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Invasive Giant Prolactinoma with Loss of Therapeutic Response to Cabergoline: Expression of Angiogenic Markers

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Abstract The present study reports the case of a 70-year-old Caucasian man who was referred to the Military Hospital of Buenos Aires for evaluation of a giant sellar-extrasellar mass with extension in the right temporal lobe and compression of the third ventricle. Patient was initially responsive to cabergoline with reduction of prolactin levels and shrinkage of tumor burden for at least 36 months. Thereafter, prolactin levels and tumor size increased even though cabergoline dosage was increased. Transcraneal surgery was performed at 56 months of treatment. Prolactin levels and tumor proliferation did not subside and the patient died 14 months later. High GH and IGF-I levels were observed in the late stages of tumor development, with no evidence of acromegalic features. Immunohistochemistry of the excised tumor revealed strong immunoreactivity for VEGF and FGF-2, two potent angiogenic factors, and CD31 (an endothelial marker) indicating high vascularization of the adenoma.

Keywords prolactinoma · dopamine agonist resistance · VEGF · FGF · Ki67 · IGFI

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Introduction

Prolactin-secreting adenomas are the most frequent type among pituitary tumors. Patients with prolactinoma usually present with endocrinological symptoms resulting from hyperprolactinemia and, less commonly, with visual defects due to compression of the optic chiasm. Macroprolactinomas are benign, slowly proliferating tumors, although they may be locally highly aggressive, particularly in males, and invade adjacent structures.

Giant prolactinomas (tumor volume exceeding 4 cm in diameter and/or with prolactin levels higher than 3,000 ng/ml and mass effect [1, 2]), a rare subcategory of prolactinomas, remain one of the greatest challenges in neurosurgery. Because of invasive growth, giant adenomas can compress or destroy adjacent structures, resulting in neurological dysfunction such as visual loss, and cavernous sinus compression. Pharmacological therapy with a dopamine agonist remains the mainstay of treatment. This therapy is effective in more than 85% of patients with prolactin-secreting pituitary tumors. A minority of patients will show no response to either bromocriptine or cabergoline [3] and development of dopamine agonist resistance in an initially responsive prolactinoma is unusual [4–6].

The formation of new blood vessels within neoplasms, termed angiogenesis, provides the tumor tissues with oxygen and basic energetic compounds. Invasive macroprolactinomas are significantly more vascular than noninvasive macroprolactinomas [7], and, in general, neovascularization occurs during the formation and growth of solid tumors. Inhibitors of angiogenesis were effective in the suppression of growth of experimental prolactinomas [8–10]; and, in angiographic studies, the presence of additional arteries (which were not part of the portal system) were found in 66% of patients with pituitary adenomas [11]. Nevertheless, the role of angiogenesis

in pituitary tumor development has been questioned, as the normal pituitary is a highly vascularized gland [12].

Experiments over the past decade indicate that vascular endothelial growth factor-A (VEGF) is a central regulator of angiogenesis in endocrine glands. VEGF enhances vascular permeability, participates in angiogenesis and tumorogenesis, wound healing, embryo organ development, and in reproductive functions in the adult [13]. Fibroblast growth factor 2 (FGF-2) is also a potent angiogenic growth factor, which was first described in pituitary tissues [14]. Levels of VEGF and bFGF are elevated in the peripheral blood of patients with pituitary adenomas [15], even though the expression of these two growth factors in pituitary tumors has been variable [16–19].

We report the case of a man who presented an initially dopamine-responsive giant prolactinoma, followed by the unusual development of late dopamine agonist resistance, marked proliferation of the tumor, and late secretion of GH, with no acromegalic features. The unusual clinical course prompted us, in retrospect, to make a detailed analysis of angiogenic factors in the tumor.

Case Report

A 70-year-old Caucasian man was referred to the Endocrinology Service of the Central Military Hospital of Buenos Aires for evaluation of a giant sellar-extrasellar mass. He suffered from severe frontal headache, sleep and satiety disorders, attention deficit, and an unsteady gait with tendency to veer to the left. He had a history of hypothyroidism as a consequence of hyperthyroidism treatment with methimazole and was therefore medicated with levothyroxine (125 μg/day).

Brain magnetic resonance imaging (MRI) revealed a voluminous sellar-extrasellar mass with extension in the right temporal lobe and partial compression of the third ventricle (tumor volume 202 cm^3, maximum diameter 7.5 cm [2]; (Fig. 1a).

