Cribiform-Morular Variant of Papillary Thyroid Carcinoma

Molecular Characterization of a Case With Neuroendocrine Differentiation and Aggressive Behavior

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Key Words: APC; Cribiform-morular variant; Familial adenomatous polyposis; Neuroendocrine differentiation; Papillary carcinoma; RET-PTC, p53; β-Catenin; Thyroid cancer; CD10

Abstract

We describe an especially aggressive case of cribriform-morular variant (C-MV) of papillary thyroid carcinoma (PTC) in a 42-year-old man with familial adenomatous polyposis who died with lung and brain metastases 17 months after thyroidectomy. The angioinvasive neoplasm combined a mixture of trabecular, solid, cribriform, and follicular patterns of growth with CD10+ morules. Follicles were devoid of colloid, and the nuclear features typical of PTC were present in some areas and missing in others. Tumor cells were positive for thyroid transcription factor-1 and, in 40% of the tumoral mass, also were positive for chromogranin and synaptophysin and were negative for thyroglobulin and calcitonin. Strong nuclear staining for β-catenin was found in all tumor cells, as was positivity for p53 and cyclin D1. In addition to the germline heterozygous APC Ex 2-3 duplication mutation, a somatic homozygous silent p.Thr1493Thr gene variant was found in the neoplastic cells along with RET/PTC rearrangement. This tumor represents the first case of C-MV of PTC showing neuroendocrine differentiation.

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder characterized by numerous adenomatous colorectal polyps that have an intrinsic tendency to progress to adenocarcinoma.1 It is caused by a germline mutation in the adenomatous polyposis coli (APC) gene, which is located on the long arm of chromosome 5 (5q21-22), although in up to 5% of families, the genetic defect causing FAP is not known.1-3 Extracolonic manifestations include epidermoid cysts, dental abnormalities, congenital hypertrophy of the retinal pigment epithelium, desmoid tumors, gastric and upper intestinal adenomas and carcinomas, hepatoblastomas, osseous tumors, brain tumors, and other tumors.1 In 1949, Crail4 made the first case report of malignancies arising in the rectum, brain, and thyroid gland, and, in 1968, Camiel et al5 suggested for the first time the relationship of FAP with thyroid carcinoma. A study from the St Mark’s Hospital Polyposis Registry,6 London, England, revealed an association between FAP and thyroid carcinoma showing that young women with FAP had approximately 160 times more risk of developing thyroid cancer than did healthy people. The prevalence of thyroid carcinoma in different FAP registries has been reported to be 1% to 2%.7,8 Recently, however, a 12% overall prevalence of thyroid carcinoma in a cohort of 51 patients with FAP was reported,9 probably related to increased detection of subclinical disease, a true increased incidence of thyroid carcinoma, or both.

In 1994, Harach et al10 first characterized the thyroid carcinoma developing in patients with FAP as a distinct follicular cell tumor in view of its histologic differences from papillary and follicular carcinoma. They also suggested that these unusual histologic features in a thyroid tumor, especially if multicentric, should alert physicians to the possibility of FAP with its implications for family screening.
Other studies confirmed the findings of Harach et al. The term cribriform-morular variant (C-MV) of papillary thyroid carcinoma (PTC) was coined by Cameselle-Teijeiro and Chan in 1999 to describe the sporadic counterpart of FAP-associated thyroid carcinoma. These authors reported this tumor type as morphologically indistinguishable from most thyroid carcinomas that arise in the setting of FAP and as a peculiar variant of PTC. The term cribriform-morular variant is now generally used to describe this tumor type when it occurs as a sporadic tumor (often solitary) and in the setting of FAP (often multicentric). Herein we describe a case of C-MV of PTC with 2 peculiar previously unreported features: neuroendocrine differentiation and very aggressive behavior.

