Light and Electron Microscopic Features of a Pituitary Adenoma in Nelson’s Syndrome

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ABSTRACT

Kovacs, Kalman, Horvath, Eva, Kerenyi, Norbert A., and Sheppard, Robert H.: Light and electron microscopic features of a pituitary adenoma in Nelson’s syndrome. Am J Clin Pathol 65: 337-343, 1976. Electron microscopy of an amphophil pituitary adenoma surgically removed from a 51-year-old woman who had Nelson’s syndrome revealed that the tumor was composed of melanocorticotroph cells. This finding is consistent with the view that in the human pituitary gland one single cell type produces both adrenocorticotropic hormone (ACTH) and melanocyte-stimulating hormone (MSH). In contrast to the ultrastructure of pituitary adenomas associated with Cushing’s syndrome, no or only very few microfilaments were detected in the cytoplasm of the tumor cells, suggesting that adrenocortical steroids are required for the formation of microfilaments. The presence or absence of microfilaments in the tumor cells may be regarded as a distinguishing ultrastructural feature between Cushing’s syndrome and Nelson’s syndrome. It appears that changes in the level of circulating corticoids may affect the ultrastructural features of melanocorticotroph cells not only in normal human pituitaries but also in adenohypophyseal adenomas. (Key words: Pituitary; Pituitary ultrastructure; Pituitary adenoma; Pituitary pathology; ACTH; MSH.)

Nelson’s syndrome is characterized by the presence of adrenocorticotropic hormone–melanocyte-stimulating hormone (ACTH–MSH)-secreting adenoma of the pituitary gland in patients who have undergone adrenalectomy for pre-existing Cushing’s disease. By light microscopy the pituitary tumors are found to consist of basophil, acidophil, amphophil, or chromophobe cells. Although numerous cases have been reported since the first description of the syndrome by Nelson and associates in 1958, studies of the morphology of the pituitary tumors failed to provide an unequivocal answer with regard to structure–function relationships. In the present work, a light- and electron-microscopic investigation was undertaken on a pituitary neoplasm surgically removed from a 51-year-old woman who had Nelson’s syndrome in order to shed some light on the morphologic features of the ACTH–MSH-secreting cell of the anterior pituitary.
Report of a Case

Clinical Findings: A 51-year-old housewife had been found to have Cushing’s syndrome and had undergone bilateral total adrenalectomy at the age of 43 years. The removed adrenal glands were enlarged (14 Gm.) but otherwise failed to show gross or histologic abnormalities. The postoperative course had been uneventful, and the patient was kept on cortisone (12.5 mg. per day, per os). About 6–8 months after adrenalectomy, the color of her skin had turned darker, and gradually the skin had become deeply pigmented. This progressed to the point where the patient had spotty melanin pigmentation all over her body, including her face. There was no other symptom related to the pituitary gland. In 1974, 8 years after removal of the adrenals, examination disclosed the presence of pituitary tumor. Skull x-ray and pneumoencephalogram showed a markedly enlarged sella turcica. Fasting blood ACTH level was 2100 pg. per ml. Other investigations, including the measurements of various pituitary hormones, were not contributory. In 1974, with the patient under general anesthesia, a transnasal-transsphenoidal hypophysectomy was performed, and from the paper-thin sella turcica a mushy pituitary tumor was removed. Postoperatively, transient diabetes insipidus developed, but resolved spontaneously. The patient felt very well after operation, and it was noteworthy that the skin pigmentation strikingly decreased.

Methods

For light microscopy, tumor tissue was fixed in 10% buffered formalin and embedded in paraffin. Sections 4–6 μm. thick were stained with hematoxylin and eosin, hematoxylin–phloxine–saffron, PAS, Goldberg-Chaikoff’s trichrome, Mann’s, aldehyde fuchsin, aldehyde thionin, orange G, and light green technics.