Visual field could not be achieved because of lack of cooperation. The endocrine tests showed: serum prolactin 8,800 ng/ml (normal range (NR) 2.5–13 ng/ml), low LH, FSH, and testosterone levels: LH, 0.21 uUI/ml (NR, 1.2–8.6); FSH, 0.79 uUI/ml (NR, 1.2–19.2); testosterone, 0.37 ng/ml (NR, 1.75–7.8); morning cortisol, 18.3 ng/dl (NR, 8.7–22.4); FT4, 0.68 ng/ml (NR, 0.58–1.63); and TSH, 0.66 uUI/ml (NR, 0.49–4.6).

With diagnosis of invasive giant prolactinoma, cabergoline was prescribed; this therapy was initiated with 1 mg/week and the dose was increased up to 3.5 mg/week during the first year of treatment (Fig. 2a). The patient presented an overall improvement of symptoms, prolactin level decreased to 25 ng/ml and MRI revealed reduction of tumor volume to 6.8 cm^3 and of maximum diameter to

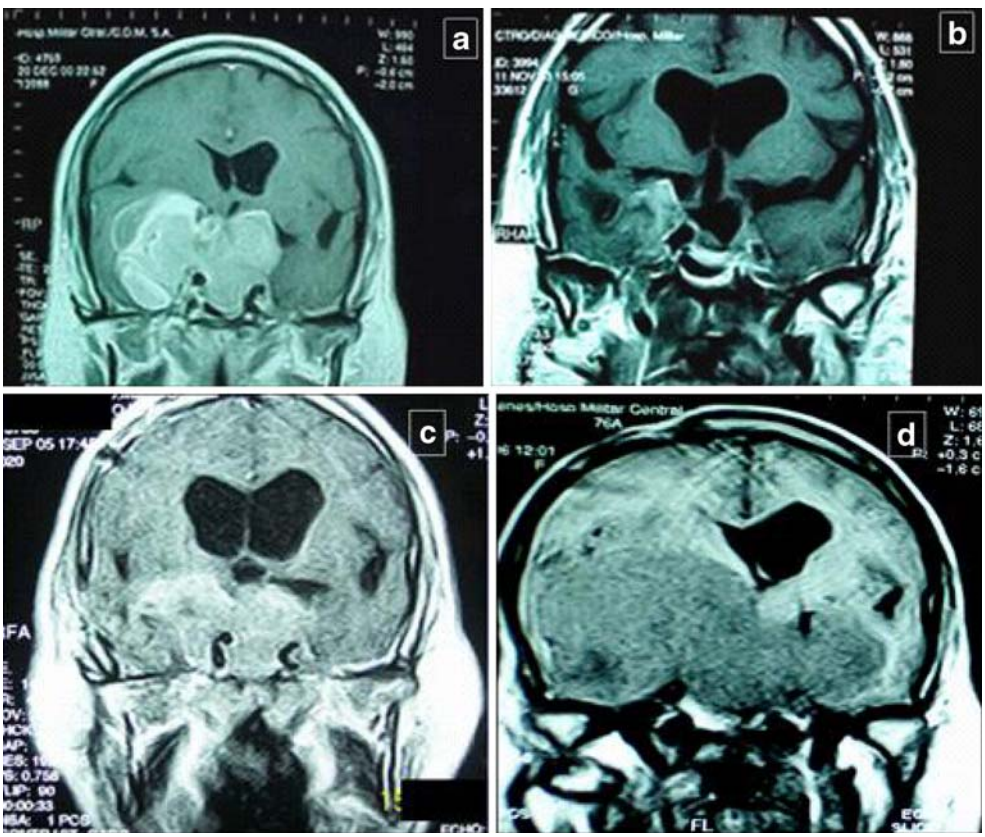

Fig. 1 MRIs from the patient **a** at the moment of the diagnosis, **b** 26 months of treatment, **c** immediate post-surgery, 57 months of treatment, **d** 6 months after surgery

