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Case report

Atypical, invasive, recurring Crooke cell adenoma of the pituitary

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ABSTRACT

We report the case of a 49-year-old woman presenting with Cushing disease and visual disturbance. An atypical, aggressive, invasive pituitary tumor regrew despite several surgeries. Detailed morphologic investigation by histology, immunohistochemistry and electron microscopy documented a Crooke cell adenoma, a rare form of ACTH-producing pituitary tumor. Recognition of such adenomas is of importance given their aggressive behavior and tendency to recur. More studies are needed to explain the pathobiology of this not invariably functional pituitary adenoma.

Key words: Crooke cell adenoma, Histology, Immunohistochemistry, Neoplasm, Ultrastructure

INTRODUCTION

Pituitary tumors producing ACTH are a biologically disparate group of adenomas. Several subtypes can be distinguished on the basis of clinical, histologic, immunohistochemical and ultrastructural features.[1-5] Their correct classification is of importance given differences in biologic behavior, therapy and prognosis.[6-13] Endocrinologically functional ACTH adenomas are associated with Cushing disease or Nelson syndrome.[14,15] Clinically nonfunctioning tumors are classified as "silent corticotroph adenomas" of subtypes 1 and 2.[3,4] Both pituitary carcinoma and the recently described pituitary blastoma often produce excess ACTH with resultant Cushing disease.[16-19] Crooke cell adenoma is relatively rare but not invariably functional.[20]

Herein, we report the case of a 49-year-old woman presenting with Cushing disease and visual disturbance. The tumor invaded the cavernous sinus and was only partly removed by transsphenoidal surgery. The tumor regrew and several surgeries were performed. No metastases were documented. Detailed morphologic investigation including histology, immunohistochemistry and electron microscopy revealed the characteristic features of Crooke cell adenoma.

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CASE REPORT

The patient, a 49-year-old woman, presented at

the age of 43 with Cushing disease and visual field deficit. Magnetic resonance imaging (MRI) showed a large, multi-lobed sellar tumor, an unusual finding since only 8% of patients with Cushing disease present with mass effects including visual symptoms.[3,20]

In 2004, despite three separate transsphenoidal resections for recurrent tumor, the patient's initial complaint of visual deficit persisted. After much of the tumor was removed, mild improvement of Cushing disease was noted. Follow-up consisted of radiotherapy, subsequent treatment of temozolomide (85 mg p.o. daily) and serial neuroimaging. The tumor responded to temozolomide. Treatment was discontinued for a short interval after which symptoms resurfaced, prompting cabergoline (1 mg weekly) treatment, however with no effect. Five years after initial surgery, the patient presented with rapidly progressing left temporal visual field loss, ataxia and proximal muscle weakness. MRI revealed a sellar, right parasellar and suprasellar tumor measuring 1.5cm TR x 1.5 cm AP x 1.3 cm SI (Figure 1). The parasellar tumor was stable. The tumor encased and constricted the left carotid artery. A stereotactically guided, endoscopic, subtotal resection of the tumor was performed. Adjuvant therapy consisted of fractionated stereotactic radiotherapy (50 Gy in 25 fractions) and subsequent temozolomide chemotherapy (85 mg p.o. daily). No metastases were apparent. Positron emission tomography (PET) scan and cytology on the cerebrospinal fluid were not performed.