For electron microscopy, pieces of tumor tissue were fixed in 10% buffered formalin for 20 hours and then transferred to 2.5% glutaraldehyde in 0.15 M Sorensen’s buffer, postfixed in 1% osmium tetroxide in Millonig’s buffer, dehydrated in graded ethanol, and embedded in epoxy resin. Thick sections were cut with a Porter Blum MT-2 ultramicrotome and stained with toluidine blue to select areas for electron microscopic study. Ultrathin sections were stained with uranyl acetate and lead citrate and investigated with a Philips 300 electron microscope.

Morphologic Findings

Gross Findings: The specimen consisted of multiple fragments of reddish tissue. Size and weight of the tumor were not determined.

Light-microscopic Findings: The tumor appeared to be fairly cellular and was intermingled with blood. It was composed of relatively large cells arranged in cords or strands, in some areas manifesting an alveolar pattern (Fig. 1). There was no significant variation in shape or size among the tumor cells, and mitotic figures were not common. The nuclei were usually round and rich in chromatin. The cytoplasm was quite prominent and showed positive staining with PAS or aniline blue, as well as with orange G, eosin, phloxine, or light green. Aldehyde fuchsin or aldehyde thionin failed to give definite coloration in the tumor cell cytoplasm.

Electron-microscopic Findings: The tumor cells were polyhedral or angular (Fig. 2). The nuclei were oval or lobulated, with clumping of chromatin substance, and were usually located eccentrically. Nucleoli, when seen, were small and very dense. Mitotic figures were also encountered. The rough-surfaced endoplasmic reticulum (RER) was moderately or well developed, consisting either of an interconnected network of cisternae or of randomly scattered short profiles studded with ribosomes. Free...
ribosomes and polysomes were numerous. Most adenoma cells possessed relatively few, small, oval to rod-shaped mitochondria with a moderately dense matrix and lamellar cristae. A few cells, however, contained more mitochondria that were larger. Occasionally cells manifesting advanced oncocytic transformation were also detected. The Golgi apparatus was moderately developed in the smaller adenoma cells. These cells had a lighter cytoplasm, with only a few RER membranes and secretory granules. In the larger, organelle-rich, well-granulated cells, however, prominent, hypertrophied Golgi zones with several vesicles and immature secretory granules were seen (Fig. 3).

All adenoma cells contained varying numbers of secretory granules. A few cells possessed only granules about 250 nm. in diameter. These secretory granules showed little variation in density, they had a well-recognizable limiting membrane and were lining up along the cell membrane. A few other cells, manifesting cytoplasmic features similar to those of normal ACTH-MSH cells, contained large numbers of granules varying markedly in electron densities and with diameters of 300–600 nm., most of them measuring 300–400 nm. A few larger bodies, as much as 600–700 nm. in diameter, were also found in a few cells. The larger granules showed marked differences in electron density. Some of them were very pale, with a frequently incomplete limiting membrane. Both the pale and the dark granules seemed to be released from the Golgi complex. The plasma membranes of neighboring tumor cells were generally closely apposed. Despite the definite margination of secretory granules along cell borders, neither granule extrusions, nor pits on the membranes suggesting earlier extrusions, were seen. Intercellular junctions of the adherent type were infrequently observed. Type I microfilaments
Fig. 2. Electron micrograph, showing several adenoma cells containing a fair number of mitochondria and well-developed Golgi complexes. Note the striking differences in electron densities of secretory granules. ×14,000.
and large secondary lysosomes, the regular components of ACTH-MSH cells, were only occasionally encountered. The capillary endothelium showed partial loss of fenestration, as well as focal thickening and swelling.

**Discussion**

Correlations between pituitary-cell morphology and hormone secretion are not yet fully clarified. To establish a proper classification of various cell types of the human pituitary gland involved in the production of different hormones, animal studies are of only limited value, because due to considerable species differences no firm extrapolation from animal to man is justified. There is also no need to stress the difficulties when one wishes to collect human pituitary glands for sophisticated morphologic studies. Autopsy material is obviously not suitable for electron microscopic evaluation, and it always remains uncertain as to how closely surgical biopsy specimens taken from non-tumorous adenohypophyseal tissue reflect conditions that prevail in the normal pituitary gland. The light- and electron-microscopic investigation of surgically removed pituitary tumors with known endocrine activity seems to provide the best opportunity to achieve a better understanding of structure-function relationships.

