Original Article

Null Cell Adenomas, Oncocytomas, and Gonadotroph Adenomas of the Human Pituitary: An Immunocytochemical and Ultrastructural Analysis of 300 Cases

George Kontogeorgos, M.D., Kalman Kovacs, M.D., Ph.D., Eva Horvath, Ph.D., and Bernd W. Scheithauer, M.D.

Abstract

The immunocytochemical profile of 300 clinically nonsecreting pituitary adenomas was investigated. All tumors were diagnosed, classified, and separated into null cell adenomas, oncocytomas, and gonadotroph adenomas according to their ultrastructural morphology. The immunocytochemical analysis was based on the semiquantitative proportional estimates of positive cells immunostained for all known peptide and glycoprotein pituitary hormones including alpha-subunit. The majority of tumors (87%) were to some extent immunopositive for various hormones. Glycoprotein hormones were most frequently encountered. Usually, particularly in males, more than one subunit was present in the same tumor. In 97 tumors (32%) more than 25% of adenoma cells were immunoreactive for glycoprotein hormones. Fifty-five tumors (18%) contained occasional cells immunopositive for growth hormone (GH), prolactin (PRL), and adenocorticotropin (ACTH) in addition to glycoprotein hormones. Given the significant proportion of immunoreactive cells for gonadotropins and alpha-subunit, in tumors characterized as null cell adenomas and oncocytomas, immunocytochemistry may provide valuable information to the pathologist and clinical endocrinologist contributing to the evaluation of this heterogeneous group of tumors. **Endocr Pathol 4:20–27, 1993.**

Department of Pathology, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada (GK, KK, EH); and Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota (BWS).

Address correspondence to Bernd W. Scheithauer, M.D., Department of Pathology and Laboratory Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

Somewhat more than one-third of operated pituitary adenomas secrete no hormones in excess and are unassociated with endocrine hypersecretion syndromes. Clinically, such tumors are termed "nonsecreting adenomas" [15, 17, 18]. Early observers, utilizing simple histological stains, classified these adenomas as "chromophobic." Lack of endocrine activity as well as staining of adenoma cells was at first attributed to absence of cytoplasmic granules. It was McCormick and Halmi (1971) who, in a histochemical study of a large series of pituitary adenomas, first found that truly agranular adenomas do not exist [23]. Subsequent ultrastructural observations confirmed the presence of secretory granules, albeit in small number, in the cells of chromophobic adenomas [18, 19]. The substantial majority of clinically nonsecreting adenomas are known to correspond to null cell adenomas, oncocytomas, or gonadotroph adenomas [3, 7, 10, 11, 12, 15, 16, 17, 18, 24, 25, 28]. In producing various glycoprotein hormones or their alpha-subunit, these lesions share common histological features and biochemical properties. As a result, it is not always possible to draw distinctions between them [7, 13, 15]. The term "null cell adenoma" has been applied to tumors lacking specific immunocytochemical, ultrastructural, or biochemical markers. Although cytoplas-

mic organelles, particularly those involved in hormone synthesis and release, are present in null cell adenomas and their oncocytic variant, they are typically scant, consisting of only occasional profiles of rough endoplasmic reticulum, small Golgi complexes, and sparse, minute secretory granules. The cellular origin of null cell adenomas is obscure [18]. The same is true of oncocytomas, which are characterized by cytoplasmic accumulation of mitochondria [16, 17, 20]. Oncocytic change also occurs, albeit less frequently, in gonadotroph tumors. The latter, in 50% of cases and particularly in men, are ultrastructurally identical to null cell adenomas [7, 13, 30].

Although studies employing improved immunohistochemical techniques have contributed to the more frequent recognition of gonadotroph adenomas [4, 7, 8], their relationship to null cell tumors remains unclear. Null cell adenomas and oncocytomas may contain small numbers of thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and/or alpha-subunit immunoreactive cells [15, 17]. It is not clear, therefore, whether null cells represent dedifferentiated gonadotrophs, undifferentiated precursors, or resting cells capable of multidirectional differentiation not only toward glycoprotein-producing cells but also occasionally to other cell types [14, 15].

