REVIEW ARTICLE

Clinically Non-Functioning Human Pituitary Adenomas

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ABSTRACT: Clinically non-functioning pituitary adenomas are morphologically classified into two groups, those which have hormone immunoreactivity and ultrastructural features of known adenohypophysial cell types but are clinically silent, and those composed of cells that do not resemble nontumorous adenohypophysial cell types. Among the fomer are the silent somatotroph adenomas, silent corticotroph adenomas and silent gonadotroph adenomas; the latter include the silent type III adenomas, null cell adenomas and oncocytomas. We review their histological, immunohistochemical and ultrastructural features, the results of *in situ* hybridization to determine hormone synthesis by these tumors and data obtained from tissue culture characterizing their hormone release *in vitro*. Non-functioning adenomas represent a heterogeneous group. The discrepancies between morphology, immunoreactivity and lack of endocrine activity of silent adenomas are not clear. Oncocytomas are variants of null cell adenomas. We suggest that null cell adenomas and oncocytomas originate in uncommitted pluripotent precursor cells capable of undergoing multidirectional differentiation. The progenitor cells differentiate most frequently toward FSH / α-subunit producing cells; the mechanism of preferential differentiation is obscure.

RÉSUMÉ: Adénomes pituitaires humains cliniquement silencieux. Les adénomes pituitaires cliniquement silencieux sont classifiés en deux groupes, selon leur morphologie : ceux qui ont une immunoréactivité hormonale et des caractéristiques ultrastructurales de types cellulaires de l'antéhypophyse tout en étant cliniquement silencieux et ceux qui sont composés de cellules qui ne ressemblent pas à des types cellulaires non tumoraux de l'antéhypophyse. Parmi les premiers, on retrouve les adénomes somatrophes silencieux, les adénomes corticotrophes silencieux et les adénomes gonadotrophes silencieux; les seconds incluent les adénomes silencieux de type III, les adénomes à cellules nulles et les oncocytomes. Nous revoyons leurs caractéristiques histologiques, immunohistochimiques et ultrastructurales, les résultats d'hybridation *in situ* pour déterminer la synthèse hormonale de ces tumeurs et les données obtenues de cultures cellulaires caractérisant leur libération hormonale *in vitro*. Les adénomes non-fonctionnels représentent un groupe hétérogène. Leur cytogenèse et les discordances entre leur morphologie, leur immunoréactivité et leur absence d'activité endocrinienne ne sont pas claires. Les oncocytomes sont des variantes de l'adénome à cellules nulles. Nous suggérons que les adénomes à cellules nulles et les oncocytomes originent de cellules précurseurs pluripotentes dont la différenciation n'est pas encore amorcée et qui sont capables de subir une différenciation multidirectionnelle. Les cellules progénitrices se différencient le plus souvent ves des cellules produisant de la FSH/sous-unitéd. Le mécanisme de la différenciation préférentielle est obscur.

Can. J. Neurol. Sci. 1992; 19: 228-235

Human pituitary adenomas are generally classified by the clinical syndromes with which they are associated or by the biochemical manifestations of hormone production by the tumor. Approximately 25-30% of patients with pituitary adenomas have no clinical or biochemical evidence of homone excess; this is the group of patients with "clinically non-functioning" pituitary adenomas.

Morphologic studies of clinically non-functioning pituitary adenomas have shown that they are composed of cells which have the organelles necessary for hormone synthesis.[1] Based on histologic, immunohistochemical and ultrastructural characteristics, these tumors are classified into two main categories: those which resemble known adenohypophysial cell types (the silent somatotroph adenomas,[2] silent corticotroph adenomas[1] and silent gonadotroph adenomas[1]), and those which are composed of cells that have no specific markers and do not resemble known adenohypophysial cell types (the silent "subtype III" adenomas,[3] null cell adenomas and oncocytomas.[1] By electron

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Received April 1, 1991. Accepted in final form October 18, 1991
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microscopy the latter always possess cytoplasmic secretory granules signifying endocrine differentiation; the endocrine function and cellular origin of these adenomas, however, is not clear. The scarcity of knowledge regarding their derivation is reflected in the confusion related to terminology. Several terms, such as fetal, embryonal, chromophobic, non-functioning, undifferentiated and precursor cell adenomas were used to separate them into a distinct entity. The term null cell adenoma of the pituitary[4] was introduced to stress the most obvious feature of these tumors: the absence of specific markers which would permit the disclosure of their cellular origin. Oncocytomas are characterized by increased number and volume density of cytoplasmic mitochondria. The existence of similarities in clinical and biochemical features as well as morphological overlap between null cell adenomas and oncocytomas indicate that non-oncocytic null cell adenomas can transform to oncocytomas and they are regarded as variants of the same entity.[1,5]

Tissue culture has revealed that many clinically non-functioning adenomas release gonadotropins or their subunits into culture media.[6] Northern blot or *in situ* hybridization analyses have shown that the vast majority of clinically non-functioning adenomas express messenger RNA (mRNA) for α-subunit of pituitary glycoprotein hormones and a significant number express mRNA for the β-subunits of the gonadotropins;[7,8] a small proportion contain mRNA for growth hormone (GH) or prolactin (PRL),[8,9] and a few express mRNA for adrenocorticotropin (ACTH).[7-9] Receptor localization has shown that many of these tumors contain receptors for a number of hypothalamic peptides.[10-16] In many of these studies, morphologic analysis was not performed and the results do not clarify whether the hormone release, gene expression or receptors correlate with the morphologic parameters of classifiable tumors.

