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Case study

Tubulopapillary adrenocortical adenoma in a patient with familial adenomatous polyposis: a morphologic, ultrastructural, and molecular study☆

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Summary Patients with familial adenomatous polyposis have a higher incidence for developing adrenal neoplasms, most of which are nonfunctioning with conventional histologic appearance. We report a patient with a history of multiple colon polyps who developed an adrenocortical adenoma with unusual morphology. The tumor showed a tubulopapillary architecture and plasmacytoid cytomorphology that were distinct from conventional adrenocortical adenomas. β-Catenin stain showed aberrant nuclear positivity in the tumor, suggesting an altered β-catenin–related pathway. The unusual morphology prompted molecular characterization, and sequencing demonstrated the patient to be germline heterozygous for a 5-base-pair *APC* deletion at codon 1309 with loss of heterozygosity in the tumor. Our study provides further evidence of genetic predisposition to extraintestinal tumors in the familial adenomatous polyposis population.

1. Introduction

Studies have demonstrated a higher incidence of adrenal neoplasms in patients with familial adenomatous polyposis (FAP) compared with the general population [1,2]. Most adrenal neoplasms found in FAP patients are nonfunctioning adrenocortical adenomas (ACAs) with conventional morphology. Unusual morphologic features have rarely been reported [3,4]. Although adenomatous polyposis coli (APC) gene mutations are commonly found in FAP patients, the molecular profiles of the ACAs in this population are very limited. We recently encountered an ACA in a 26-year-old female FAP patient that showed very distinct morphology. Histologically, the tumor exhibited tubulopapillary and plasmacytoid features that differ from conventional ACAs. We also performed immunohistochemical (IHC), electron microscopic, and molecular studies on this tumor for further characterization.

2. Materials and methods

2.1. Case presentation

The patient was a 26-year-old woman with a history of adenomatous polyposis and chronic hepatitis C. Her prior medical record was notable for numerous polyps in the gastrointestinal

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tract, with biopsy findings demonstrating multiple tubular adenomas. Her initial germline testing did not identify any clinically significant gene mutations, but she did meet clinical diagnostic criteria for FAP. During evaluation before a total colectomy, she was found to have a left adrenal mass by abdominal computed tomographic scan, whereas her right adrenal gland appeared normal. The mass grew from 1.7 to 3.2 cm in 3 years. Her biochemical evaluation and hormonal workup were within normal range. No history of malignancy was known. No family history of FAP or adrenal cortical tumor was reported. A left laparoscopic adrenalectomy was performed on the patient, and the postoperative course was uneventful.

2.2. Morphologic and IHC examination

Gross examination was performed on the resected specimen. Microscopic examination was performed on formalin-fixed, paraffin-embedded tissues stained with hematoxylin and eosin (H&E). For IHC studies, formalin-fixed, paraffin-embedded sections were immunostained with the following antibodies using standard protocol: inhibin, CD56, and synaptophysin from Cell Marque, Rocklin, CA; β-catenin (β-catenin-1), vimentin, cytokeratin AE1/AE3, chromogranin, Wilms tumor 1, and mindbomb E3 ubiquitin protein ligase 1 (MIB-1) from Dako, Glostrup, Denmark; Melan-A from Ventana, Tucsan AZ; CD99 from BioLegend, Dedham MA; and steroidogenic factor-1 (SF-1) from Santa Cruz, Dallas, TX. The protocol for forkhead box L2 (FOXL2) stain was performed as previously described [5].

2.3. Electron microscopic examination

Electron microscopy was performed in a Hitachi H600 transmission electron microscope from formalin-fixed tissue that was postfixed in glutaraldehyde, embedded in EPON, and routinely processed for ultrastructural examination.

2.4. Molecular studies

Available H&E slides from the patient were reviewed, including normal adrenal gland, normal gastrointestinal tissue, tubular adenomas, and the adrenal tumor, to identify the block(s) most suitable for molecular analysis. Unstained slides were prepared, and regions of lesional and normal tissues were manually macrodissected separately before DNA extraction following the Pinpoint (Zymo) protocol from each tissue type. Spectrophotometry methods quantified DNA concentrations.