The purpose of this study is to investigate the immunocytochemical features of a large group of clinically nonsecreting pituitary adenomas, all of which were removed by surgery and were ultrastructurally characterized as null cell adenomas or oncocytomas, some with glycoprotein differentiation or gonadotroph adenomas. Our aim is to assess the intimate relationship and apparently overlapping characteristics of the adenoma types and to better define them according to their immunocytochemical profile.

Material and Methods

Cases

There were 724 cases, including 266 pituitary tumors morphologically diagnosed as null cell adenoma, 217 as oncocytoma, and 241 as gonadotroph adenoma, which were retrieved from approximately 3,000 reports of surgically removed pituitary adenomas in the consultation files of two of the authors (K.K. and E.H.) at St. Michael's Hospital. Derived in part from patients undergoing surgery at Mayo Clinic, the study group consisted of 100 null cell adenomas, 100 oncocytomas, and 100 gonadotroph adenomas. In each subgroup, male and female patients were included so as to be equally represented. All 300 tumors, including the gonadotroph adenomas, had been classified exclusively on the basis of their electron-microscopic features, though all had been studied by routine histochemistry as well as immunocytochemistry for adenohypophyseal hormones.

Methods

For light microscopy, 10% buffered formalin-fixed and paraffin-embedded tissues were used. Sections of 4–6 μm thickness were stained by the hematoxylin and eosin (H&E) and the periodic acid–Schiff (PAS) methods.

For immunocytochemistry either the peroxidase-antiperoxidase method (PAP) or the avidin–biotin–peroxidase complex (ABC) technique was utilized [8, 29]. Consecutive microsections were pretreated with 5 mg/100 ml pronase E (Sigma, St. Louis, MO) for 10 minutes at room temperature, incubated overnight at 4°C with antisera directed against the following hormones or hormone fragments: growth hormone (GH; DAKO, Santa Barbara, CA), prolactin (PRL; donated by Dr. H. Friesen, Winnipeg, MB), adrenocorticotropin (1-39 ACTH; donated by Dr. S. Raiti, NIADDK, Bethesda, MD), monoclonal β-thyroid stimulating hormone (β-TSH; Chemicon Inc., El Segundo, CA), β-follicle-stimulating and β-luteinizing hormone (β-FSH and β-LH; donated by Dr. S. Raiti, NIADDK, Bethesda, MD), and monoclonal α-chorionic gonadotropin (α-HCG; Biogenex Laboratories, Dublin, CA). Working dilutions ranged from 1:200 to 1:2,000. The specificity of immunostains was verified by replacing primary antisera with phosphate-buffered saline or with normal rabbit or mouse serum. The chromogen 3'3'-diaminobenzidine (DAB)

was used for visualization of antigen-antibody binding sites.

For electron microscopy, 2.5% glutaraldehyde-fixed and osmicated tissues were embedded in an Epon-Araldite mixture. Ultrathin sections stained with uranyl acetate and lead citrate were examined on either a Philips 300 or a 410-LS electron microscope.

Immunoreactive Cell Estimation

All immunostained sections were reevaluated utilizing a semiquantitative estimate of the proportion of immunopositive cells based on a five-step scale: e.g., − = 0%, + = <5%, ++ = 5%–15%, +++ = 15%–25%, ++++ = >25%. Immunoreactive cells in peripheral portions of the tumor fragments were disregarded as were cells arranged in nests the size of normal pituitary cords. The latter were regarded as nontumorous adenohypophyseal cells trapped by tumor.

Classification Criteria

Ultrastructural features were the basis of adenoma classification. The diagnosis of null cell adenomas was based on the following ultrastructural features: absence of specific features of differentiation (Fig. 1); small, sparse secretory granules less than 200 nm in diameter; and numbers of mitochondria not exceeding 15% of the cytoplasmic volume (Fig. 1). Null cell adenomas exhibiting pronounced mitochondrial accumulation—that is, occupying approximately 30% of cytoplasmic volume in 50% of cells or more—were classified as oncocytomas (Fig. 2). Gonadotroph adenomas in males were defined as tumors with specific ultrastructural features including polar cells with well-developed membranous organelles and uneven distribution of small, approximately 200 nm diameter, secretory granules, either subplasmalemmal or within cytoplasmic processes (Fig. 3). In female patients these features of gonadotroph differentiation were accompanied by "honeycomb" transformation of the Golgi apparatus, a specific marker of gonadotroph adenomas of "female type" [7, 13] (Fig. 4).