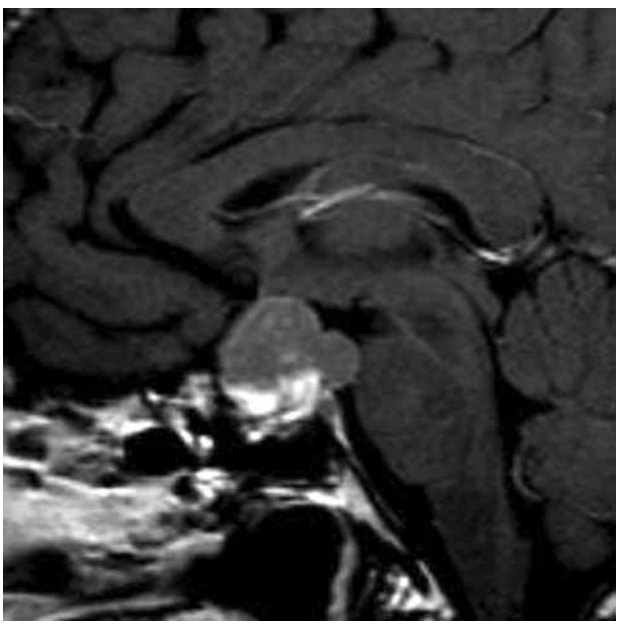

Figure 1. Magnetic resonance imaging (MRI) demonstrates a large suprasellar tumor. Sagittal view.

MORPHOLOGIC METHODS

The majority of the specimen was fixed in 10% buffered formalin, routinely processed for light microscopy and paraffin-embedded. Hematoxylin-eosin, periodic acid-Schiff and Gordon-Sweet silver stains were applied. Five-micron sections were subjected to immunohistochemical (streptavidin-biotin-peroxidase complex method) staining for the entire spectrum of adenohypophysial hormones including: growth hormone (GH) (Ventana, Tucson, AZ; polyclonal; 1:50); prolactin (PRL) (Ventana: SPM108; 1:1000); adrenocorticotropic hormone (ACTH) (Ventana, Tucson, AZ; polyclonal; 1:200); luteinizing hormone (LH) (Ventana, Tucson, AZ; ZAL11; 1:3000); follicle-stimulating hormone (FSH) (Ventana, Tucson, AZ; INN-hFSH-60; 1:3000); thyroid-stimulating hormone (TSH) (Ventana, Tucson, AZ; QB2/6; 1:400); and α-subunit (Biogenex, Sanraman, CA; monoclonal; 1:50). Antibody sources, clonality and dilution have been previously described.[21,22] Additional antibodies applied included: MIB-1 (Dako, Carpinteria, CA; MIB-1; 1:20); p53 (Dako, Carpinteria, CA; DO7; 1:2000); p27 (Dako, Carpinteria, CA; SX53G8; 1:50); topoisomerase-2 alpha (Dako, Carpinteria, CA; Ki-S1;1:100); CAM 5.2 (Ventana, Tucson, AZ;); O(6)-Methylguanine-DNA methyltransferase (MGMT) (Thermofisher, Fremont, CA; MG3.1; 1:50); and vascular endothelial growth factor (VEGF) (Santa Cruz Biotechnology, Santa Cruz, CA; A-20; 1:50). For electron microscopy, tissue was fixed in 2.5% glutaraldehyde, osmicated, dehydrated in graded ethanol, processed through propylene oxide, embedded in epoxy resin and studied on a Hitachi H7650 digital electron microscope.[21,22]

PATHOLOGIC FINDINGS

Based upon the morphologic features, a diagnosis of Crooke cell adenoma was made. The tumor resected in 2009 showed features similar to those of the tumor initially resected in 2004. Histologically, it

consisted of amphophilic, polygonal cells exhibiting a diffuse growth pattern and featuring perinuclear ring-like hyaline material. The cells of the tumor were PAS-positive and showed no significant cellular or nuclear pleomorphism (Figure 2). There was a loss of the acinar structure and the reticulin fiber network, a characteristic feature of pituitary tumors. No mitotic figures, necrosis, inflammatory cells or calcification were observed in the 2004 specimen. The 2009 specimen featured large zones of necrosis. The cells of all specimens showed extensive Crooke hyalinization. Cytoplasmic immunoreactivity for ACTH and perinuclear low molecular weight keratin staining were evident (Figures 3, 4). The tumor cells were negative for other adenohypophysial hormones. The MIB-1 nuclear labeling index ranged from 5% to 8%. Almost all tumor cell nuclei were immunopositive for topoisomerase-2 alpha. The tumor cells showed 80% immunoreactivity for p27, few cells showing p53 nuclear immunostaining (Figure 5). Nearly all the tumor cell nuclei were immunonegative for MGMT. Cytoplasmic reactivity for low molecular weight keratin and VEGF was noted in nearly all tumor cells (Figure 6).