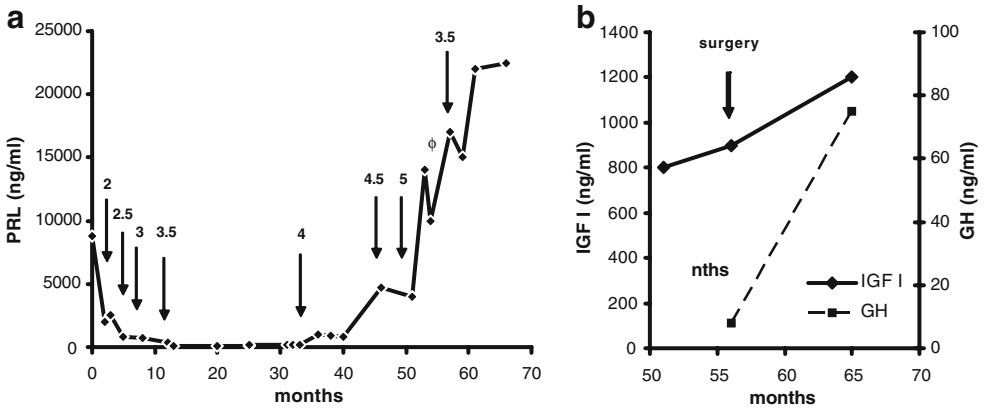

Fig. 2 **a** Serum PRL levels measured by Quimioluminiscence. *Arrows* indicate cabergoline treatment in mg/week. *Φ* indicates surgery. **b** Serum IGFI levels before and after surgery; serum GH levels after surgery

3.1 cm, 36 months after the beginning of therapy (Figs. 1b and 2).

Forty-four months after starting the treatment prolactin levels increased to 3,912 ng/ml, therefore cabergoline dose was elevated to 4.5 mg/week. Nonetheless, 5 months later, MRI showed tumor enlargement (53.9 cm^3 and maximum diameter 6.2 cm), and very high prolactin levels (16,000 ng/ml), therefore, cabergoline dose was increased to 5 mg/week. The patient presented episodic amnesia and visual loss of the right eye. IGF-I dosage was performed as pre-surgical protocol screening yielding 974 ng/ml (NR, 70–360). No acromegalic signs were present.

Given the accelerated tumor growth associated with failure of dopamine agonist response, visual symptoms and high IGF-I, surgery was decided. Partial resection was performed via transcraneal approach. Pathology reported a pituitary adenoma of solid growth, composed by uniform cells with slight eosinophilic and polygonal cytoplasms; nuclei showed moderate anisocariosis. No mitotic figures were present. Epithelial proliferation adopted a diffuse pattern and in some sectors, acinar organization could be evidenced. Most areas were densely vascularized and a fibrillar hyaline stroma could be found especially in perivascular areas. Immunohistochemistry examination showed densely granulated prolactin positive cells (Fig. 3a) and scattered groups of GH positive cells (Fig. 3b). The proliferation marker Ki-67 was moderately high: 4% of total cells. We evaluated P53, a nuclear phosphoprotein whose immunohistochemical accumulation has served as an unfavorable prognostic factor for a wide range of human neoplasms, by Western blot and immunohistochemistry using anti-p53 (Santa Cruz, abDOI). No p53 expression was found.

Four days after surgery, the patient's serum prolactin levels fell slightly to 10,000 ng/ml. The severe frontal headache subsided. A follow-up MRI scan showed no reduction in tumor volume (Fig. 1c) and prolactin levels remained persistently high even though cabergoline therapy was continued throughout (3.5 mg/week; Fig. 2a).

Octeotride treatment was initiated at dose of 20 mg/month. Six months after surgery, serum prolactin levels were 16,800 ng/ml, and serum IGF-I and basal GH were elevated (Fig. 2b; IGF-I, 1,872 ng/ml; GH, 12.30 ng/ml (NR, 0–4)) in spite of octeotride treatment, brain MRI showed enlarged invasive tumor with further adjacent structure compression (Fig. 1d).

Patient died 14 months after surgery and autopsy was not performed.

Immunohistochemistry

Tumor sample obtained from the surgical intervention was analyzed by immunohistochemistry for angiogenic factors and an endothelial marker (CD31). VEGF, FGF-2, and CD31 immunostaining was performed following routine protocols, as previously described [20]. Antibodies used were anti-hVEGF (1:100, Santa Cruz Biotechnologies, Santa Cruz, CA, USA); anti-h FGF2 (1:100, Santa Cruz); and anti-CD31 (1:200, Santa Cruz). We found strong VEGF immunopositivity around vessels, in the cytoplasm, and in cell nuclei (Fig. 3c, d). There was also FGF-2 staining in the nucleus of tumor cells (Fig. 3e) and the endothelial marker CD31 revealed intense immunopositivity in the samples (Fig. 3f).

