Additional 4 cases were also regarded as ectopic ACTH.
syndrome by their clinical manifestations including high urinary excretion of 17-OHCS and by postmortem findings of diffuse adrenocortical hyperplasia (Table 2).

Control materials employed were 12 cases of lung cancer without Cushing's syndrome and 2 cases of Cushing's syndrome composed of one with pituitary basophilic adenoma and another with ectopic adrenocortical carcinoma.14

For an identification of cell patterns in the anterior pituitary, alcian blue-PAS-Masson pentachromatic stain and Herlant's tetrachromatic stain15 were used and individual cell types were counted by the Rusmussen and Herrick's method.16

For an evaluation of adrenocortical hyperplasia, width of the adrenal cortex was measured by a micrometer at 10 ideal portions in histologic specimens and an average of the cortical width was calculated.

### RESULTS

1. **Gross findings on metastasis**

   Extensive hematogenous metastases to various organs were usual finding in lung cancer (Table 1). In a case of thymic carcinoma (No. 7) metastases to the heart, prostate and testes were remarkable. But in other cases (Nos. 6, 8-12) the extent of metastasis was fairly limited. In cases with carcinoma of abdominal organs, liver metastasis of considerable amount was unexceptionally observed. Adrenal metastasis seems to have some relationship to hypersecretion of ACTH or ACTH-like substance, but it was limitedly found in 3 of 5 cases of lung cancer and in one of islet-cell carcinoma.

2. **Histologic characteristics of carcinoma**

   In cases with biochemical demonstration of an ACTH-like substance in carcinoma tissue, (lung cancer 3, thymic cancer 2, islet-cell cancer 2 and colon cancer 1), the findings were most reliable as a standard for histopathology of this disease.

   a) **Lung cancer:** All three cases had different histopathological patterns and the content of ACTH-like substance determined by Dr. Imura was high in an undifferen-
tiated adenocarcinoma (0.33 mU/g), moderate in an oat cell cancer (0.18 mU/g), and very low in an undifferentiated epidermoid carcinoma (0.06 mU/g).

These three cases had carcinoma histology which was different in basic structure but common in poor differentiation. Furthermore, adenocarcinoma seemed to be more appropriate for elaboration of an ACTH-like substance by tumor tissues than epidermoid carcinoma.

b) Thymic cancer: Case No. 6 with small cell medullary carcinoma contained a considerable amount of ACTH activity (0.45 mU/g). In Case No. 7, the tumor was previously excised without improvement of the symptom and diagnosed as thymic carcinoma composed of moderate-sized cells. The patient died 5 years after surgery and autopsy revealed a wide-spread metastasis to various organs including myocardium with a feature of undifferentiated carcinoma reduced in cell-size.

In this case, small carcinoma cells were arranged in trabecular and ribbon-like patterns resembled to islet-cell carcinoma of the pancreas. Anaplasia of carcinoma cells was also confirmed by an electron microscopy and cells with electron dense cytoplasm without particular secretory granules prevailed.

c) Pancreas cancer: In 2 cases of islet-cell carcinoma, the concentration of ACTH-like substance was generally low but adrenocortical hyperplasia mainly involving the fascicular zone was remarkable. Carcinoma cells were small and arranged in trabecular (Case No. 9) and undifferentiated medullary (Case No. 11) patterns. There was no argentaffin cell.

d) Colon cancer: Case No. 12 had a tumor in the ascending colon measuring 6 cm in largest diameter. Clinical features of the ectopic ACTH syndrome were typical and multiple metastases to the liver, up to 3 cm in diameter, were most remarkable in postmortal examinations. The concentration of ACTH in the neoplastic tissue was extraordinarily high compared with other tumors (Nos. 7 and 10) also determined by Dr. Miura.