Ultrastructural studies showed the adenoma to consist of large, ovoid cells with spherical nuclei containing macronucleioli and delicate heterochromatin. Their cytoplasm contained numerous intermediate cytokeratin filaments corresponding to Crooke hyaline, which obscured both rough endoplasmic reticulum and Golgi complexes (Figure 7). Secretory granules were both localized to the Golgi region and peripherally displaced within the cells. Most measured 100-200 nm and were smaller than those usually seen in corticotroph cells (200-350 nm). The mitochondria, although displaced by filaments, exhibited normal morphologic features.

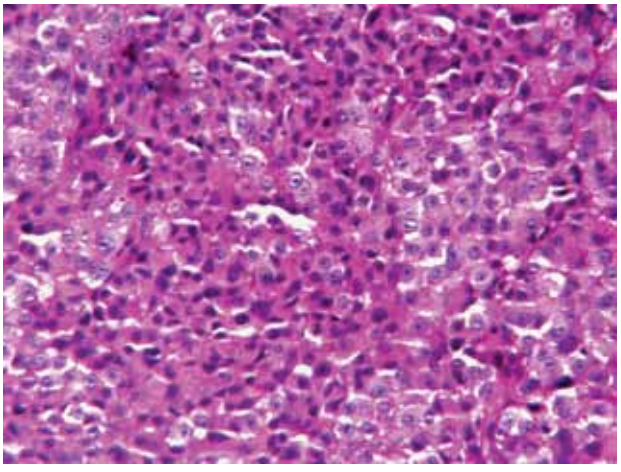

Figure 2. Light microscopy demonstrates a cellular pleomorphic, PAS positive tumor showing Crooke hyaline change. PAS immunostain. Original magnification: X250.

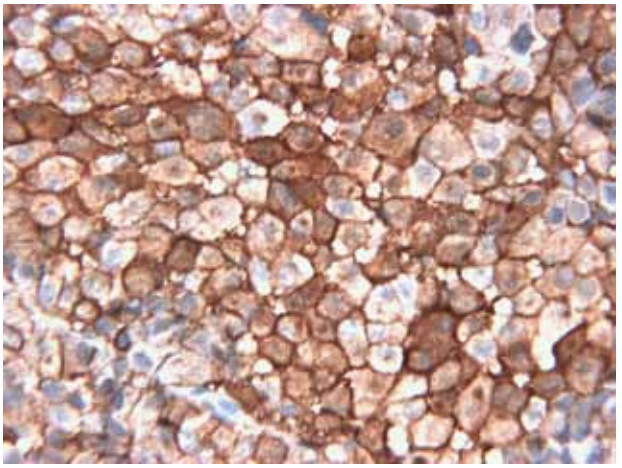

Figure 3. The tumor cells are immunoreactive for ACTH. Many tumor cells show marked Crooke hyalinization. Immunostaining for ACTH. Original magnification: X250.

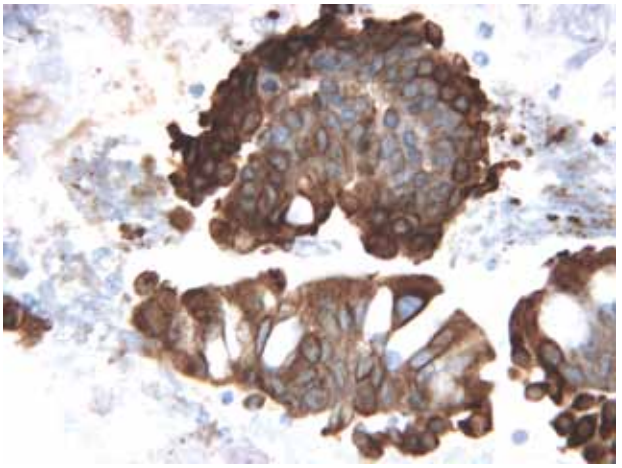

Figure 4. The tumor cells are immunopositive for low molecular weight keratin. Immunostaining for low molecular weight keratin. Original magnification: X400.