DNA from the patient's normal and tumor tissues was sequenced using the Ion Torrent AmpliSeq Cancer Hotspot V2 panel (Thermo Fisher Scientific, Waltham, MA). DNA and library concentrations were quantified by spectrofluorometry methods (Qubit). Genomic DNA was used to prepare a library following the Ion AmpliSeq Library Kit 2.0 paired with the Ion AmpliSeq Cancer Hotspot Panel v2 (207 primer pairs/pool) protocols. Ion Xpress barcode adapters were then ligated to the fragmented DNA on both 5′ and 3′ ends. Templates were prepared by emulsion polymerase chain reaction (PCR) using the Ion OneTouch 2 system. Libraries and Ion Sphere Particles (ISP) were added to the Ion OneTouch 2, and the ISPs containing template were enriched. The quality of the enriched template-positive ISPs was assessed using the Qubit 2.0 Fluorometer. Enriched ISPs were then loaded onto a 316 or 318 chip for semiconductor sequencing of the targeted region. The version 5.2 of TMAP and Torrent variant caller informatics tools were used to identify variants. Variants were further reviewed and annotated using the GenomOncology Clinical Workbench software. Validation studies conducted in the MCW Clinical and Translational Research Lab have demonstrated that the analytical sensitivity was greater than 95% and specificity was 100% for detecting single base-pair substitutions and small insertions-deletions with variant allele frequencies of greater than 3.5%.

Sanger sequencing was performed to confirm the presence of the variant in both the normal and tumor samples using M13-tailed primers to amplify a fragment of DNA flanking *APC c.3927*. The PCR amplicons were confirmed by electrophoresis in 2% agarose gel. PCR products were sequenced in both directions using the BigDye Direct Cycle Sequencing Kit according to the manufacturer's conditions. After the sequencing step, the BigDye Xterminator Purification Kit was used to clean up the dye excess. Sequence data were obtained with a 3500 genetic analyzer (Applied Biosystems, Foster City, CA). Sequencing results were analyzed using SeqScape v2.7 (Applied Biosystems).

3. Results

3.1. Histopathologic findings

3.1.1. Gross findings

The resected adrenal gland weighed 34 g and showed a 3.5 × 2.5 × 2.5-cm well-circumscribed, thinly encapsulated nodule located in the cortex. The nodule exhibited a yellow-brown, homogeneous, rubbery cut surface. No necrosis or hemorrhage was grossly identified. The remainder of the adrenal gland was unremarkable, showing a well-demarcated tan-orange cortex and tan-brown medulla, each measuring 0.1 cm in maximum thickness.

3.1.2. Histologic findings

The tumor showed an unusual morphology characterized by a striking tubulopapillary growth pattern with stromal hyalinization (Fig. 1B and C). The tumor was composed of uniform, polygonal cells with eccentrically located nuclei, speckled chromatin pattern, inconspicuous nucleoli, and abundant eosinophilic granular cytoplasm (Fig. 1D). The tumor cells showed peculiar nuclear polarization away from the delicate central fibrovascular cores, focally simulating the appearance of corded growth pattern (Fig. 1D). A few microscopic

foci showed transitions with more conventional ACA morphology, consisting of tumor cells with abundant vacuolated cytoplasm (Fig. 1A). The tumor cells were devoid of mitotic activity, and the tumor was well circumscribed and encapsulated without evidence of vascular invasion. Given the absence of necrosis, vascular and capsular invasion, cytological atypia, and the inconspicuous mitotic activity, the tumor was classified according to Weiss criteria as an unusual benign adrenal cortical neoplasm.