Materials and Methods

Study Case

A 42-year-old man with known FAP and absence of polyps in his last colonoscopy underwent a colectomy for diverticulitis and a high risk of colon cancer. During the preoperative examinations, a thyroid mass was found. There was a familial history of colon carcinoma affecting his maternal grandfather, his mother, 2 of 5 maternal uncles, and 1 cousin. His mother and his only brother also had osteomas. In previous examinations, genetic analysis of all 15 exons of the APC gene and promoter using the MLPA (multiplex ligation-probe amplification)-APC kit (MCR-Holland, Amsterdam, the Netherlands), revealed the same heterozygous APC Ex 2-3 duplication in the blood samples of the patient and his son. The familial study for congenital hypertrophy of the retinal pigment epithelium was negative. A fine-needle aspiration biopsy of the thyroid mass provided evidence suggestive of PTC, and a total thyroidectomy with central compartment clearance was performed.

After histologic diagnosis, a computed tomography (CT) scan showed bilateral lung metastases. Given the nature of the tumor, radiiodine therapy was considered inappropriate; based on the focal neuroendocrine differentiation, radiolabeled somatostatin analogue therapy was attempted. During follow-up, there was partial response to treatment; soon, however, blurred vision, multiple brain metastases evident on CT scan, and progressive impairment developed. The patient died at 17 months after thyroidectomy.

Histopathologic and Immunohistochemical Analysis

The surgical specimen was fixed in neutral, phosphate-buffered, 10% formalin, and paraffin-embedded sections were stained with H&E. Immunohistochemical studies were performed on 4-µm-thick paraffin sections using a peroxidase-conjugated dextran-labeled polymer (DAKO EnVision Peroxidase/DAB [diaminobenzidine]; DAKO, Glostrup, Denmark) to avoid misinterpreting endogenous biotin or biotin-like activity in cell cytoplasm or in nuclei as positive staining. Antibodies, dilutions, suppliers, pretreatment, and immunostaining results are listed in Table 1.

Molecular Genetic Analysis

For molecular genetic analysis, genomic DNA was extracted from the paraffin-embedded tumor tissue using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer’s instructions.

We screened for mutations in exon 11 and 15 of the BRAF gene. Exon 11 was amplified by polymerase chain reaction (PCR) using the forward primer 5'-GCATAAGGTAATGTACTTAGGGTGAA-3' and the reverse primer 5'-AACAGTGAAATTTCCCTTTGATGAT-3'; and for exon 15, the following primers were used: forward primer, 5'-TCATAATGCTTGGCTAGGAG-3'; and reverse primer, 5'-GCCAAAATTTAATCAGTGG-3'. These primers were previously designed with the Primer3 Input program (http://frodo.wi.mit.edu/cgi-bin/primer3/ primer3_www.cgi). The PCR conditions were as follows: denaturation at 94°C for 3 minutes followed by 35 cycles at 94°C for 30 seconds, 30 seconds of annealing at 60°C for exon 15 and at 55°C for exon 11, and 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. The PCR products were bidirectionally sequenced in capillary electrophoresis (ABI3730, Applied Biosystems, Foster City, CA) using the aforementioned primers.

Three sets of primers, as previously reported, were used to amplify, by PCR analysis, the MCR region of the APC gene (codons 1286-1513).

Sequences of H-RAS (exons 1 and 2) and N-RAS (exon 2) were analyzed as described by Castro et al, and CTNNB1 exon 3 analysis was performed as described by Rocha et al. The PCR mixture (25 mL) contained 2.5 mL of 10× complete PCR buffer (Bioron, Ludwigshafen, Germany), 1 mL of deoxynucleoside triphosphates (5 mmol/L each), 0.1 ng of each primer, 0.1 to 0.5 ng of genomic DNA, and 0.2 U of Taq DNA Polymerase (Bioron). After 10 minutes of initial denaturation, the PCR mixtures were subjected to 35 cycles of denaturation for 30 seconds at 95°C, annealing for 45 seconds at variable temperatures according to the amplicon, and extension for 45 seconds at 72°C. A final extension period of 10 minutes at 72°C was performed to complete the reaction. The PCR products were analyzed by direct sequencing. Samples were sequenced on both strands after enzymatic purification treatment. Sequencing was performed in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).
**Results**

**Gross, Histologic, and Immunohistologic Findings**

Diverticular disease, but no polyps or carcinoma, was found in the surgical specimen of colectomy, in concordance with an attenuated form of FAP.