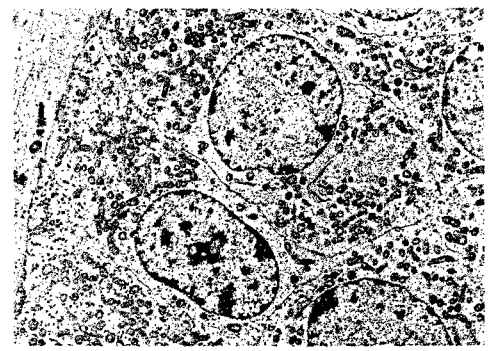


Figure 2. Oncocytoma. The ultrastructural features of this tumor are those of null cell adenoma with pronounced mitochondrial accumulation defined as approximately 30% of cytoplasmic volume in 50% or more of cells (×5,500).

Figure 1. Null cell adenoma. The tumor shows absence of specific features of differentiation, sparse small (< 200 nm) secretory granules, and mitochondria occupying less than 15% of cytoplasmic volume (×6,900).

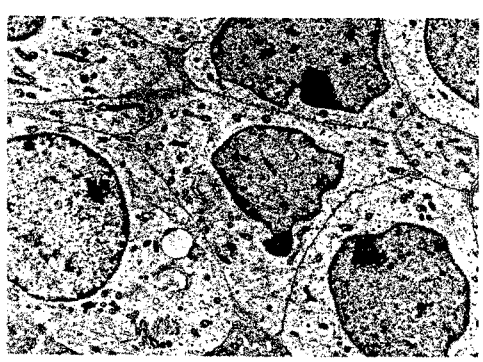
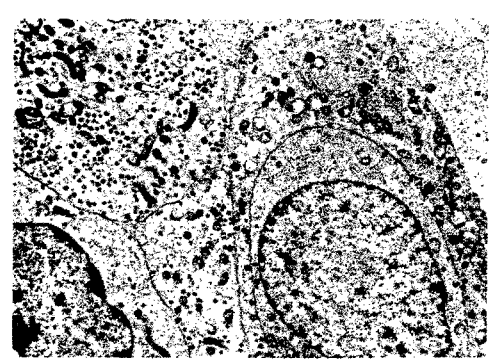


Figure 3. Gonadotroph adenoma, male type. The cells exhibit polarity, well-developed membranous organelles, and uneven distribution of small, approximately 200 nm, secretory granules, either subplasmalemmal or within cytoplasmic processes (×11,000).



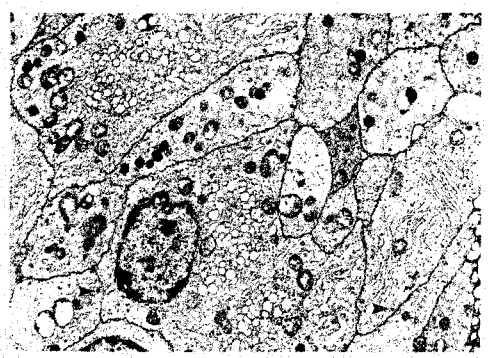


Figure 4. Gonadotrophic adenoma, female type. These tumors exhibit features of gonadotrophic differentiation, as noted in the previous feature, accompanied by "honeycomb" transformation of the Golgi apparatus, a specific marker of "female-type" gonadotroph adenomas (×10,800).

Statistics

For statistical analysis the Student's *t* test was applied. Statistical differences were considered significant when associated with *p* values of 0.01 or less.

Results

From the 724 initially retrieved cases, 266 tumors (37%) were classified according to their ultrastructural features alone as null cell adenomas, 217 (30%) as oncocytomas, and 241 (33%) as gonadotroph adenomas. None of the patients' clinical presentations were associated with endocrine manifestations. Detailed preoperative hormone levels were not available in many cases; thus this information has not been included.

Of the 300 cases constituting the study group, 261 adenomas (87%) were immunopositive for various adenohypophyseal hormones. A high frequency of immunoreactivity was observed in all three adenoma types. Although the proportion of reactive cells was slightly higher in males, no statistically significant differences in reactivity were noted between sexes or among the three adenoma groups (Table 1).