This paper reviews briefly structure-function correlations of clinically non-functioning pituitary adenomas which are morphologically classifed by immunohistochemistry and electron microscopy combined with *in situ* hybridization and analyzed for hormone release using tissue culture.

Clinical Features

Clinically non-functional pituitary tumors are detected either incidentally or when brought to medical attention due to symptoms of a sellar or parasellar mass.[17] At the time of diagnosis patients with null cell adenoma belong to the older age group and are rarely under the age of 40 years. Silent adenomas may occur at any age. Because of absence of clinical symptoms in early phases of tumor growth, these tumors are usually diagnosed at the macroadenoma stage. The patients most frequently complain of local symptoms, primarily visual disturbances and sometimes headaches and cranial nerve deficiencies. Extension and invasion into adjacent tissues is frequently seen.

From the endocrine viewpoint, various degrees of hypopituitarism, especially hypogonadism and hypothyroidism may be apparent. Lack of adenohypophysial hormone excess is confirmed by radioimmunoassay measurement of blood levels of GH, PRL, ACTH, thyrotropin (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and α-subunit of glycoprotein hormones; preoperative testing with insulin-induced hypoglycemia, TRH challenge and administration of gonadotropin-releasing hormone (GnRH), growth hormone-releasing hormone (GRH) or corticotropin-releasing hormone (CRH) may reveal abnormal responses of hormone secretion indicating hypopituitarism. In some patients, slight or moderate hyperprolactinemia can be demonstrated. The cause of elevated serum prolactin levels is claimed to be the so called "stalk section effect"; the growing tumor is thought to affect the hypothalamic production, release or transport of dopamine to the adenohypophysis. In the absence of dopamine, prolactin cells become activated and release prolactin in excess.

Imaging methods include skull x-rays, computerized tomography and magnetic resonance imaging to confirm the presence and extent of pituitary tumor.

At present, the only method to cure the patients is surgery. Since the tumors are usually large, in many cases transfrontal approach is required. The recurrence rate after surgery is considerable since the tumors are usually macroadenomas and their removal is difficult. Tumor apoplexy may also occur.

Methods of Investigation

The structural features of tumors are classified by light microscopy and by electron microscopy.[1] Immunocytochemistry is used to determine the hormonal profile of the adenomas using primary antisera directed against GH, PRL, ACTH, β-endorphin, β-TSH, β-FSH, β-LH and α-subunit of glycoprotein hormones. Ultrastructural immunocytochemistry is performed by the immunogold technique[18] in some cases. To document hormone synthesis by these tumors, *in situ* or Northern blot hybridization is used to characterize mRNA expression.[19] Hormone release and its regulation can be analyzed in tissue culture; culture medium hormone content is measured by radioimmunoassay.[6] Cultured cells can be incubated with various adenohypophysiotropic substances to study the responses of hormone release and clarify the regulation of tumor cell function.[20] In our laboratory, the nature of the cells cultured is documented by light and electron microscopy and immunocytochemistry at the termination of cultures. The reverse hemolytic plaque assay can also be performed to analyze hormone release by individual cells.[21] Receptors for various substances can be identified on tumor cell membranes and the mechanism of their actions can be determined by analyzing the effects of stimulation on cAMP and calcium levels or turnover of inositol phospholipids.[15,16]

Tumor Classification

Silent somatotroph adenomas

A small group of clinically non-functioning tumors has the characteristic ultrastructural features of sparsely granulated somatotroph adenoma with fibrous bodies.[2] Immunocytochemistry demonstrates little or no GH reactivity but *in situ* hybridization reveals GH mRNA expression in adenoma cells. Tissue culture studies disclose a low rate of GH secretion initially (15-29 ng/2×10^4 cells/24 hrs) compared to clinically functioning somatotroph adenomas (700-7000 ng/2×10^4 cells/24 hrs) and a spontaneous 10-20 fold rise after several days in culture (300-330 ng/2×10^4 cells/24 hrs). GH release increases after exposure to GRH. No other adenohypophysial hormones can be detected in culture media; electron microscopy confirms that sparsely granulated somatotrophs comprise the cultured material.

