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Pituitary Pathology in Carney Complex Patients

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Abstract

Carney complex (CNC) is a familial multiple neoplasia syndrome with features overlapping those of McCune-Albright syndrome (MAS) and multiple endocrine neoplasia (MEN) type 1 (MEN 1). Like MAS and MEN 1 patients, patients with CNC develop growth hormone (GH)-producing pituitary tumors. Occasionally, these tumors are also prolactin-producing, but there are no isolated pro-lactinomas or other types of pituitary tumors. In at least some patients with CNC, the pituitary gland is characterized by hyperplastic areas; hyperplasia appears to involve somatotrophs *only*. Hyperplasia most likely precedes the formation of GH-producing adenomas in CNC, as has been suggested in MAS-related somatotropinomas, but has never been seen in MEN 1 patients. In at least one case of a patient with CNC and advanced acromegaly, a GH-producing macroadenoma showed extensive genetic changes at the chromosomal level. So far, half of the patients with CNC have germline inactivating mutations in the *PRKARIA* gene; in their pituitary tumors, the normal allele of the *PRKARIA* gene is lost. Loss-of-heterozygosity suggests that *PRKARIA*, which codes for the regulatory subunit type 1 α of the cAMP-dependent protein kinase A (PKA) may act as a tumor-suppressor gene in CNC somatotrophs. These data provide evidence for a *PRKARIA*-induced somatotroph hyperplasia in the pituitary tissue of CNC patients; hyperplasia, in turn may lead to additional genetic changes at the somatic level, which then cause the formation of adenomas in some, but not all, patients.

Keywords

Carney complex; pituitary tumors; hyperplasia; genetics; *PRKARIA*; protein kinase A

Introduction

The complex of “spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas” or Carney complex (CNC) is a multiple endocrine neoplasia (MEN) and lentiginosis syndrome [1–4] that is inherited in an autosomal dominant manner [5], and is genetically heterogeneous [6–8]. Although growth hormone (GH) and prolactin (PRL) secretion are frequently abnormal in affected patients [9,10], clinical acromegaly or significant hyperprolactinemia and GH- or PRL-producing tumors, respectively, have been detected in less than one fifth of them [11,12]. The pattern of biochemical abnormalities of GH and PRL secretion without pituitary tumors that are detectable by common imaging modalities, and infrequent development of clinically significant acromegaly is reminiscent of the situation in McCune-Albright syndrome (MAS) [13–16]. Studies of tumors excised from patients with

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CNC had indicated that their molecular abnormality may be in the molecular pathway that involves the stimulatory α -subunit of the guanine nucleotide-binding protein ($G\alpha$) [17,18], *GNAS*— the gene responsible for MAS [13]. However, $G\alpha$ mutations were not present in an investigation of a series of CNC tumors [18].

Approximately half of the patients with CNC have germline inactivating mutations in the *PRKARIA* gene [19,20], which codes for the regulatory subunit type 1 α of the cAMP-dependent protein kinase A (PKA) [21]. Loss-of-heterozygosity (LOH) in some tumors from patients with CNC suggested that *PRKARIA*, may act as a tumor-suppressor gene in tissues affected by CNC [22,23]. However, *PRKARIA*'s role in human oncogenesis is controversial, and studies mainly from cell line-related work have not suggested a tumor-suppressor role [24].

Pituitary Findings in Patients with CNC

Clinical and histopathologic analysis

GH-producing tumors have been identified so far in several CNC patients (Table 1) with clinically diagnosed acromegaly at the National Institutes of Health [25]. The frequency of *PRKARIA* mutations in these patients is the same as in the whole group (approximately 50%); clinical details have been described elsewhere [25]. All CNC-related GH-producing tumors stained positive for PRL and occasionally for other hormones (Table 1). Most of the patients who had acromegaly as the primary manifestation of CNC had macroadenomas, as is the case for most acromegalic patients. Multiple macroscopic and microscopic tumors were seen in the pituitary gland of several patients (Fig. 1), including one with a microadenoma (data not shown). In these patients, extra-tumoral pituitary parenchyma showed evidence of GH- and PRL-producing cell hyperplasia.