The adenomas exhibited immunoreactive cells varying in number, in distribution (focal, patchy, or diffuse), and in their degree of staining intensity. Glycoprotein hormones were most frequently encountered as compared to other hormone types in all three groups. Usually positivity for more than one beta-subunit as well as alpha-subunit was noted in the same tumor. The spectrum of immunoreactivities in male and female (M/F) patients, stated in terms of the proportion of positive cells, is tabulated for each adenoma group in Tables 2, 3, and 4.

Glycoprotein hormones were significantly more frequently represented in tumors of males than in females ($p < 0.001$). Combinations of gonadotropins, particularly FSH and alpha-subunit, showed a significant preponderance in males ($p < 0.005$), LH being the exception in that it was nearly equally represented in oncocytomas of both sexes.

TSH was the least frequent glycoprotein hormone demonstrated. No adenoma-producing β-TSH alone was encountered. Only one null cell adenoma in a female patient contained more than 25% TSH-immunopositive cells. In two other cases, one null cell adenoma and one oncocytoma, 15%–25% of adenoma cells were TSH-reactive. Beta-FSH or LH staining unassociated with alpha-subunit was noted in 1 male tumor and in 4 females, respectively. In contrast, the presence of alpha unassociated with beta-glycoprotein hormone subunits was seen in 17 tumors, including 9 null cell adenomas, 4 oncocytomas, and 4 gonadotroph adenomas.

In 97 tumors (32%), 23 null cell adenomas, 28 oncocytomas, and 46 gonadotroph adenomas, according to a combined collective estimate, 25% or more of adenoma cells were immunopositive for FSH, LH, and/or alpha-subunit (Table 5). A preponderance of such tumors was observed in male patients with null cell and gonadotroph adenomas. Null cell adenomas and oncocytomas contained fewer

Table 1. Incidence of immunoreactivity in the three adenoma groups in males and females

	Null Cell Adenoma (n = 100)	Oncocytoma (n = 100)	Gonadotroph Adenoma (n = 100)
M/F	48/41	47/42	49/34
%	89	89	83

Table 2. Distribution of the immunopositivity of null cell adenomas according to sex (M/F)

	GH	PRL	ACTH	TSH	FSH	LH	Alpha-SU
+	14/1	5/1	1/0	21/3	15/9	16/8	12/12
++	0/3	0/0	0/0	8/3	12/6	13/4	11/7
+++	0/0	0/1	0/1	0/1	7/2	6/3	8/2
++++	0/0	0/0	0/0	0/1	5/3	3/1	11/7
Total M/F	14/4	5/2	1/1	29/8	39/20	38/16	42/28
Total %	18	7	2	37	59	54	70

immunopositive cells in comparison to gonadotroph adenomas, nearly half the latter containing more than 25% immunoreactive cells.

There were 55 tumors (18%), including 20 null cell adenomas, 23 oncocytomas, and 12 gonadotroph adenomas, which contained cells reactive not only for glycoprotein hormones but also for GH and/or PRL or rarely for ACTH. Immunopositivity for these hormones was limited, being found in 5% or less of adenoma cells and unevenly distributed. GH was the most common of these hormones, occurring twice as frequently in null cell adenomas and oncocytomas as in gonadotroph adenomas. Of these hormones ACTH was the least often encountered.

Discussion

The analysis of our data indicates that null cell adenomas, oncocytomas, and gonadotroph adenomas, tumors comprising the substantial majority of clinically nonsecreting adenomas, are ultrastructurally distinct lesions which exhibit immunocytochemical similarities. In immunophenotypic terms the majority of these lesions corresponded to glycoprotein-producing adenomas since a substantial number of cases were immunoreactive for alpha-subunit, FSH, LH, or TSH. Immunopositivity to FSH and alpha-subunit was expressed to a greater extent in male patients.