3.1.3. IHC findings

IHC stains revealed that the tumor cells showed diffuse nuclear positivity for SF-1; uniform cytoplasmic positivity for inhibin, Melan-A, vimentin, and synaptophysin; and strong membranous positivity for CD56 and CD99 (Fig. 2A and B). Stains for cytokeratin AE1/AE3, chromogranin, Wilms tumor 1, and FOXL2 were negative. CD10 showed faint focal positivity in the tumor cells. These IHC stain results support the adrenal cortical origin of the tumor. An MIB-1 proliferation marker showed a low proliferative rate (<5% of positive nuclei). β-Catenin stain showed aberrant nuclear positivity in the tumor cells in comparison with the expected weak membrane and focal cytoplasmic positivity in the adjacent normal adrenal gland (Fig. 2C).

3.2. Electron microscopic findings

Thin sections stained with uranyl acetate and lead citrate showed large round cells with round, slightly eccentric nuclei displaying peripheral margination of chromatin and scattered euchromatin. The cytoplasm contained abundant mitochondria with lamellar cristae admixed with scattered cisternae of smooth endoplasmic reticulum and occasional lipofuscin granules (Fig. 2D). Lipid vacuoles were scanty or absent.

3.3. Molecular findings

Considering the patient's clinical presentation of FAP, the patient was re-referred for genetic counseling and repeat germline genetic testing. The IonTorrent AmpliSeq Cancer Hotspot Panel v2 was performed on normal and tumor DNA and revealed a 5-base-pair (bp) AAAGA deletion involving a pentameric tandem repeat. The 5-bp deletion results a transcript that codes for the variant p.E1309fs*4 (c.3927_3931delAAAGA). Sanger sequencing was used to confirm this variant. The variant allele frequency was 46.8% in normal tissue and 88% in tumor tissue. Comparison of the total sequence reads indicated that relatively equivalent libraries were prepared and sequenced from both normal and tumor DNAs. However,