Adenohypophyseal hyperplasia, characterized by poorly delineated zones with increased cellularity and an expanded, somewhat irregular reticulin pattern was seen in most pituitary gland tissues excised from CNC patients (Fig. 2). A zone of probable transition from hyperplasia to adenoma, characterized by the gradual disappearance of the reticulin pattern and increasing cellularity, was also documented in these cases.

Both hyperplastic areas and adenoma tissue stained for GH and PRL in all patients. PRL-staining was less intense and more limited than GH-staining [25], although in all cases it was the same cellular population that demonstrated immunoreactivity for both GH and PRL at consecutive sections. Staining for α -subunit was also present in most tumors in the same pattern as that of PRL. Occasional staining for TSH (β -subunit) and LH was also present in diffusely and rarely present cells of some adenomas and within foci of normal cells entrapped within the tumors. ACTH and FSH staining, when obtained, could only be seen in foci of normal cells entrapped within the tumors or the hyperplasia.

Electron microscopy studies

We recently described the electron microscopy findings of two GH-producing tumors from patients with CNC (Cases 1 and 2, Table 2) [26]. One additional patient has recently been studied (Case 3, Table 2, Fig. 3).

The tumors described by Kurtkaya-Yapici et al. consisted of large, closely apposed, slightly irregular cells. The nuclei were ovoid or variably irregular and possessed a well to extensively developed nucleolus as well as small quantities of stippled heterochromatin. Rough endoplasmic reticulum was abundant and organized in parallel arrays or well-developed and randomly disposed short profiles. Golgi complexes were conspicuous, occupied a large portion of the cytoplasm and contained spherical or irregular, fused, immature secretory granules.

Outside the Golgi zone, secretory granules were scant and measured up to 350 nm in diameter, most ranging between 200–250 nm. Extrusion of secretory granules was occasionally seen. Cell membranes between neighboring cells often showed complex inter-digitation. Cytoplasmic fibrous bodies were not identified. A very few adenoma cells (1%) were densely granulated, possessed well-developed rough endoplasmic reticulum and very prominent Golgi complexes. Their secretory granules measured up to 500 nm in diameter, most ranging between 350–400 nm. Granule extrusion was seen. Based on immunoelectron microscopy (see below), these cells were considered mammosomatotrophs.

In the first tumor [26], many of the tumor cells had lactotrophic characteristics, but secretory granules in their Golgi areas labeled for both PRL and GH. The large secretory granules of the densely granulated cell component were either bi-hormonal or mono-hormonal, labelling either for GH and/or PRL. Thus, the immunore-activities in this case were not necessarily true to the ultrastructural phenotype.

In the second tumor, both nontumoral adenohypophysis and adenoma were seen [26]. The adenoma featured large irregular cells with eccentric, sometimes markedly irregular nuclei of which containing moderately developed nucleoli and small amounts of stippled heterochromatin. Rough endoplasmic reticulum was poorly to moderately developed and Golgi complexes were inconspicuous. Secretory granules were small (less than 250 nm) and sparse in some adenoma cells, numerous and measured up to 450 nm in others. Several tumor cells contained a prominent fibrous body. The non-tumoral adenohypophysis was normal. Immunogold labeling for only GH was noted in this tumor [26].

A third tumor has been investigated recently (case 3 in Table 2, Fig. 3). Its features were similar to the above two tumors (Table 2, Fig. 3) but, in addition, LH- and GH-staining was seen in several tumor cells.

Overall, the electron microscopy features of CNC-associated pituitary tumors are not consistent but appear to be highly variable and not very different from those of GH-secreting, sporadic (non-syndromic) pituitary adenomas [26].

Genetic analysis of CNC pituitary tumors

Comparative genomic hybridization (CGH) analysis of 3 CNC-associated microadenomas showed no significant changes over normal DNA [25]. In contrast, analysis of the most aggressive tumor, an invasive macroadenoma, showed multiple changes, including losses of chromosomal regions 6q, 7q, 11p, 11q, and gains of 1pter-p32, 2q35-qter, 9q33-qter, 12q24-qter, 16, 17, 19p, 20p, 20q, 22p, 22q [25]. The greatest contiguous changes were losses of the long arm of chromosome 6 and the entire chromosome 11.