Coexistence of various glycoprotein subunits in the same tumor was more frequently noted in male patients. Of interest, given the nosologic tendency to clearly distinguish gonadotroph from thyrotroph cell adenomas, is the frequency with which TSH reactivity was encountered in association with gonadotropins. Indeed, in this series immunoreactivity for TSH was always coexpressed with FSH and LH. Nearly always, only scattered (less than 5%) TSH-positive cells were demonstrated. This necessarily raises two questions. Are such tumors simply glycoprotein adenomas producing the full spectrum of glycoprotein hormones, an entity not accommodated in present classifications of pituitary adenomas? Or is TSH positivity a

Table 3. Distribution of the immunopositivity of oncocytomas according to sex (M/F)

	GH	PRL	ACTH	TSH	FSH	LH	Alpha-SU
+	7/8	3/1	1/1	18/11	19/16	19/13	13/14
++	1/0	1/0	0/0	2/3	10/7	10/15	11/3
+++	0/0	0/0	0/0	0/1	9/5	3/8	8/4
++++	0/0	0/0	0/0	0/0	5/5	3/2	7/11
Total M/F	8/8	4/1	1/1	20/15	43/33	35/38	39/32
Total %	16	5	2	35	76	73	71

Table 4. *Distribution of the immunopositivity of gonadotroph adenomas according to sex (M/F)*

	GH	PRL	ACTH	TSH	FSH	LH	Alpha-SU
+	2/3	2/2	1/3	17/8	7/6	8/4	6/14
++	1/2	0/0	0/0	6/1	10/5	3/6	3/9
+++	0/0	0/0	0/0	0/0	5/2	10/4	9/5
++++	0/0	0/0	0/0	0/0	21/7	18/5	22/4
Total M/F	3/5	2/2	1/3	23/9	43/20	39/19	40/32
Total %	8	4	4	32	63	58	72

manifestation of cross-reactivity, perhaps with FSH, one of the most frequently concurrent hormones? It is notable that none of the TSH-containing tumors in this series was associated with hyperthyroidism, a positive manifestation of TSH-producing adenomas, and that none of these lesions exhibited the characteristic ultrastructural features of TSH cell adenoma. At present the questions raised by these observations cannot be answered conclusively, but consideration should be given to the possibility that glycoprotein-producing adenomas do not fall into gonadotroph and thyrotroph cell categories.

A decade ago, on the basis of ultrastructural features, a significant proportion of chromophobic, clinically nonfunctioning tumors were classified as null cell adenomas [18]. Subsequently, the continued use of electron microscopy, supported by immunocytology, led to the characterization of the poorly understood gonadotroph adenomas. Typical ultrastructural features included the cell polarity, a full complement of synthetic organelles, and accumulation of small secretory granules within cytoplasmic processes. Subsequently, a novel feature, the so-called honeycomb Golgi, came to be recognized as a unique attribute of gonadotroph adenoma in female patients. These morphological observations were put into perspective by the immunocytochemical demonstration of gonadotropins within adenoma cells [7, 13, 17]. The integration of these methods necessarily underwent a process of evolution. Early studies were plagued by wide antibody cross-reactivity, nonspecific background staining, and frequent failure of hormone demonstration owing to lack of antibody sensitivity. Currently, improved immunocytochemical techniques, the introduction of more specific and sensitive antibodies, and the development of enzyme pretreatment methods for the unmasking of hidden antigenic sites have all contributed to the recognition of glycoprotein hormone-containing adenomas [4, 7, 8].

It is remarkable that in the first descriptive study of null cell adenoma, only 10 (18%) of 56 tumors were found to be focally immunopositive for glycoprotein hormones [18]. Years later immunocytochemical studies found a significant proportion of null cell adenomas and oncocytomas (79%) to be immunoreactive, particularly for various glycoprotein hormones [15]. In the present series, 89% of null cell adenomas and oncocytomas showed some degree of hormone immunoreactivity. It is evident from these studies that the substantial majority of null cell and oncocytic tumors possess immunocytochemical markers indicative of glycoprotein differentiation. It has, in fact, been suggested that null cell adenoma might be classified as gonadotroph adenoma when an appreciable proportion, perhaps 25% of the tumor

Table 5. *Tumors containing more than 25% adenoma cells immunoreactive for FSH, LH, or alpha-subunit*

	Null Cell Adenoma (n = 100)	Oncocytoma (n = 100)	Gonadotroph Adenoma (n = 100)
M/F	14/9	14/14	38/8
Ratio	1.5/1	1/1	4.5/1

cells, are immunoreactive for FSH, and/or alpha-subunit [15]. If this assumption is valid, 23% of null cell adenomas and 28% of the oncocytomas in our series could be considered gonadotroph adenomas.