Discussion

Dopamine agonist medication can normalize prolactin levels in more than 90% of cases of microprolactinomas and 77% of macroprolactinomas and reduce tumor volume in approximately 85% of cases. Its main mechanism involves the activation of dopamine D2 receptors on cell membrane of prolactin cells. Dopamine agonist resistant tumors have been recognized to have a particularly severe clinical course [21] and are generally more frequent in men [22]. Tumor size or the pretreatment serum prolactin levels are not predictive of the response to bromocriptine therapy

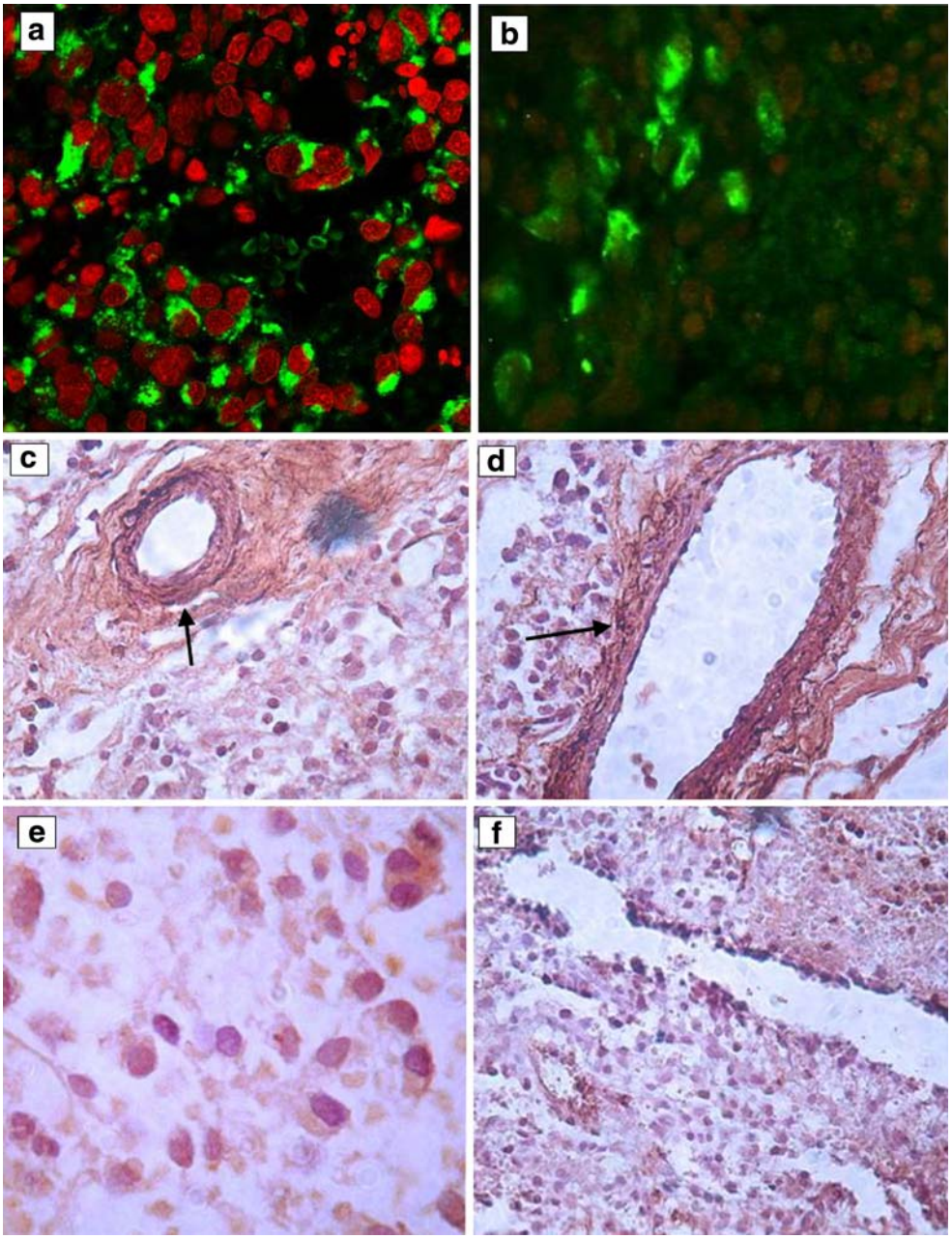

Fig. 3 **a** Immunofluorescence for PRL in a tumor section (*green* prolactin, *red* cell nuclei counterstained with propidium iodide; objective lens magnification ×60/1.4 oil). **b** For GH (labeled with FITC, *green*; objective lens magnification ×40/1.4 oil). **c** and **d** VEGF immunohistochemistry (positive reaction evidenced in *brown* (DAB), magnification ×40), *arrows* indicate enlarged vessels. **e** Immunohistochemistry for FGF-2 (positive reaction in *brown*, DAB, nuclei were counterstained with hemotoxylin, magnification ×100). **f** CD31 expression (positive reaction in *brown*, DAB, magnification ×20)

and microadenomas have been reported to be even less sensitive to bromocriptine than macroadenomas [23].