DISCUSSION

Crooke hyaline change was first described in 1935

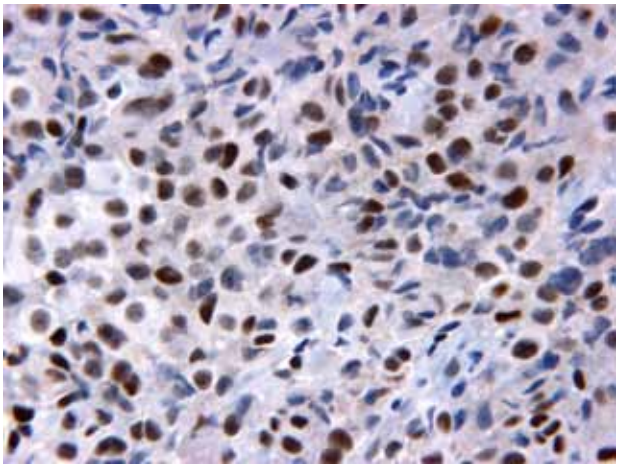

Figure 5. Many tumor cell nuclei are immunopositive for p27. Immunostaining for p27. Original magnification: X400.

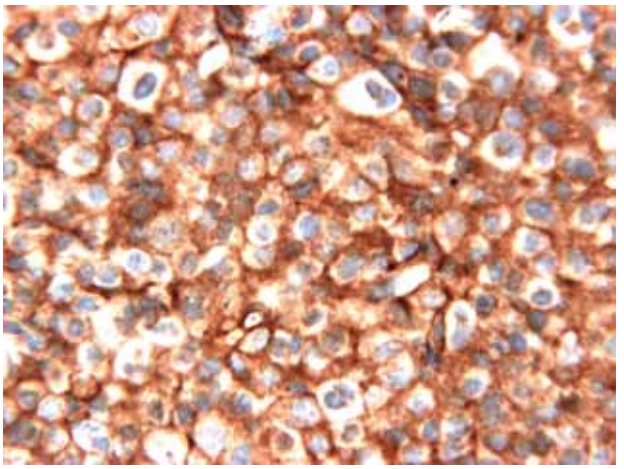

Figure 6. Almost all tumor cells show cytoplasmic immunopositivity for VEGF. Crooke hyalinization is prominent. Immunostaining for VEGF. Original magnification: x400.

by the endocrinologist A. C. Crooke who, while studying with Dorothy Russell at the London Hospital, reported his findings in 12 pituitaries of patients with Cushing disease.[23] It affects non-neoplastic corticotrophs in any setting of glucocorticoid excess, including that due to the administration of glucocorticoids.

Corticotrophs of the anterior pituitary normally contain small numbers of perinuclear cytokeratin filament bundles. Persistently elevated blood cortisol levels result in accumulation of these cytokeratin filaments with reduction, displacement of cytoplasmic organelles and secretory granules. Cytoplasmic