The pathophysiology of silent somatotroph adenomas is uncertain. Previous reports of patients with elevated levels of GH and somatomatomedin C but no acromegaly have postulated abnormal biological activity of GH or somatomedins.[22] The presence of abundant GH mRNA in tumors without detectably elevated blood levels of GH suggests that the post-translational processing of the gene product might be defective, perhaps yielding an immunologically altered GH, but this is not supported by the behaviour *in vitro*. Alternatively, there may be release of only small amounts of hormone *in vivo*; the low levels of release initially and the intact responsiveness of tumor cells to stimulation by GRH in culture suggest that lack of hormone excess *in vivo* may be attributable to lack of stimulation or inhibition of GH release.

Silent corticotroph adenomas

A few clinically silent pituitary adenomas have basophilic staining qualities, strong periodic acid-Schiff (PAS) positivity, immunoreactivity for derivatives of proopiomelanocortin (POMC), and ultrastructural parameters characteristic of corticotrophs.[1,23-27] Several subtypes have been described based on the degree of resemblance to nontumorous corticotrophs. Silent corticotroph adenomas subtype I are basophilic, show strong positivity with PAS, lead hematoxylin and immunostains for ACTH (Figure 1a), and have ultrastructural features indistinguishable from adenomas associated with Cushing's disease or Nelson's syndrome (Figure 1b). Subtype II adenomas can be chromophobic, may exhibit only a moderate or slight staining with PAS or lead hematoxylin and have varying degrees of positivity for ACTH and related peptides; ultrastructurally, while the cells resemble corticotrophs, they are almost devoid of the characteristic cytoplasmic filaments found in that cell type. Recent studies have documented the presence of POMC mRNA in tumor cells.[28,29] Tissue culture studies show that ACTH release by these tumors (25-250 pg/2×10^4 cells/24 hrs) is significantly less than released by corticotroph adenomas associated with Cushing's disease or Nelson's syndrome (2500-16000 pg/2×10^4 cells/24 hrs). Preliminary studies have also documented release of β-endorphin by these morphologically classified cultured tumors. A single report has implicated production of abnormal ACTH-related POMC products by a silent corticotroph adenoma.[30]

The pathophysiology of silent corticotroph adenomas has been the subject of speculation. Several authors have postulated production of small amounts of hormone, abnormal hormone, or other substances.[1,23-27] The possibility that β-endorphin is the