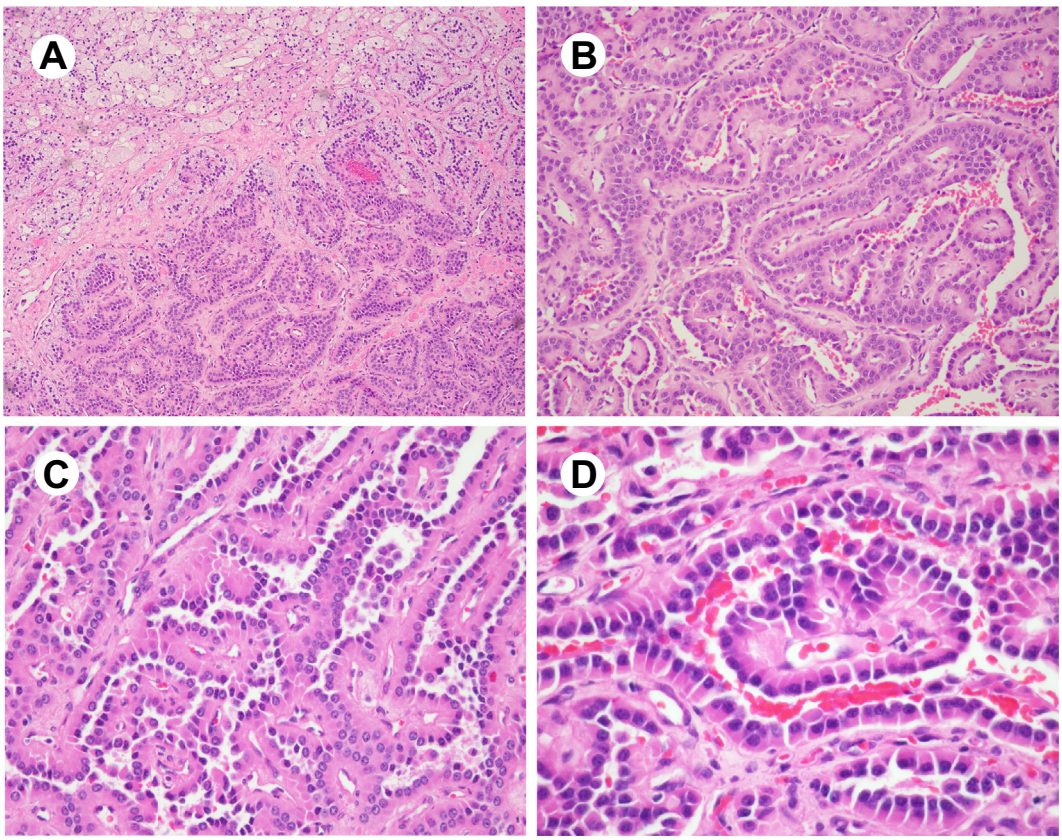

Fig. 1 Microphotographs of the ACA. A, Areas showing ACA with conventional histology (upper left) and tubulopapillary architecture (lower right). E; ×10 objective. (;B-D, Higher-power views demonstrated uniform, large, and polygonal tumor cells with abundant plasmacytoid eosinophilic cytoplasm. H&E (B) ;, original magnifications ×10 objective (A), ×20 objective (B), ×40 objective (C), and ×100 objective (D).

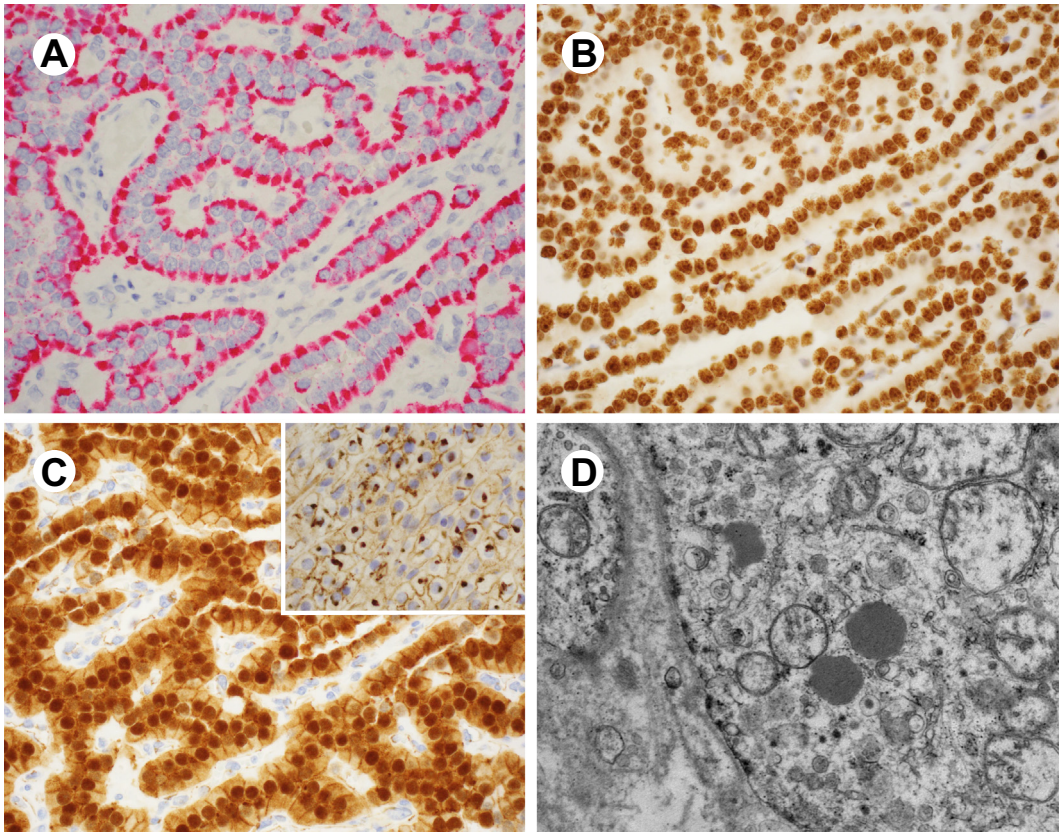

Fig. 2 IHC stains and electron microscopic figures. Melan-A showed cytoplasmic positivity (A), whereas SF-1 showed diffuse nuclear positivity in the tumor cells (B; ;original magnification ×40 objective). C, β-Catenin showed aberrant nuclear staining of the tumor cells, whereas normal adrenal gland showed weak membranous staining pattern (inset; ×40 objective). D, Electron microscopic figures showed the cytoplasm of the tumor cells with abundant mitochondria, scattered cisternae of smooth endoplasmic reticulum, and occasional lipofuscin granules.