The CNC tumors studied to date from patients with inactivating *PRKARIA* mutations have demonstrated LOH for the 17q22–24 *PRKARIA* locus [19]. There have been no studies of pituitary tissue from patients with CNC that appear to have other genetic defects.

Overview

Acromegaly is usually characterized by a slow, progressive course. In CNC this course is even slower and acromegaly is often unmasked by adrenalectomy for primary pigmented nodular adrenocortical disease (PPNAD) [27]. In our prospective evaluation of patients with CNC, we have had the opportunity to observe the “development” of pituitary adenomas, so far in 4 patients. In these cases, biochemical abnormalities of GH and PRL secretion were present in advance of radiological detection of the tumor [9,10]. The incidence of GH-producing pituitary tumors in CNC has been estimated at less than 15% [1,6,11], whereas GH “paradoxical”

responses to various stimuli [such as to thyrotropin-releasing hormone (TRH)] or IGF-I elevation, without a detectable tumor, maybe present in up to 80% of affected patients [25]. The finding of probable hyperplasia in pituitary tissue from patients with CNC, who underwent TSS for acromegaly, is consistent with these clinical observations.

The GH-secreting adenoma in all cases where adequate tissue was available for examination appeared to be surrounded by regions with expanded irregular reticulin structure, and GH-, PRL-, and occasionally α -subunit immunoreactive cells. It is noteworthy that in these patients, multiple tumors were also seen both macroscopically and microscopically. PRL staining was not present in all the GH-stained areas; accordingly, PRL levels in the peripheral blood were not markedly elevated in most patients with CNC, in contrast to GH or IGF-I levels in the same patients. Electron microscopy studies showed significant differences between the two CNC-related adenomas [26], suggesting that not all GH-producing adenomas are the same and these lesions are potentially pleiomorphic.

The genetic investigation complemented the above findings by suggesting that in its evolution, the largest and most aggressive CNC-associated tumor [25] had accumulated a series of genetic changes; in contrast, the small adenomas had normal CGH results. These findings are in agreement with the hypothesis that pituitary tumors develop from clonal expansion of transformed somatic cells [28,29]. They are also consistent with observations in patients with MAS [14–16] and some patients with MEN 1 [30]. CNC and MAS are genetic conditions that share skin pigmentation abnormalities, adrenocortical hyperplasia, thyroid tumors and even myxomas. However, in most tissues, the lesions are histologically and clinically different in the two conditions: skin lentigines and blue nevi *versus* café-au-lait spots, micronodular and pigmented dysplasia *versus* adrenocortical macronodular hyperplasia, hormonally silent nodules or cancer *versus* thyroid hormone hypersecretion, and skin *versus* intramuscular myxomas [1,6,14,31,32]. Mammosomatotroph hyperplasia may be the only lesion that is in fact common in CNC and MAS [33]. Clinically, too, both share a “pro-acromegalic” state [34], which only rarely leads to the detection of an adenoma [10,14–16,34]. Similar, long-standing somatotroph hyperplasia, which only occasionally leads to pituitary adenoma has been seen in several other patients, albeit GHRH-induced [35,36].

The genetic changes required for the formation of an adenoma in the background of benign hyperplasia are not known but appear to be multiple. As other investigators have shown in tumors with Gs α mutations or allelic losses of the *MEN 1* locus [37,38], pituitary genetic changes tend to increase in number and significance in parallel with the clinical behavior of the neoplasm [28,29]. Pituitary tumorigenesis in CNC patients may follow the pattern of mutation accumulation that has been suggested for other neoplasms [39,40]. The extensive genetic instability of cells cultured from CNC tumors suggests that secondary “hits” underlie tumor formation in CNC, the first “hit” being the germline *PRKARIA* mutation, in at least these patients that have a *PRKARIA* defect. This corresponds to Knudson’s hypothesis [41].

Is PRKAR1A Mutated in Other Pituitary Tumors?