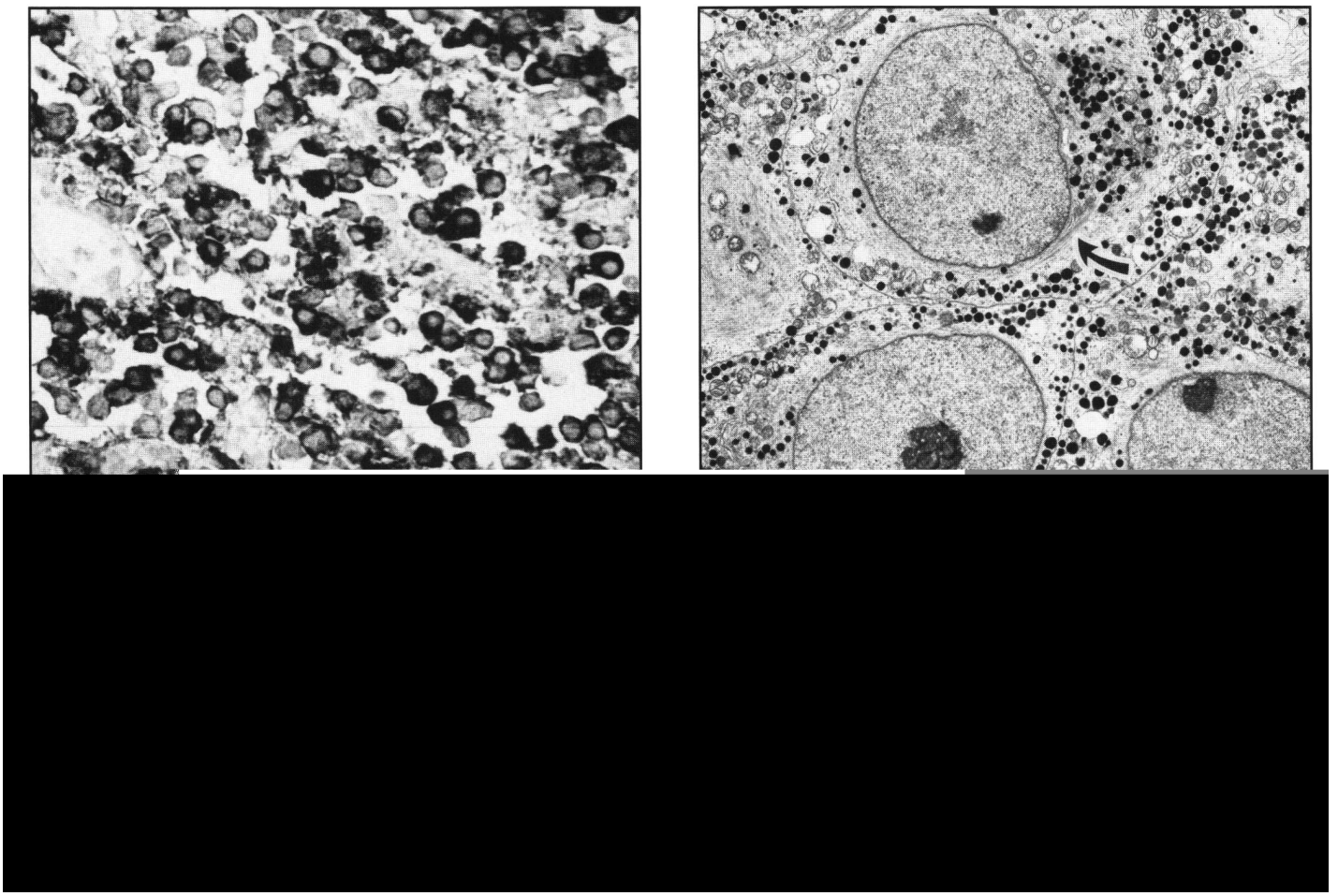


*Figure 1 — **(A)** A silent corticotroph adenoma (type I) contains intense positivity for ACTH in the cytoplasm of tumor cells. (Avidin-biotin-peroxidase complex technique; magnification × 340). **(B)** This tumor type has all the ultrastructural features of a functioning corticotroph adenoma including numerous secretory granules of variable size, shape and electron density and juxtanuclear bundles of intermediate filaments (arrows) that are the characteristic marker of corticotrophs. (Magnification × 3,600).*

https://doi.org/10.1017/S0317167100042311 Published online by Cambridge University Press

main hormonal product of this tumor type[23,26] is supported by the striking association of hyperprolactinemia with even small tumors in which the hormone excess cannot be attributed to "stalk section effect". Lysosomal accumulation with crinophagy has been described in a single case,[27] suggesting a defect in the release of secretory granules and their intracellular lysosomal disposal.

Silent gonadotroph adenomas

A number of clinically non-functioning pituitary adenomas contain immunoreactivity for the β-subunits of FSH and/or LH and α-subunit of glycoprotein hormones (Figure 2a) and the tumor cells have ultrastructural features of gonadotrophs (Figure 2b). Although the blood FSH and LH levels are normal for the patient's gonadal status, these tumors are nevertheless morphologically classified as gonadotroph adenomas.[1,31] Tissue culture studies document release of FSH, LH and α-subunit by these tumors.[6] The hormone levels in culture media are significantly lower than those of tumors which give rise to elevated levels of gonadotropins *in vivo*:[32] FSH secretion by silent tumors averages 1473 ng/10^4 cells/24 hrs and ranges from 130-3240 ng/10^4 cells/24 hrs, in contrast with functioning tumors which release 13350 (12330-14364) ng/10^4 cells/24 hrs; LH release by silent tumors averages 314 (83-942) ng/10^4 cells/24 hrs, whereas functioning tumors release 4565 (3148-5983) ng/10^4 cells/24 hrs; α-subunit release by silent tumors averages 6.4 (0.2-18.4) ng/10^4 cells/24 hrs in contrast to functioning tumors which release 32.0 (28.0-35.8) ng/10^4 cells/24 hrs. The reverse hemolytic plaque assay shows that among these adenomas, only a small percentage of tumor cells form plaques with antisera against the α- or β-subunits of gonadotropic hormones and the mean sizes of plaques are smaller than those of clinically functioning adenomas studied for comparison.[21]

The reason for clinical silence of some gonadotroph adenomas has been attributed to difficulty in the detection of elevated gonadotropin levels,[33,34] as for example, elevated blood levels of FSH or LH are physiological in women beyond the menopause. In other instances, failure of detection has been attributed to production of biologically and/or immunologically altered hormone.[35,36] Our tissue culture studies and results of the reverse hemolytic plaque assay suggest that the lack of hormone excess *in vivo* may be attributable to production of smaller amounts of hormone than those required to cause elevation of blood hormone levels.[6,21]