It has been demonstrated by immunocytochemistry that clinically nonsecreting adenomas may be positive not only for glycoproteins but, in a few cases, for other types of adenohypophyseal hormones [5, 15, 18, 26]. These observations are in keeping with results of tissue culture and more advanced studies employing the reverse hemolytic plaque assay as well as of specific probes for demonstration of the mRNA of various hormones [2,. 8, 21, 22, 27, 31]. Recently a case of a null cell adenoma exhibiting ultrastructural and immunocytochemical evidence of plurihormonality and plurimorphous differentiation has been reported [14]. In the present series, 55 tumors were immunopositive for GH, PRL, and less frequently for ACTH in addition to glycoprotein hormones. Such observations are of particular interest in the light of recent molecular biological studies, which, in applying clonal analysis to clinically nonfunctioning pituitary adenomas, indicate the monoclonal origin of such tumors [1, 6, 11]. These observations favor the hypothesis that pituitary adenomas originate from mature adenohypophyseal cells or precursor cells which undergo somatic mutation and are capable of differentiating not only toward gonadotrophs but, less frequently, toward other adenoma cell types [14]. Several factors such as oncogenes, interleukins, and growth factors may be implicated in neoplastic development and multidirectional differentiation. Although hypothalamic disturbances in the genesis of pituitary neoplasms are questionable and have yet to be elucidated, they may, in conjunction with other factors, contribute significantly to the development of some adenomas [1, 6].

Ultrastructural examination of pituitary adenomas assisted by immunocytochemistry continues to occupy a central place in the study of clinically nonsecreting pituitary adenomas. The term "null cell" should, at least for the present, be retained. Perhaps, with the introduction of new methods and more defined diagnostic criteria, the ever-constricting category of null cell adenoma will someday find a place in the nosology of pituitary adenomas under the term "chromophobic" adenoma.

Acknowledgments

This work is supported in part by grant MT-6349 awarded by the Medical Research Council of Canada and the generous contribution of Mr. and Mrs. Francis A. Wittern, Sr., as well as Mr. and Mrs. Francis A. Wittern, Jr. Authors wish to thank Mrs. Jane Karpenko, Mrs. Nancy Ryan, and Dr. Zi Cheg for their contribution to the immunocytochemical studies and Ms. Christine G. Kontogeorgos for organizing the material.

References

1. Alexander JM, Biller BMK, Bikkal H, Zervas NT, Arnold A, Klibanski A. Clinically nonfunctioning pituitary tumors are monoclonal in origin. J Clin Invest 86:338–340, 1990.
2. Asa SL, Gerie BM, Singer W, Horvath E, Kovacs K, Smyth HS. Gonadotropin secretion *in vitro* by human gonadotroph adenomas and oncocytomas. J Clin Endocrinol Metab 62:1011–1019, 1986.
3. Black PMcL, Hsu DW, Klibanski A, et al. Production of glycoprotein hormone subunits in clinically nonfunctioning pituitary adenomas. J Neurosurg 66:244–250, 1987.
4. Denk H, Radaszkiewicz T, Weirich E. Pronase pretreatment of tissue sections enhances sensitivity of the unlabeled antibody-enzyme (PAP) technique. J Immunol Methods 15:1663–1667, 1977.
5. Heitz PU. Multihormonal pituitary adenomas. Horm Res 10:1–13, 1979.
6. Herman V, Fagin J, Gonsky R, Kovacs K, Melmet S. Clonal origins of pituitary adenomas. J Clin Endocrinol Metab 71: 1427–1433, 1990.
7. Horvath E, Kovacs K. Gonadotroph adenoma of the human pituitary: sex-related fine dichotomy. A histologic and electron microscopy study of 30 tumors. Am J Pathol 117:429–440, 1984.
8. Hsu SM, Raine L, Fanger H. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. Am J Clin Pathol 75:816–821, 1981.
9. Jameson JL, Klibanski A, Black PMcL, et al. Glycoprotein hormone genes are expressed