The thyroid gland weighed 75 g and showed a solid, fleshy, yellowish, and hemorrhagic tumor measuring 87 mm in maximal dimension. Histologic examination revealed a circumscribed, highly cellular, and angioinvasive neoplasm partially divided into lobules by sclerotic septa. The tumor combined a mixture of trabecular, solid, cribriform, and follicular patterns of growth with scattered squamoid islands (morules) **Image 1** and **Image 2**. The luminal spaces were devoid of colloid. The tumor cells were columnar or cuboidal but in some areas formed solid fascicles or whorls.
**Image 1** Cribriform-morular variant of papillary thyroid carcinoma showing an infiltrative margin (A) and vascular invasion (B). The tumor combined a mixture of growth patterns, including cribriform (C), trabecular, solid (F), and follicular (D). All of the patterns can sometimes be interspersed with morules (D). In some areas, an adamantinous-like pattern (E), atypical mitotic figures (F), and hyaline globules (thanatosomes; E) were also found (A, H&E, ×100; B-F, H&E, ×200).
Cribriform-morular variant of papillary thyroid carcinoma. Tumor cells are negative for thyroglobulin (A), but immunoreactivity for thyroid transcription factor-1 (B), β-catenin (E), and p53 (G) was detected. Positivity for synaptophysin (C) and chromogranin (D) was found in cells of some tumor lobules. Strong CD10 immunoreactivity was found in morules (F) (A, B, and D, ×200; C and F, ×100; E and G, ×400).
of spindle-shaped cells, sometimes in an adamantinous-like pattern (Image 1). The nuclei were crowded, stratified, round to oval, and pale, with only occasional grooves and very occasional pseudoinclusions. In morules, the nuclei were frequently filled with lightly eosinophilic, homogeneous (biotin-rich) inclusions (Image 1D). Frequently, the nuclear features typical of papillary carcinoma were replaced by more atypical vesicular nuclei with more prominent nucleoli (Image 1F). Alcian blue–negative hyaline globules (thanatosomes) were found in one area of the tumor (Image 1E), and there was an average of 7 mitotic figures per 10 high-power fields. No psammoma bodies, irregular calcifications, necrosis, or lymph node metastases were found.

The results of the immunohistochemical study are summarized in Table 1. The tumor cells showed reactivity for thyroid transcription factor-1 (TTF-1) (Image 2B), cytokeratins (CKs; clone AE1/AE3, CK7, and CK19), vimentin, Hector Battifora mesothelial cell-1, galectin-3, progesterone receptor, α-estrogen receptor, bcl-2, p27KIP1, p53 (Image 2G), and cyclin D1. Cytoplasmic and strong nuclear staining for β-catenin was observed in almost every tumor cell (Image 2E). Positivity for chromogranin and synaptophysin was found in cells of some tumor lobules in about 40% of the neoplasm (Images 2C and 2D). Faint, patchy E-cadherin expression and very faint and/or focal immunoreactivity for carcinoembryonic antigen, CA 19.9, CD5, β-estrogen receptor, and androgen receptor were found. Strong CD10 immunoreactivity was found in morules (Image 2F) and in a few scattered cellular groups. No immunoreaction was noted for thyroglobulin (Image 2A), thyroperoxidase, calcitonin, CK20, S-100 protein, epidermal growth factor receptor, c-kit (CD117), or Wilms tumor 1. Some S-100+ dendritic cells were scattered throughout the tumor but not in the normal thyroid tissue. Specifically, intravascular tumor cells were also positive for TTF-1 and CK7 with negativity for CK20. The proliferative index in the neoplasia, evaluated with the immunostaining for MIB-1, was 60%.