Histologically, the tumor was composed of rather small cuboidal cells with scanty cytoplasm arranging in trabecular and ribbon-like patterns and the finding somewhat resembled cylindroma, islet cell carcinoma or malignant carcinoid tumor. Postmortal electron microscopic examinations of the tumor revealed intraplasmic granules of a particular pattern. They were 0.08-0.2 microns in diameter and resembled serotonin granules from the viewpoints of distinct limiting membrane and electron dense core in the centrum. However, no argentaffin cells were confirmed in paraffin sections. In the periphery of primary tumor, findings of papillary adenocarcinoma including goblet cells and cells with brush borders were observed, and this fact was also against the diagnosis of carcinoid tumor.

In general, these tumors with biochemical evidence for elaboration of an ACTH-like substance by tumor tissues showed features of undifferentiated carcinoma of small cell or middle-sized cell type, and no adenocarcinoma or epidermoid carcinoma of well differentiation was observed.
3. **Cases without assay for ACTH**.

In the remaining 4 cases (Nos. 4, 5, 8 and 11) the concentration of an ACTH-like substance in tumor tissues was not determined, but the diagnosis of this disease was undoubtful from clinical and postmortal findings. There were 2 cases of lung cancer and each one case of thymic and pancreas carcinoma. A lung cancer (No. 4) and a thymic carcinoma (No. 8) were composed of small spindle cells, and a pancreas cancer was of small undifferentiated acinar cells resembling cells of islet cell carcinoma.

An exceptional case of lung cancer (No. 5) was colloid carcinoma exhibiting mucin-producing papillary adenocarcinoma. The patient had clinical features of Cushing's syndrome including gynecomastia and his urinalysis showed a high gonadotropin excretion. Serum potassium level was low but within normal range. Autopsy revealed slight adrenocortical hyperplasia of the Cushing type prevailing in the fascicular zone, while the Crooke cells were scarcely encountered in the pituitary gland.

4. **Adrenocortical hyperplasia**

In general, the adrenals showed bilateral cortical hyperplasia, diffuse and distinctive. Lipid droplets were decreased diffusely in most cases, but in some cases the lesions were irregularly intermingled with nodular foci of lipid preservation.

The severity of adrenocortical hyperplasia seemed to have no relation to the age of patient. Histometrical values of cortical width in cases with the ectopic
ACTH syndrome were generally high as compared with those in control cases of lung cancer without endocrine manifestations (Fig. 1).

Histologically, diffuse hyperplasia was observed mainly in the fascicular zone and somewhat in the glomerular and reticular zones. Clear cell hyperplasia rich in lipid was found occasionally in the reticular zone.

As a sequence of ACTH hyperactivity, various lesions by cortical cell-stimulation were observed; colloid formation, large compact cells with bizarre nuclei and abundant eosinophilic cytoplasm, invasion of cortical tissues into the periairrenal adipose tissue and focal necrosis in the cortex associated with inflammatory cell infiltration. As to invasion of cortical cells, some investigators interpreted this as transformation of brown fat cells into adrenocortical cells as a sequence of ACTH stimulus. Focal inflammatory cell infiltration, which was known as myeloid metaplasia or myeloilipoma, may be induced by a latent septicemic condition, but it seems most likely that the above cell infiltration is due to local cell damages by a high level of ACTH.

5. Crooke cells

The pituitary gland was examined in 8 cases. Each of the cell types was calculated by Rusmussen's method and Crooke cells were identified in comparison with the pituitary gland in a case of Cushing's syndrome with adrenocortical carcinoma in the testicular region. Basophilic cells were classified into beta 1 and beta 2 types, or R and S cells, by aldehyde-thionin-PAS staining method (Ezrin and Murray). These two types could also be identified by alcian blue-PAS-Masson's pentachromatic staining. Four cases of lung cancer showed relatively high counts of beta 2 cell (Fig. 2).
Appearance of considerable numbers of typical Crooke cells was apparently characteristic for this disease as shown in Table 3. The number and feature of Crooke cells seemed to have some relation to the activity of ACTH-like substance, which was elaborated by carcinoma tissues. For example, among cases in which ACTH concentrations in the tumor tissue were determined by Dr. Imura, Case No. 9 (islet-cell carcinoma) with a high ACTH activity had a high incidence of Crooke cells, while Case No. 1 (lung cancer) with a low activity contained almost no typical Crooke cells in the anterior pituitary. Lung cancer had another exceptional case (No. 5) with almost negative incidence of Crooke cells. In these two cases adrenal metastasis was indistinct, while it was distinct in other two cases containing several typical Crooke cells.
In general, it might be accepted that the high incidence of typical Crooke cells was observed in cases with tumors containing more than 0.1 mU/g of an ACTH-like substance. Furthermore, the adrenocortical width and Crooke cell counts were closely related to each other (Fig. 3). An extremely high incidence of Crooke cells (81.5%) was found in a 9-year-old girl with islet-cell carcinoma, in which adrenal cortex was very thick.