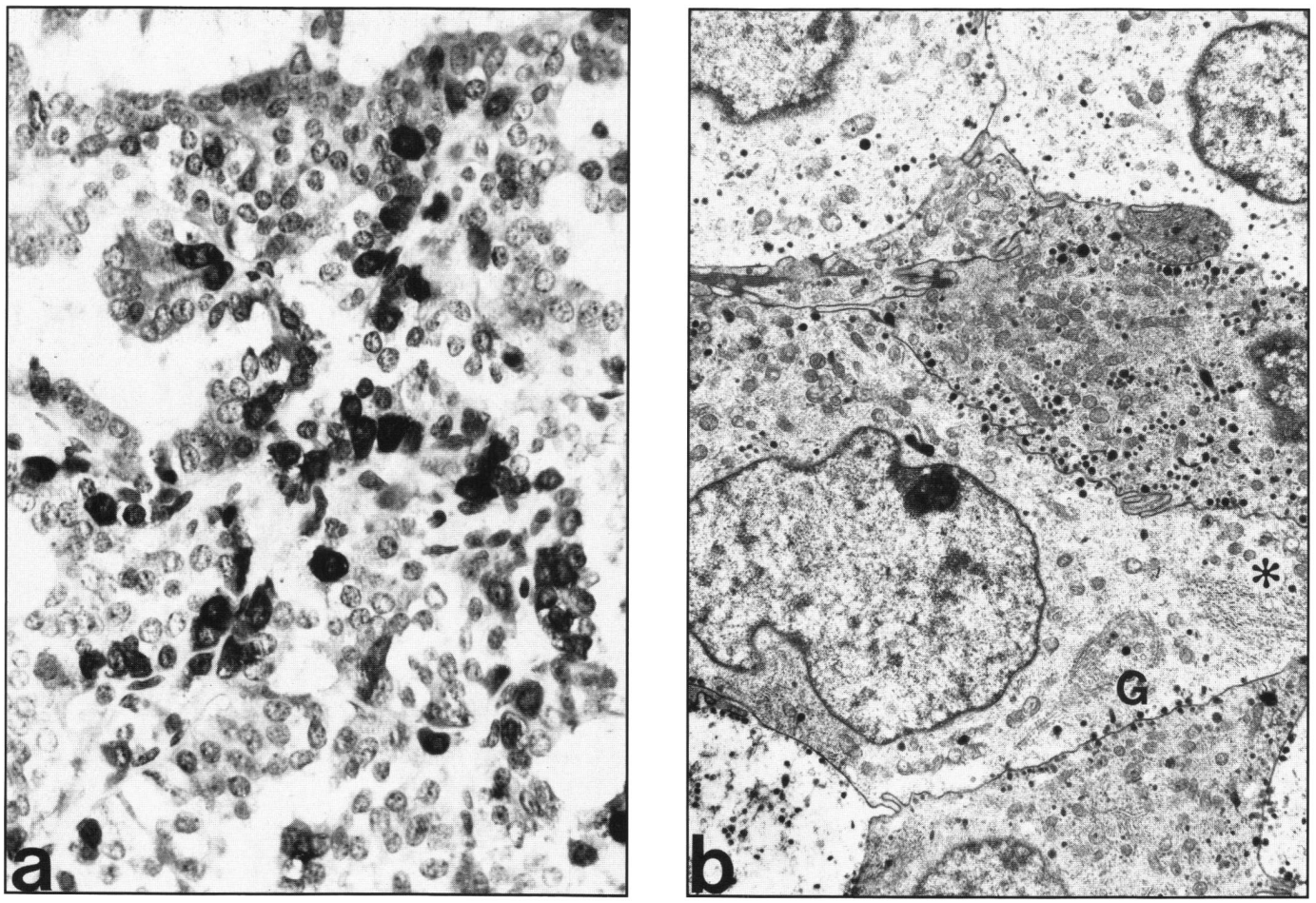


*Figure 2 — **(A)** A silent gonadotroph adenoma has numerous cells that stain for β-FSH. (Avidin-biotin-peroxidase complex technique; magnification × 400). **(B)** By electron microscopy, tumor cells of a silent gonadotroph adenoma are elongated and polygonal with distinct polarity; they have short profiles of rough endoplasmic reticulum (*), well developed Golgi complexes (G) and numerous small secretory granules which tend to line up along the cell border. (Magnification × 7,300).*

https://doi.org/10.1017/S0317167100042311 Published online by Cambridge University Press

Silent "subtype III" adenomas

A type of clinically non-functioning tumor containing focal positivity for POMC-derived peptides was initially described as silent corticotroph adenoma subtype III, however, the immunoreactivities were not consistent and the ultrastructural features of this subtype, although indicative of active hormone synthesis, bore little resemblance to those of corticotroph adenomas. Subsequently, the term corticotroph was deleted.[3] Some of these tumors contain immunoreactivity for one or more adenohypophysial hormones; however, the majority of adenoma cells remain negative for known hormones. The ultrastructural features are similar to those of glycoprotein hormone-producing cells, especially thyrotrophs. Tissue culture studies reveal the presence of very small amounts of several adenohypophysial hormones with no consistent pattern.

Although the cytogenesis of this tumor type is unknown, it has been postulated that the tumors may represent multidirectional differentiation of a stem cell; alternatively, the fine structural features suggest that they may be derived from glycoprotein hormone-producing cells and finally it may be that these tumors are primarily involved in the production of other, as yet unknown, substances.[3]

Null cell adenomas and oncocytomas

By histology, null cell adenomas are benign, chromophobic or acidophilic tumors. The acidophilic granules in the cytoplasm of oncocytomas represent mitochondria and not hormone-containing secretory granules. The tumor cells are arranged usually in a diffuse or sinusoidal pattern, with frequent pseudorosettes around vessels.