coverage of the reads containing the *APC* 5-bp deletion was 1.5× higher in the normal library compared with the tumor library, suggesting that the patient was germline heterozygous for the 5-bp *APC* deletion with acquired loss of heterozygosity (LOH) in the tumor.

In addition, genetic germline testing had been previously performed in 2013 at a commercial reference laboratory using a next-generation sequencing (NGS) panel targeting the following genes: *APC*, *BMPR1A*, *CDH1*, *CHEK2*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *PMS2*, *PTEN*, *SMAD4*, *STK11*, and *TP53*. This germline testing did not identify any clinically significant gene mutations at that time. Based on our results, we requested reanalysis of these initial NGS data with a fresh sample and current NGS methodology and bioinformatics analysis, and this confirmed a low-level deletion signal at position c.3927 in the *APC* gene at a heterozygous ratio consistent with a heterozygous change, identical to that found by AmpliSeq and Sanger sequencing. The deletion was detected at levels below the threshold that this reference laboratory used for follow-up confirmation at the time the testing was originally performed. Therefore, this variant was identified by 3 independent sequencing assays in 2 College of American Pathologists/Clinical Laboratory Improvement Amendments–certified laboratories.

4. Discussion

Our present case is an apparent de novo FAP patient who was found to have a growing left adrenal mass during a preoperative evaluation. She underwent adrenalectomy, and histopathologic examination revealed an ACA with unusual morphology. Adrenal neoplasms are a frequently occurring extraintestinal manifestation in patients with FAP, partly because these patients often undergo abdominal imaging and have a higher likelihood of adrenal masses being identified. In a retrospective study, Marchesa et al [1] found a 7% prevalence of adrenal "incidentalomas" in patients with FAP compared with 3% in the general population. The prospective study by Smith et al [2] on 107 FAP patients reported the prevalence of adrenal masses to be 13%. So far, more than 50 cases have been reported in the literature, many of which were asymptomatic [6]. Although ACAs are commonly found in FAP patients, adrenal carcinomas are rare, with only a few cases being reported [1].

Although adrenal cortical tumors have been reported frequently in FAP patients, the morphology of most tumors is that of a classic ACA [3]. The present case has unique features compared with the previous reports. The histologic appearance

of this tumor is unlike that of conventional adrenal cortical neoplasms because of the unusual tubulopapillary architecture and plasmacytoid features (Fig. 1). ACAs with unusual histologic appearance have been reported before, but they did not share the same features as in our case. The case reported by Wakatsuki et al [3] documented an adrenocortical tumor in the setting of FAP, in which they described polygonal cells that formed trabeculae and/or islands. In a study of an FAP patient with ovarian steroid cell tumor, Hu et al [4] also reported an adrenal nodule containing cells with eosinophilic cytoplasm arranged in a corded, trabecular pattern. In both cases, there was striking heterogeneity of the tumor cell nuclei. The present case differs in that the tumor cells were monotonous and plasmacytoid, and the architecture was distinctly tubulopapillary. In addition, the prominent nuclear localization of β-catenin was not seen in the case reported by Hu et al. Wakatsuki et al mentioned the similarity of the morphology of their case to a gonadal sex–cord stromal tumor. For comparison, we performed FOXL2 IHC stain in our case. FOXL2 IHC has been recently shown to be a sensitive marker for sex-cord differentiation [5]. The negative IHC staining for FOXL2 in our case argues against differentiation toward sex-cord stromal neoplasm in the current tumor.