in clinically nonfunctioning pituitary adenomas. J Clin Invest 80:1472–1748, 1987.
10. Klibanski A. Nonsecreting pituitary tumors. Pituitary tumors: diagnosis and management. Endocrinol Metab Clin North Am 16:793–804, 1987.
11. Klibanski A. Editorial: further evidence of somatic mutation theory in the pathogenesis of human pituitary tumors. J Clin Endocrinol Metab 71:1415A–1415C, 1990.
12. Klibanski A, Ridgway EC, Zervas NT. Pure alpha subunit-secreting pituitary tumors. J Neurosurg 59:585–589, 1983.
13. Kontogeorgos G, Horvath E, Kovacs K. Sex-linked ultrastructural dichotomy of gonadotroph adenomas of the human pituitary. An electron microscopic analysis of 145 tumors. Ultrastruct Pathol 14:475–482, 1990.
14. Kontogeorgos G, Kovacs K, Horvath E, Killinger WR, Smyth HS. Null cell adenoma of the pituitary with features of plurihormonality and plurimorphous differentiation. Arch Pathol Lab Med 115:61–64, 1991.
15. Kovacs K, Asa SL, Horvath E, Ryan N, Smyth HS, Scheithauer BW, Ebersold MJ. Null cell adenomas of the pituitary: attempts to resolve their cytogenesis. In: Lechago J, Kameya T, eds. Endocrine pathology update, vol I. Philadelphia: Field and Wood, 1990:17–31.
16. Kovacs K, Horvath E. Pituitary "chromphobe" adenoma composed of oncocytes. Arch Pathol 95:235–239, 1973.
17. Kovacs K, Horvath E. Tumors of the pituitary. Atlas of tumor pathology. Fascicle XXI, 2nd series. Washington DC: Armed Forces Institute of Pathology, 1986.
18. Kovacs K, Ryan N, Horvath E, Ezrin C. Null cell adenoma of the human pituitary. Virchows Arch [A] 387:165–174, 1980.
19. Landolt AM. Ultrastructure of human sella tumors. Correlation of clinical findings and morphology. Acta Neurochir (Wien) Suppl 22:1–167, 1975.
20. Landolt AM, Oswald UW. Histology and ultrastructure of an oncocytic adenoma of the human pituitary. Cancer 31:1099–1105, 1973.
21. Lloyd RV, Jin L, Fields K, Chandler F, Horvath E, Stefaneanu L, Kovacs K. Analysis of pituitary hormones and chromogranin A mRNA in null cell adenomas, oncocytomas, and gonadotroph adenomas by in situ hybridization. Am J Pathol 139:553–564, 1991.
22. Mashiter K, Adams EF, Van Noorden S. Secretion of LH, FSH, and PRL shown by cell culture and immunocytochemistry of human functionless pituitary adenomas. Clin Endocrinol 15:303–312, 1981.
23. McCormick WF, Halmi NS. Absence of chromophobe adenomas from a large series of pituitary tumors. Arch Pathol 92:231–238, 1971.
24. Oppenheim DS, Arnold RK, Jangbir SS, Klibanski A. Relevance of alpha-subunit hypersecretion in patients with pituitary tumors: clinical nonfunctioning and somatotroph adenomas. Clin Endocrinol Metab 70:859–864, 1990.
25. Ridgway EC, Klibanski A, Ladenson PW, et al. Pure alpha-secreting pituitary adenomas. N Engl J Med 304:1254–1259, 1981.
26. Saeger W, Gunzl H, Meyer M, Schulze C, Ludecke DK. Immunohistological studies on clinically silent pituitary adenomas. Endocr Pathol 1:37–44, 1990.
27. Sakurai T, Seo H, Yamamoto N, et al. Detection of mRNA of prolactin and ACTH in clinically nonfunctioning adenomas. J Neurosurg 69:653–659, 1988.
28. Snyder PJ. Gonadotroph adenomas of the pituitary. Endocr Rev 6:552–563, 1985.
29. Sternberger LA, Hardy PH Jr, Cuculis JJ, Mayer HG. The unlabeled antibody enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase–antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333, 1970.
30. Yamada S, Asa SL, Kovacs K. Oncocytomas and null cell adenomas of the human pituitary: morphometric and *in vitro* functional comparison. Virchows Arch [A] 413:333–339, 1988.
31. Yamada S, Asa SL, Kovacs K, Muller P, Smyth HS. Analysis of hormone secretion by clinically nonfunctioning human pituitary adenomas using the reverse hemolytic plaque assay. J Clin Endocrinol Metab 66:73–80, 1989.