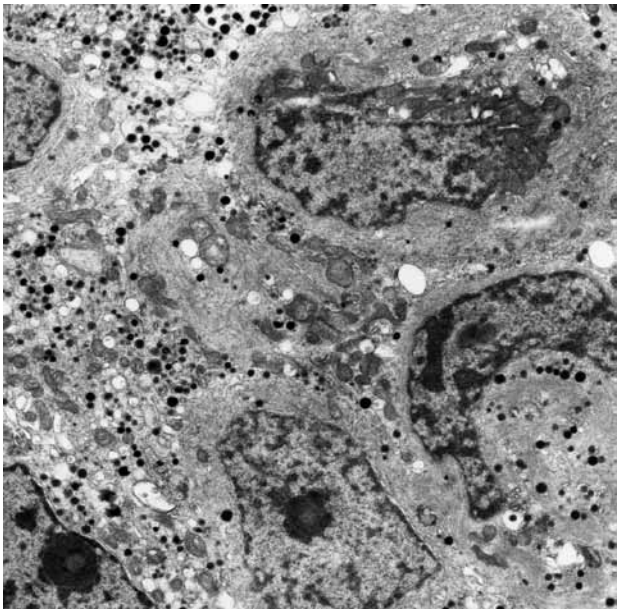

Figure 7. Electron microscopy shows the presence of many microfilaments in the cytoplasm of tumor cells. Transmission electron micrograph. Original magnification: X3000.

accumulation of filaments results in a distinctive cellular alteration known as Crooke hyaline change in which a circumferential ring of keratin gives the cell cytoplasm a distinctly hyalinized appearance on hematoxylin-eosin and PAS stains.[23-28]

On rare occasions, Crooke change also occurs in ACTH-producing pituitary adenomas.[24-26] Such adenomas display variable but often extensive Crooke hyalinization, as demonstrated in our case. Patients with Crooke cell adenoma may have previously shown evidence of hypercortisolism and Cushing disease.[29,30] In other instances, the tumor is functionally silent and unassociated with Cushing disease.[26,31,32] In clinicopathologic terms, Crooke cell tumors are unique and should not be confused with typical endocrinologically active corticotroph adenomas.

Although first described by Felix et al,[24] it was the large series of George et al[26] that clearly defined the endocrine, radiologic and behavioral profile of Crooke cell adenoma. Innately aggressive, nearly all are atypical macroadenomas, being invasive and prone to recurrence, and are associated with significant morbidity.[6,8-10,26,33-37] Rare examples of Crooke cell carcinomas have also been reported.[17,18,34-36] By definition, such tumors undergo cerebrospinal and/

or systemic metastases.[38-40]

Cell proliferation markers are routinely used to assess tumor aggressiveness. In the present case, increased MIB-1 nuclear labeling was accompanied by surprisingly low p53 expression. This is unusual in that p53 staining is associated with large tumor size, invasiveness and aggressive behavior.[9,33,41] p53 is a tumor suppressor protein playing an important role in cell response to DNA injury. p53 activation may result in cell cycle arrest, DNA repair and apoptosis.[42] In our case, strong and diffuse immunopositivity for topoisomerase 2 alpha and VEGF was also apparent. Both of these biomarkers have been found to be good predictors of tumor aggressiveness as well as indicators of poor or unfavorable prognosis.[43,44] An unexpected finding in this study was the high percentage of cell nuclei expressing p27, a protein that plays a regulatory role in the cell cycle by inhibiting the activity of cyclin dependent kinases (CDKs), resulting in suppression of cell cycle progression and causing G1 arrest. Lloyd et al, showed that there is a marked decrease in the expression of p27 in malignant endocrine neoplasms compared to normal tissues. This suggests that rapid cell proliferation should be accompanied by a decrease in the number of p27 immunoreactive tumor cell nuclei.[45] We cannot provide a conclusive explanation as to why p27 expression was high in the aggressive Crooke cell tumor investigated by us. It was recently reported that p27 may have an opposing role as well: it may function as a tumor suppressor but may also have an oncogenic effect.[46] Aside from low p53 and high p27 stainings, our findings are consistent with the view that biomarkers are useful in predicting tumor behavior.[9,33,47-50]

On balance, studies of Crooke cell adenomas pose more questions than answers. No explanation can be provided as to why Crooke hyalinization is rare in corticotroph tumors and why Crooke cell adenomas are sensitive to glucocorticoid excess, are aggressive and in some cases secrete large amounts of ACTH despite massive cytoplasmic accumulation of microfilaments, a feature of suppressed activity in normal corticotrophs.

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