Molecular Genetic Analysis

In the study of the APC gene, in addition to the germline heterozygous APC Ex 2-3 duplication mutation, we detected a homozygous, nondeleterious, c.4497G/G genotype at codon 1493 in normal thyroid tissue, along with a homozygous c.4497A/A genotype at the same codon, which was detected only in the thyroid tumor cells. RET/PTC rearrangement was observed in 11% of the 200 nuclei analyzed. No PAX8-PPARγ rearrangement was found, nor were CTNNB1, BRAF, N-RAS, or H-RAS gene mutations detected.

Discussion

According to the criteria at our disposal, the tumor herein described fits with the diagnosis of the CM-V of PTC. This tumor type is characterized by a prominent
cribriform pattern of growth with interspersed squamoid islands (morules) that frequently harbor nuclei filled with lightly eosinophilic, homogeneous, biotin-containing inclusions. Closely packed follicles, papillae, and trabeculae are commonly admixed. Characteristically, the luminal spaces are devoid of colloid. The tumor cells are columnar or cuboidal, and the nuclei are often chromatin-rich; nuclear grooves, pale or clear nuclei, and intranuclear cytoplasmic inclusions are often seen focally. Some tumor cells can be plump and spindle-shaped, forming fascicles or whorls. The neoplasia is often circumscribed or even encapsulated, with or without capsular and/or vascular invasion.

Because of its peculiar features, CM-V of PTC presents several major differential diagnoses. Tall cell variant of PTC\textsuperscript{33} is a neoplasm with columnar cells, but in contrast with our case, is usually a highly papilliferous tumor composed of cells whose height is at least 3 times their width; the nuclei are mostly basally located and not different from those of conventional PTC, and the cells are always immunoreactive for thyroglobulin.

Columnar cell carcinoma\textsuperscript{33} of the thyroid also shows significant morphologic overlap with the CM-V of PTC (pseudostratified columnar cells, solid areas, and elongated, empty follicles resembling tubular glands). In fact, a case classified as a columnar cell carcinoma occurring in a patient with FAP\textsuperscript{34} probably represents a FAP-associated CM-V of PTC. However, tumor cells in columnar cell carcinoma have striking nuclear hyperchromasia, may contain supranuclear and subnuclear cytoplasmic vacuoles reminiscent of those of early secretory endometrium, and are thyroglobulin+.

Poorly differentiated carcinoma\textsuperscript{35} has a solid-trabecular-insular pattern of growth and a high mitotic index (≥2 mitotic figures per 10 high-power fields) and lacks the conventional features of PTC, partially mimicking the present case; however, at variance with poorly differentiated carcinoma, our case displays a cribriform pattern of growth with CD10+ morules and negativity for thyroglobulin.

The peculiar morphologic appearance of CM-V of PTC is related to the permanent activation of the Wnt pathway, with aberrant nuclear expression of β-catenin, as a consequence of germline mutation of the APC gene,\textsuperscript{11,14-17,19,22,36,37} somatic APC gene mutation,\textsuperscript{24} and/or somatic mutations of the β-catenin gene (CTNNB1).\textsuperscript{26} The familial data, nuclear translocation of β-catenin, and APC mutations strongly support this diagnosis in the present case. Morules are different from squamous metaplasia and also represent a diagnostic clue to C-MV of PTC.\textsuperscript{26} The results we obtained with CD10 immunostaining support the recent proposal of this marker as a useful tool for identifying morules in the “biotin-rich, optically clear nuclei” family of tumors that occur in different organs, all sharing alterations in the APC/β-catenin pathway.\textsuperscript{38} These findings also make the designation “cribriform-morular” variant more appropriate for such tumors than “cribriform” variant, which appears in the World Health Organization classification of endocrine tumors.\textsuperscript{33}