Immunocytochemical studies initially revealed the absence of immunoreactive adenohypophysial hormones in these tumors;[4] however, with the use of improved methodology, the substantial majority of null cell adenomas exhibit scattered immunopositivity for one or more adenohypophysial hormones, most commonly β-FSH and the α-subunit of glycoprotein hormones (Figure 3a), less frequently β-LH and β-TSH, rarely PRL or GH, and occasionally ACTH.[5,37] The variability of immunoreactivity has been attributed to suboptimal tissue fixation and embedding which can lead to substantial loss of antigenicity, particularly among glycoprotein hormones.

Electron microscopic investigation reveals that null cell adenomas are composed of small, polyhedral cells with oval or irregular, kidney-shaped or deeply indented nuclei and moderately developed nucleoli (Figure 3b). The scanty cytoplasm con-

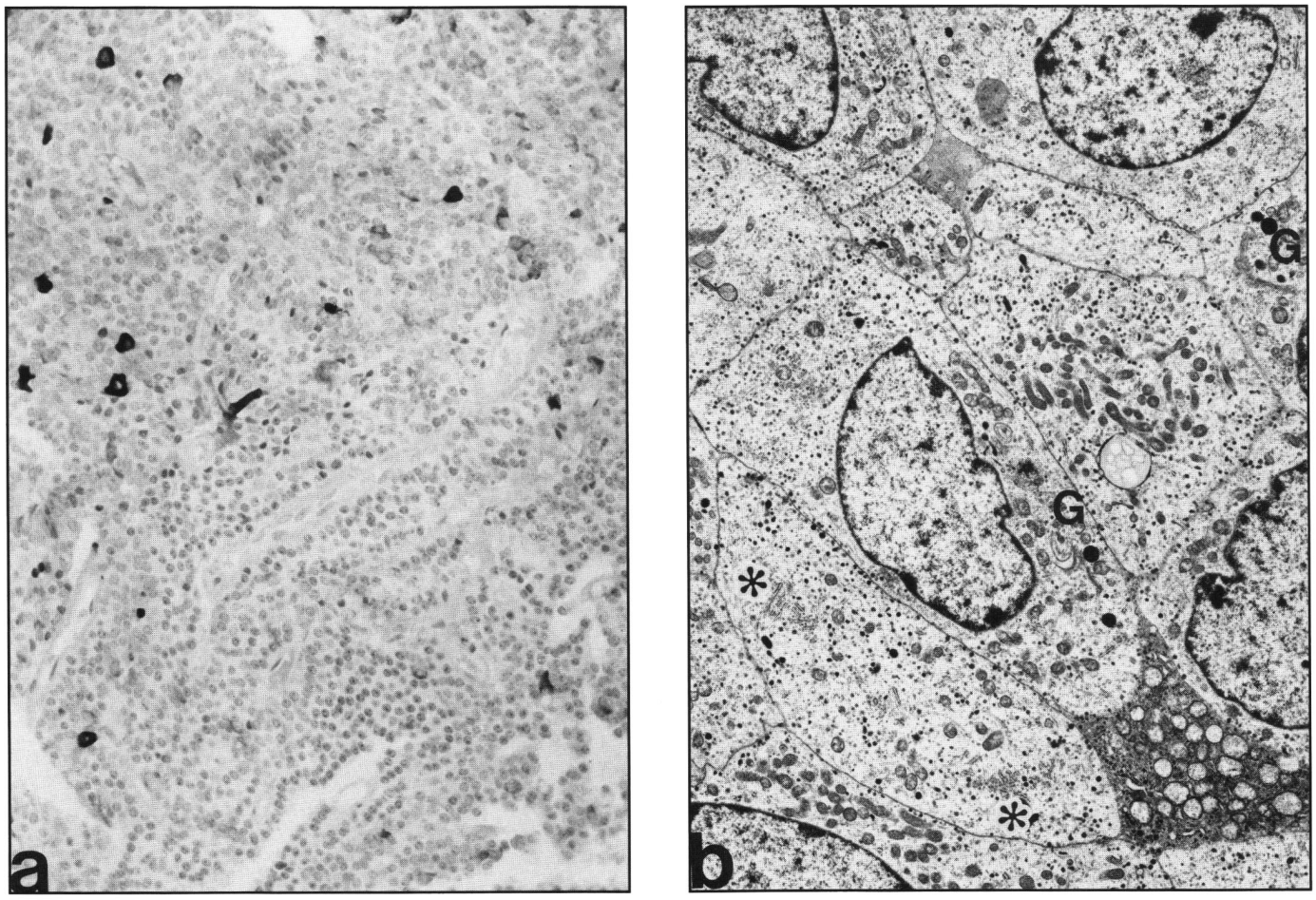
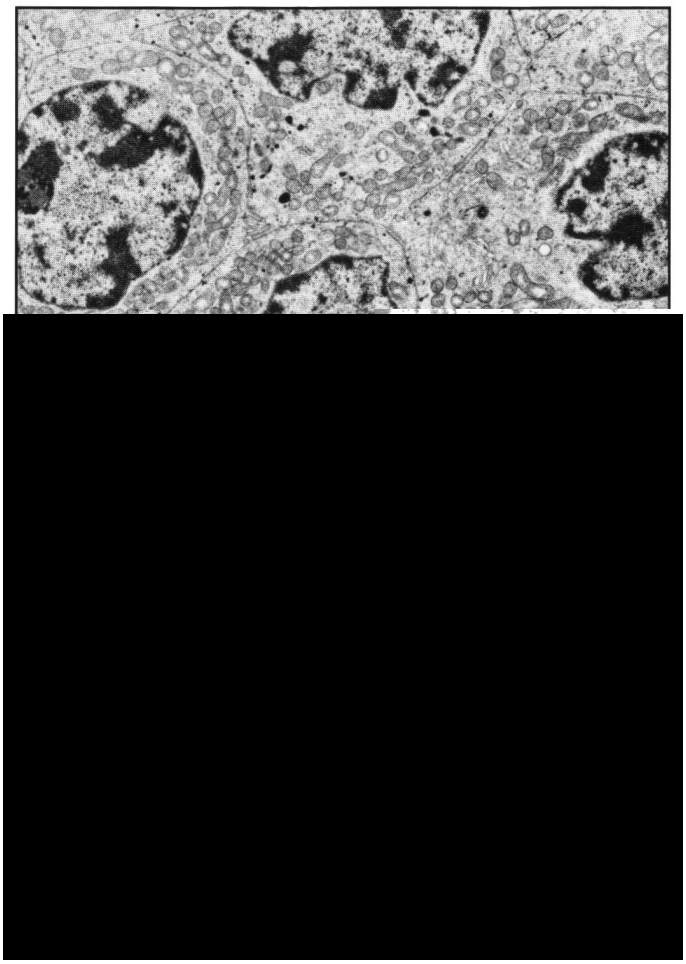