**Discussion**

Eight cases of undoubtful ectopic ACTH-secreting tumors, which were confirmed by activities of an ACTH-like substance in carcinoma tissues, have provided several findings to make contributions to pathology of these tumors. They were unexceptionally composed of poorly differentiated small or middle-sized carcinoma cells, and there were no differentiated patterns of adenocarcinoma or epidermoid carcinoma. These features were identical with the description of previous reports that undifferentiation of small cells was common in such tumors associated with Cushing's syndrome as oat cell carcinoma of lung, malignant carcinoid tumor, thymic carcinoma and a pattern of ovarian carcinoma. It was suggested that adenocarcinoma was more appropriate to the ACTH elaboration by tumor tissues than epidermoid carcinoma, but there was no definite relation between carcinoma cell patterns and degrees of ACTH-activity. Further studies in cooperation between pathologists and endocrinologists should be required so that we can establish the relation between the ACTH concentration and histology of tumor tissues.

A question why morphologically undifferentiated tumor cells are possessed of such highly differentiated function of hormone production remains unsolved. Our electron microscopic observations revealed that particular intraplasmic granules resembled serotonin ones in an undifferentiated adenocarcinoma of colon, but failed to demonstrate them in thymic carcinoma. To detect the cytological characteristic of ACTH secretion, ultrastructural and cytochemical investigations should be further performed on several examples with or without clinical manifestations because asymptomatic production of ACTH was confirmed in lung cancer by radioimmunoassay.

The adrenals showed diffuse cortical hyperplasia of Cushing type. Diffusely thickened cortex with elongation of cell columns in the fasciculata was remarkable in comparison with control cases of lung cancer without Cushing's syndrome.

Neville and Symington described that islands of clear cell throughout the adrenal cortex and remarkable hypertrophy of both clear and compact cells were characteristic in adrenocortical hyperplasia of Cushing's syndrome by bronchial carcinoma. Such findings were not rarely observed in our ectopic ACTH-secreting tumors, particularly in a case with colon carcinoma. Additional findings in the adrenal cortex, e.g., intraplasmic colloid formation, focal necrosis and inflammatory round cell infiltration, were also ascribed to the sequence of intensive adrenocortical stimulation by massive ACTH-like substance.

Appearance of typical Crooke cells in considerable number was remarkable in
the anterior pituitary. Pituitary basophilism with Crooke's hyaline changes was classically described as a characteristic finding of Cushing's syndrome.\textsuperscript{17} After that, these Crooke cells were observed in cases with carcinoma of thymus, adrenals, ovaries and pancreas. Hence they seemed to be nonspecific for Cushing's syndrome.\textsuperscript{2} But, according to our recent knowledge these various carcinomas except for adrenal tumors seem to be ectopic ACTH-secreting tumors.

It is generally accepted that ACTH is secreted by a beta 1 cell or R cell in the anterior pituitary, but the problem of the significance of Crooke cell still remains unsolved. These cells appear in cases with prolonged administration of corticosteroids as Sasano et al.\textsuperscript{22} previously observed. It also remains unsolved whether Crooke cells are induced by a high serum corticosteroid level or by a reduced secretion of endogenous ACTH. In the present study, a high incidence of typical Crooke cells was frequently seen in tumors with high ACTH activities. But the ratio of Crooke cells to beta 1 cells had a parallel relation to the width of the adrenal cortex rather than to the value of ACTH-activities in the tumor tissue. A study of serum ACTH level was performed in some cases, but the result was not enough for a comparison with lesions of pituitary cells.