We speculated above that the germline CNC mutation causes a predisposition towards other molecular events that are necessary for pituitary tumor formation in CNC patients. Although the evidence suggests that this may be the case in CNC, it is not known whether *PRKARIA* can cause sporadic pituitary tumors when mutated at the somatic level. Three recent studies [42–44] have demonstrated that *PRKARIA* is an unlikely molecular etiology of non-familial pituitary tumors, not unlike the case of *menin*, the MEN 1 gene [45].

Conclusion

Occasional CNC patients with known *PRKARIA*-inactivating mutations have somatomammotroph hyperplasia and/or adenomas. Additional genetic changes at the somatic level are triggered by the hyperplasia, which in turn cause the formation of GH-producing adenomas in some, but not all, patients. Molecular investigation of *PRKARIA*'s effects on cellular signalling and the cell cycle, as well as mouse model studies, are expected to shed light on this gene's role in pituitary tumorigenesis. Although epigenetic modification of its function is not unlikely, somatic *PRKARIA* mutations are not frequent in sporadic pituitary tumors.

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Fig. 1. Hyperplasia (and tumor tissue) in the pituitary gland from a CNC patient with acromegaly and a GH-producing pituitary adenoma (case 3, Table 2): immuno-stainings with antibodies specific for (A) reticulin; (B) GH; (C) PRL; and (D) LH β -subunit (all at $\times 20$).

B

Fig. 2.

Panels A and B are from case 1 (Table 1): (A) Abnormally expanded and irregular reticulin pattern, consistent with adenohypophyseal cell hyperplasia ($\times 100$); (B) disrupted reticulin pattern in the adenomatous tissue from the same patient ($\times 40$).

A

B

C

D

Fig. 3. Electron microscopy images from the same patient (case 3, Table 2) with GH- and LH-producing tumorlets [A and B] (7,682 $\times$ and 5,380 $\times$ respectively). Secretory granules are identified by GH-specific antibody [C] (14,200 $\times$) and double immunostaining for both GH and LH [D] (18,200 $\times$).

Table 1
Clinical presentation and histology of pituitary adenomas from patients with CNC

	Clinical manifestation	Tumor size	Adenoma staining					Hyperplasia
			GH	PRL	α -sub	β -TSH	β -LH	
1	Presented with acromegaly	Macro	+	+	NA	+	+	Yes
2	High IGF-I	Micro	+	+	+	+	-	NA
3	High IGF-I	Micro	+	+	NA	NA	NA	NA
4	High IGF-I	Micro	+	+	-	+	-	Yes
5	Presented with acromegaly	Macro	+	+	+	NA	+	Yes
6	Presented with acromegaly	Macro	+	+	NA	NA	NA	Yes
7	Presented with acromegaly	Micro	+	+	+	-	+	Yes
8	High IGF-I	Micro	+	+	-	NA	NA	NA

Abbreviations GH = growth hormone, IGF-I = insulin-like growth factor type I, LH = luteinizing hormone, Macro = macroadenoma (greatest diameter over 1 cm), Micro = microadenoma (greatest diameter less than 1 cm), NA = not available, PRL = prolactin, TSH = thyroid stimulating hormone.

Table 2
Clinical and electron microscopy findings of pituitary adenomas from 3 CNC patients

	Clinical manifestation	Tumor size	Adenoma staining					EM
			GH	PRL	α-sub	β-TSH	β-LH	
1	19-year-old, female with acromegaly	Macro	+	+	±	–	–	Abundant RER; immature, fused secretory granules; double GH and PRL staining.
2	28-year-old, male with acromegaly	Macro	+	±	NA	±	–	Poorly developed RER; small and sparse secretory granules; fibrous bodies in several tumor cells; immunogold staining only for GH.
3	35-year-old, male with acromegaly	Macro	+	+	±	–	+	Variable morphology between tumor cells; prominent RER and Golgi; double immunogold staining for GH and LH in several tumor cells but not in all.

Abbreviations: α-sub=alpha-subunit; GH=growth hormone; LH=luteinizing hormone; Macro=macroadenoma; PRL=prolactin; RER=rough endoplasmic reticulum; TSH = thyroid stimulating hormone.