*Figure 3 — **(A)** A null cell adenoma contains scattered cells with immunopositivity for α-subunit of glycoprotein hormones. (Avidin-biotin-peroxidase complex technique; magnification × 250). **(B)** The ultrastructural features of a null cell adenoma are not distinctive enough to allow classification as any known adenohypophysial cell type, but they do contain the organelles required for hormone synthesis and secretion: rough endoplasmic reticulum (*), Golgi complexes (G) and secretory granules. (Magnification × 4,470). **(C – opposite page)** An oncocytoma has the same subcellular organelles as those found in null cell adenomas but the cytoplasm is almost filled with numerous mitochondria. (Magnification × 4,520).*

https://doi.org/10.1017/S0317167100042311 Published online by Cambridge University Press

tains a few small profiles of rough endoplasmic reticulum (RER), varying number of free ribosomes, slightly or moderately developed Golgi apparatus and few immature forming granules. The sparse secretory granules measure 100-250 nm. They are usually spherical or oval, may be more numerous in the cytoplasmic processes and are often located in the subplasmalemmal cytoplasm. The mitochondria are small and rod shaped; their cytoplasmic volume density does not exceed 15 percent. In oncocytic cells the cytoplasm is larger and the mitochondria are increased in number (Figure 3c). Despite marked mitochondrial abundance, RER cisternae, Golgi complexes and small secretory granules are invariably recognizable in oncocytic cells. The degree of oncocytic transformation varies from one tumor to another. In the diagnosis of oncocytoma, electron microscopy is indispensable.

Tissue culture studies of null cell adenomas and oncocytomas reveal consistent release of FSH, LH, and α-subunit by the vast majority of these tumors.[5,6,20] Only trace quantities of other pituitary hormones can be detected in media from a few cultured tumors. There is no significant difference of hormone release between null cell adenomas and onocytomas and there is no correlation of hormone release *in vitro* with the patient's age or sex. The quantities of gonadotropins released *in vitro* by these tumors are low, comparable to those obtained in cultures of clinically silent, morphologically classified, gonadotroph adenomas.[6] The reverse hemolytic plaque assay shows that only a small percentage of tumor cells form plaques with antisera against α- or β-subunits of gonadotropins;[21] the percentage of plaque-forming cells and the mean size of plaques are smaller than those of clinically functioning adenomas studied for comparison. Incubation with GnRH results in increased release of LH, FSH, and α-subunit[20,38,39] in the majority of tumors; secretion of gonadotropins by some tumors is also stimulated by TRH,[20,38] as well as by GRH and CRH.[20] Functional GnRH and TRH receptors were found by studying increases in inositol phospholipid turnover in response to those substances.[15] Analysis of calcium and cAMP levels has provided evidence of tumor cell modulation by a large number of hypothalamic peptides.[16]