Recognition of the unusual morphology seen in our tumor is of importance for differential diagnosis of the lesion. The presence of a striking tubulopapillary growth pattern and the monotonous population of plasmacytoid cells may initially introduce difficulties for diagnosis and raise the possibility of a metastasis from a papillary carcinoma from another organ. Negative staining for cytokeratin should be the first indication that the tumor does not represent a metastasis from a papillary carcinoma; moreover, positive staining for inhibin and Melan-A will help support the primary adrenal cortical origin of the tumor. Identification of this peculiar growth pattern in a primary adrenal cortical neoplasm should prompt genetic testing for the *APC* gene.

Using NGS technique, LOH at the *APC* gene locus was observed in the ACA. Despite original germline testing reporting negative results, subsequent analysis revealed a pathogenic frameshift mutation in the *APC* gene caused by a 5-bp deletion (p.E1309fs*4). Although codon 1309 is one of the hotspots for *APC* gene mutations [7,8], it has never been reported in adrenocortical neoplasms found in FAP patients. The biallelic inactivation of the *APC* gene reported in ACAs in FAP patients was first described in 1992 [9], and so far, only a few variants have been reported—codons 1061 [3], 1542 [10], 1577 [11], and 1981 [12]. Therefore, our findings add to the database of a candidate for *APC* mutation associated with adrenal neoplasms. Of note, in our present case, only the ACA displayed LOH for the *APC* mutation, whereas the normal adrenal gland, other normal tissue samples, and gastrointestinal tubular adenomas were found to be heterozygous. The LOH functioning as a "second hit" was consistent with prior observations that germline variants in *APC* between codons 1194 and 1392 are strongly associated with a second allelic loss rather than truncating mutation in the development of colonic adenocarcinoma in FAP patients [8].

The IHC findings of our case support the adrenal cortical origin of this tumor, and electron microscopic examination demonstrated features of a nonsecreting tumor (Fig. 2D). IHC stain for β-catenin was performed to investigate the effect of *APC* gene mutation on the tumor, in which nuclear localization of β-catenin was observed in the tumor cells but not in the normal adrenal gland (Fig. 2C). *APC* has been shown to regulate the subcellular localization and turnover of β-catenin [13,14]. Germline LOH mutations of the *APC* gene result in accumulation and altered localization of β-catenin, and constitutive activation of the Wnt/β-catenin signaling pathway [2] and the β-catenin/T-cell factor signaling pathway [15,16], leading to tumorigenesis. *CTNNB1* is another major mutation contributing to β-catenin nuclear translocation in ACA [17], but this mutation was not found in our current case. β-catenin nuclear translocation has been implicated in both benign and malignant adrenocortical tumors [18] and may be associated with a less favorable prognosis [19]. Although the frequency of β-catenin nuclear translocation is found to be correlated with the proliferation activity [20], our case is an exception in that the MIB-1 showed only a ;less than 5% proliferation rate. Interestingly, β-catenin has been shown to physically associate with SF-1 and increase its transactivation of several gene promoters, raising the possibility that β-catenin also plays important roles in adrenocortical function through the modulation of SF-1 action [21,22]. SF-1, which stains strongly positive in the tumor (Fig. 1D), is an orphan nuclear receptor expressed extensively in the adrenal gland and gonads that plays an important role in the development and function of the organs [23]. Therefore, the altered β-catenin–SF-1 pathway in FAP patients resulting from *APC* mutations may suggest a mechanism for the genetic predisposition to ACAs in this population.

In conclusion, the present case is among the few ACAs in FAP patients with unusual morphology described in the literature so far. The tumor showed a unique tubulopapillary and plasmacytoid histologic appearance, with β-catenin nuclear translocation. It also highlights the importance of careful histologic assessment in prompting molecular workup and reanalysis of genetic testing data. In addition, it is the first report of *APC* codon 1309 variant involved in the development of ACA found in an FAP patient. Our report provides further evidence that germline *APC* mutations contribute to higher incidence of extraintestinal tumors in FAP patients.

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