The existence at the APC gene of the common genetic variant c.4497A/A (p.Thr1493Thr) found in homozygosity in the present case is particularly puzzling. Subramanian et al\textsuperscript{22} reported the homozygous genotype c.4497G/G in all tumoral components of a case of C-MV of PTC but not in the normal tissue. Although these changes are considered to be silent (common genetic variants that are inconsequential in patients with FAP-associated tumors), the presence of the changes at the same locus and limited only to the tumor cells in our patient and the patient described by Subramanian et al\textsuperscript{22} could suggest a possible pathogenetic role in this tumor type.

\(RET/PTC\textsuperscript{-1}\) and \(RET/PTC\textsuperscript{-3}\) rearrangements were previously reported in thyroid carcinoma associated with FAP.\textsuperscript{11,39} We know that the most distinctive molecular features of PTC are a series of functionally similar and mutually exclusive alterations such as \(BRAF\) mutation, \(RET/PTC\) and \(TRK\) rearrangements, or \(RAS\) mutation.\textsuperscript{33,40} At variance with PTC, follicular carcinoma has a high prevalence of \(RAS\) mutations and \(PAX8-PPAR\gamma\) rearrangements.\textsuperscript{33,40} In the present case, reactivity for CK19, down-regulation of E-cadherin expression, S-100 protein+ dendritic cells, and \(RET/PTC\) rearrangements, combined with the absence of \(PAX8-PPAR\gamma\) rearrangement, support the cribriform-morular tumor being a subtype of PTC.\textsuperscript{33,40}

With regard to the existence of areas with neuroendocrine differentiation, the diagnosis of medullary carcinoma or a mixed medullary-follicular carcinoma can be excluded based on the negativity for calcitonin. The presence of nuclear translocation of β-catenin in all tumoral cells makes a collision tumor also improbable. A trabecular arrangement reminiscent of hyalinizing trabecular tumor has been reported as typical of C-MV of PTC,\textsuperscript{23} and colloid is scant or absent in both tumor types.\textsuperscript{23,33} A case of hyalinizing trabecular carcinoma of the thyroid was reported in a woman with FAP.\textsuperscript{41} It is interesting that dual endocrine and neuroendocrine differentiation has been described in hyalinizing trabecular tumor,\textsuperscript{42,43} and a very close histogenetic relationship between hyalinizing trabecular tumors and PTC has been proposed based on the similar nuclear features, CK19 expression, and the presence of \(RET/PTC\) somatic translocations in both lesions.\textsuperscript{33,42} The dual differentiation in the present case fits well with the idea of common pathogenetic mechanisms involved in the development of all these tumors.

In addition to the neuroendocrine differentiation, the rapid fatal outcome in the present case is also an intriguing finding. Conventional C-MV of PTC has a striking female predominance (female/male ratio ≈ 17:1) with a mean age at diagnosis of the thyroid tumor about 28 years, sometimes pre-dating the diagnosis of FAP, and is generally associated with
a good prognosis; however, 6 patients were found to have died of the neoplasia of a total of 126 cases reported in the literature. At variance with most of the cases on record, the present case occurred in a man older than 40 years; despite the association of these features with worse prognosis, we think they do not explain the dreadful evolution of the present case. On the other hand, histologic and histochemical findings provide some clues that may be related to clinical aggressiveness. The tumor was very angioinvasive and displayed hyaline globules (thanatosomes), which have recently been proposed as a histologic marker of enhanced cell turnover and/or ischemic injury. High mitotic activity and positivity for p53, normally restricted to aggressive thyroid lesions, together with the overexpression of cyclin D1, are also associated with tumor progression, thus fitting with a less differentiated (poorly differentiated carcinoma), more aggressive, form of the C-MV of PTC.

References