One case reported to date has had a different profile of both hormone content and ultrastructural differentiation. A tumor which was classified as a null cell adenoma contained scattered cells positive for GH, PRL and ACTH as well as glycoprotein hormones by immunocytochemistry; electron microscopy revealed the presence of occasional cells showing differentiation towards somatotrophs, lactotrophs and corticotrophs.[40]

It is an intriguing observation that many features of null cell adenomas and gonadotroph adenomas overlap and it is difficult if not impossible to decide whether a tumor should be called null cell adenoma or gonadotroph adenoma. The problem is compounded by the often uneven distribution of immunoreactivities, since false conclusions could be drawn from the study of a small sample. In addition the number of hormone-containing adenoma cells depends considerably on tissue processing and the immunocytochemical methods used. Since transition exists between null cell adenomas and gonadotroph adenomas, tumors with null cell ultrastructure may show immunoreactivity for FSH/LH/α-subunit and vice versa, adenoma cells which appear as gonadotrophs by ultrastructural analysis[41] may not be immunopositive for glycoprotein hormones.[42]

The tissue culture findings[5,6,20] suggest that null cell adenomas and oncocytomas which show little morphologic differentiation are frequently able to release small quantities of glycoprotein hormones, usually FSH and α-subunit. It remains uncertain whether these tumors secrete hormones *in vivo*. It appears from the results of the tissue culture studies that there may be a spectrum from the morphologically unclassified null cells to the morphologically classifiable gonadotroph adenomas which do not secrete sufficient homones to cause elevation of circulating gonadotropins, to the well differentiated gonadotroph adenomas which secrete significantly more gonadotropins *in vitro* and result in elevated gonadotropin levels *in vivo*.[32]

The close relationship between null cell adenomas and gonadotroph adenomas has been further emphasized by those studies which documented the presence of mRNA encoding FSH/LH/α-subunit in many clinically non-functioning pituitary adenomas. In one study, expression of glycoprotein hormone genes was found in 79 percent of clinically non-functioning pituitary adenomas.[7] In another report, prolactin and ACTH mRNA were described in some clinically non-functioning pituitary adenomas.[9] In these investigations, morphologic classification was not performed to clarify structure-function correlations; however, a recent study with more accurate morphologic classification confirmed these data.[8]

The cytogenesis of null cell adenomas is not clear. It may be that these neoplasms arise in non-neoplastic null cells which

maintain their null cell features. Alternatively, the cells of null cell adenomas can be regarded as differentiated cells exhibiting immunohistochemical and ultrastructural signs of endocrine hypoactivity. Daneshdoost and colleagues recently suggested that the majority of clinically non-functioning pituitary tumors derive from gonadotroph cells.[34] We believe, however, that one should not classify all null cell adenomas as gonadotroph adenomas. Some null cell adenomas do not express glycoprotein hormone genes, do not contain immunoreactive FSH/LH/α-subunit and show no gonadotroph features by electron microscopy; they do not exhibit the distinctive ultrastructural sexual dimorphism characteristic of gonadotroph adenomas.[41]

These tumors, which have been shown to be monoclonal,[43] may derive from pluripotential precursor cells which are capable of differentiation into gonadotrophs or into other cell types; alternatively, these tumors may represent hypoactive differentiated cells which are committed to a single line of differentiation. Differentiation may be functional as well; thus adenomatous null cells may acquire the ability to produce hormones, most frequently FSH/LH/α-subunit, rarely other adenohypophysial hormones. It is reasonable to assume that null cells are uncommitted and pluripotent: they are more prone to differentiate toward gonadotrophs, but transformation may occur to another cell line. The factors which cause the regulate differentiation and transformation are completely unknown at present. These tumors are found predominantly in the elderly, suggesting that they may grow slowly and remain undetected until later in life; alternatively, they may have their onset later than other pituitary adenomas, raising the possibility that there is some association with gonadal failure. The factors underlying oncocytic transformation are also uncertain; it is intriguing that gonadotropin-producing adenomas have a greater propensity to undergo this change than do tumors of other cell types.

Conclusion

The morphologic classification of clinically non-functioning pituitary adenomas has shown that these tumors are not a homogenous clinicopathologic entity. The importance of morphologic and dynamic studies in the accurate diagnosis of these tumors has been illustrated; the advances in our understanding of pituitary adenoma pathology have been summarized. Numerous questions remain, particularly concerning cytogenesis and pathophysiology. Further studies should build on the understanding obtained by a multidisciplinary approach.

Acknowledgements

This work was supported in part by Grants MA-10215 and MT-6349 awarded by the Medical Research Council of Canada. The authors gratefully acknowledge the technical help of Mrs. D. Lietz.

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