In our case, the tumor was regarded clinically to represent an ACTH-MSH-secreting neoplasm. By light microscopy it was found to consist of amphophil cells that showed cytoplasmic PAS positivity and manifested affinity towards

**FIG. 3.** Prominent Golgi complex (G) in an adenoma cell containing several secretory granules of various electron densities in the sacculi. ×15,100.
various basic as well as acidic dyes but failed to stain with aldehyde fuchsin or aldehyde thionin. In previous case reports pituitary neoplasms associated with increased ACTH and MSH production were composed of basophil, acidophil, amphophil, or chromophobe cells.\textsuperscript{2,4,8,11,13,18,19}

The ultrastructural identification of the cell type that secretes ACTH and/or MSH in the human pituitary gland is not unequivocally resolved. Under the name “ACTH-producing cells,” different cell types have been described by various investigators,\textsuperscript{5,7,9} and it would not serve a useful purpose to review at length the previous literature on this subject. In our case the ultrastructural appearances of the tumor cells closely resembled those of cells detected in non-tumorous pituitary glands and called “adrenocorticotrophs” by Moriarty.\textsuperscript{9} Our findings are also very similar to those of Saeger,\textsuperscript{22} who reported the fine-structural features of four cases of pituitary adenomas associated with Nelson’s syndrome.

In our case there were conspicuous differences among the individual tumor cells that might have been the result of variations in actual secretory activity. It was evident, however, that the tumor consisted of only a single cell type, indicating that this cell type is capable of secreting both ACTH and MSH. This view is consistent with the light-microscopic findings of Phifer and associates,\textsuperscript{14,15} who delineated the melanocorticotroph cell by using immunoperoxidase stainings and postulated that this cell is the source of both ACTH and MSH. It remains to be seen whether the dense and less dense secretory granules revealed in the cytoplasm of the tumor cells correspond to these two chemically similar, nevertheless distinct, hormones, or whether they represent only one granule population in different stages of evolution. The investigations of Moriarty and Moriarty\textsuperscript{10} support the latter possibility. These authors found by electron-microscopic immono-

peroxidase technic that ACTH and MSH are present in a single cell and probably in the same secretory granule within the same cell. Hence, this finding seems to indicate that variations in electron densities of secretory granules reflect the differences in granule maturity, and if the pace of discharge of secretory products is accelerated, granules can be released in an immature phase, manifesting reduced electron density.

The presence of microfilaments is a characteristic feature in the cytoplasm of the non-tumorous melanocorticotroph cells.\textsuperscript{5,9} These structures are also apparent in the cells of pituitary adenomas associated with Cushing’s syndrome,\textsuperscript{1,17} i.e., in those cases that are accompanied by elevated levels of circulating corticosteroids. When exogenous corticoids are administered or endogenous corticoids are secreted in excessive amounts for a protracted period in the ACTH–MSH-producing cells, the so-called “Crooke’s change,” which also consists of extensive accumulation of microfilaments,\textsuperscript{3,5,6,16,23} develops. In contrast, no or only very few microfilaments were detected in the cytoplasm of the tumor cells in our case and in the four cases of Saeger.\textsuperscript{22} These results seem to indicate that the presence and quantity of microfilaments may depend upon the level of circulating corticoids.

Hence, it is easy to understand why pituitary adenomas associated with Cushing’s syndrome differ in ultrastructure from those related to Nelson’s syndrome, despite the fact that the tumors consist of cells actively secreting ACTH–MSH in both conditions. In Cushing’s syndrome hyperfunctioning adrenal cortices that secrete adrenocortical steroids in excessive amounts are present, whereas in Nelson’s syndrome the adrenals have been removed. Based on these considerations, it is conceivable that adrenocortical steroids can influence the ultrastructure of ACTH–MSH-producing pituitary neoplasms.
References