A particular characteristic of this patient was late resistance to cabergoline. Late resistance to dopaminergic agents is not common and may occur because after drug administration, tumor fibrosis becomes progressive and, to a certain extent, the tumor can shrink no further. On the other hand, absence, lower expression level, or a post-receptor defect of the dopamine D2 receptors may be causally related [24]. Caccavelli et al. [25] considered drug resistance to be strongly associated with the decrease in D2 receptor gene transcription, resulting in a fourfold decrease in the number of these receptors. Finally, silent abnormal endocrine cells within the tumor may continue proliferating even though secreting lactotropes are inhibited by dopamine agonists and contribute to tumor growth. Rarely, there may be malignant transformation of a prolactinoma.

In the present case, late resistance was associated with increasing IGF-I levels. In the immunohistological analysis of the excised tumor, few clustered groups of GH-positive cells were evidenced. But there were no acromegalic features in the patient associated to these findings. Significant excess of GH was a relatively new occurrence in the patient, either because tumor cells started secreting GH late, or because the tumor was inefficient in producing GH and therefore a very large tumor burden was required to cause biochemical evidence of excess GH.

We studied angiogenic factors expressed by the excised tumor. Pituitary adenoma growth, as with all tumors, depends on adequate vascularization, and enhanced VEGF expression has been associated with several human vascular tumors, including brain, colon, gastrointestinal tract, ovary, breast, and others [26].

In humans, Schechter et al. demonstrated that arteries are present in pituitary tumors but not in the normal pituitaries [27]. Therefore it seems possible that VEGF plays a role in human prolactin-secreting adenomas, as in animal models. It has been described that macroprolactinomas are significantly more vascular than microprolactinomas [28] and Turner et al. demonstrated a significantly higher degree of vasculature of invasive pituitary prolactinomas [7]. VEGF has been detected in all types of human pituitary adenomas, but primarily in those of somatotrophic or corticoptrophic type [29, 30], and has been associated to intratumoral hemorrhage [31]. Lloyd et al. reported decreased expression of VEGF in pituitary adenomas relative to non-tumorous pituitaries [29], even though carcinomas had high VEGF expression. Nevertheless, VEGF in invasive and noninvasive or dopamine-resistant prolactinomas, has not been systematically studied. Our present case report of a giant invasive prolactinoma, with loss of response to dopamine agonist therapy, presented strong immunoreactivity for VEGF and FGF-2, two potent angiogenic factors, and for CD31 (an endothelial marker) indicating high vascularization of the adenoma.

A relation of the dopaminergic D2 receptor (D2R) with endothelial cell proliferation within tumors has been put forth. It has been described that dopamine has antiangiogenic activity mediated through the D2Rs, inhibiting malignant tumors as well as the vascular permeabilizing and angiogenic activities of VEGF [32, 33]. In a transgenic mouse with targeted deletion of the D2R, which has been used as an experimental model for dopamine agonist resistance in prolactinomas [34], we have recently described that VEGF mRNA and protein were increased in the spontaneous prolactinomas harbored by this knockout mouse [17]. Therefore, VEGF overexpression may be linked to dopamine resistant tumors.

We found a relatively high Ki-67 index, and according to Gaffey et al., primary tumors that exhibit increased mitotic activity (Ki-67>3%) should be termed “atypical adenomas” to denote their aggressive potential and the possibility of future malignant transformation [35]. We found no expression of p53. The presence of p53 protein is a rare event in pituitary adenomas [36] and appears to be associated with invasiveness, though there is no indication of its efficacy as a routine marker of tumor aggressiveness [37].

Each pituitary tumor of clonal origin, represents the multifactorial result of failure of different regulatory events. In this regard, pro- and anti-angiogenic growth factors may determine the final angiogenic phenotype of pituitary tumors, and thus subsequent tumor behavior. We believe that the study of angiogenic factor expression in aggressive prolactinomas with resistance to dopamine agonists will yield important data in the search of therapeutical alternatives.

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