**CONCLUSION**

The present series of 12 autopsy cases may be the largest collection available in the literature, and in 8 cases of them the values of ACTH activities in the tumor tissue were very useful for histopathological analysis. Histological characteristics of the tumors prevailing in the literature were confirmed in actual cases including colon carcinoma, but materials for electron microscopy from 2 cases were not enough to determine the evidence for ectopic ACTH production. In the pituitary-adrenal system, there was a relation between the incidence of Crooke cells and severity of adrenocortical hyperplasia, but several problems still remain unsolved. A joint work of endocrinologists and pathologists will be necessary for further progress in this field.

**Acknowledgment**

We are indebted to Dr. S. Ishikawa, National Cancer Center in Tokyo for his advice and help for collecting materials as many as possible from cases available in Japan. Autopsy materials were generously allowed to use for the present study by Departments of Pathology, Aichi Cancer Center (Case 1), Kyoto University Hospital (Case 2), Toranomon Hospital (Case 3), Nagasaki University Hospital (Cases 4 and 5), Kyushu University Hospital (Case 6), Osaka Red Cross Hospital (Cases 8 and 10), Nagoya University Hospital (Case 9) and Osaka University Hospital (Control 1). Co-operations of Dr. K. Miura, Tohoku University Hospital, Dr. H. Imura, Kyoto University Hospital, and Dr. N. Shimizu, Tokyo University Hospital, are greatly acknowledged.
References


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Fig. 4. Thymic carcinoma (Case 7) of surgical materials shows nesting patterns of moderate-sized cells. Hematoxylin and eosin stain. × 60.

Fig. 5. Thymic carcinoma (Case 7) of autopsy materials shows medullary carcinoma of spindle cells. Hematoxylin and eosin stain. × 100.

Fig. 6. Islet-cell carcinoma (Case 9) with trabecular and alveolar arrangements of small carcinoma cells associated with slight pleomorphism. Hematoxylin and eosin stain. × 250.

Fig. 7. Islet-cell carcinoma (Case 11) with medullary growth composed of trabecular and ribbon-like patterns of small anaplastic cells. Hematoxylin and eosin stain. × 60.
Fig. 8. Colon carcinoma (Case 12). A part of major tumor shows moderately well differentiated adenocarcinoma. Hematoxylin and eosin stain. × 100.

Fig. 9. A prevailing pattern in colon carcinoma (Case 12) composed of small adenocarcinoma cells somewhat resembling carcinoid tumor. Hematoxylin and eosin stain. × 100.

Fig. 10. Diffuse and nodular hyperplasia of the adrenal cortex distinct in Case 12.

Fig. 11. Clear cells rich in lipid are remarkable in the inner cortex in Case 12. A gross picture of a histologic slide stained with hematoxylin and eosin.

Fig. 12. Diffuse hyperplasia of clear cortical cells is particularly evident in the fasciculata in Case 9. Hematoxylin and eosin stain. × 100.
Fig. 13. Elongation of cell columns in the fasciculata with localized lesions of necrosis and hemorrhage in Case 11. Masson-Goldner stain. × 60.

Fig. 14. A non-capsulated mass of cortical cells in the periadrenal adipose tissue in Case 7. Hematoxylin and eosin stain. × 60.

Fig. 15. A) Typical Crooke cells are numerous in the anterior pituitary in Case 12. B) Finely granular substance positive with PAS is compressed in the periphery of cytoplasm. Alcian blue-PAS-Masson pentachromatic stain. × 600 (a) and × 1,000 (b).

Fig. 16. A transition from beta 1 cell (R cell) to Crooke cell is observable in Case 11. Alcian blue-PAS-Masson pentachromatic stain. × 600.
Figs. 17 and 18. Specific granules resembling neurosecretory granules with distinct limiting membrane and electron-dense core are frequently observed in carcinoma cells in Case 12. As the material was obtained in 3 postmortal hours, autolytic changes in cell organelles and nuclei are unavoidable but the structure of above granules is well preserved.
