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A novel *BAP1* mutation is associated with melanocytic neoplasms and thyroid cancer

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Abstract

Germline mutations in the tumor suppressor gene, BRCA-1 associated protein (*BAP1*), underlie a tumor predisposition syndrome characterized by increased risk for numerous cancers including uveal melanoma, melanocytic tumors and mesothelioma, among others. In the present study we report the identification of a novel germline *BAP1* mutation, c.1777C>T, which produces a truncated BAP1 protein product and segregates with cancer. Family members with this mutation demonstrated a primary clinical phenotype of autosomal dominant, early-onset melanocytic neoplasms with immunohistochemistry (IHC) of these tumors demonstrating lack of BAP1 protein expression. In addition, family members harboring the *BAP1* c.1777C>T germline mutation developed other neoplastic disease including thyroid cancer. IHC analysis of the thyroid cancer, as well, demonstrated loss of BAP1 protein expression. Our investigation identifies a new *BAP1* mutation, further highlights the relevance of *BAP1* as a clinically important tumor suppressor gene, and broadens the range of cancers associated with *BAP1* inactivation. Further study will be required to understand the full scope of *BAP1*-associated neoplastic disease.

Keywords

Cancer genetics; BAP1; tumor predisposition syndrome; thyroid cancer; melanoma

Introduction

BRCA-1 associated protein (*BAP1*) functions as a tumor suppressor gene by modulating a number of cellular processes including cell cycle progression, cell growth, DNA repair and stem cell dynamics (1–5). Germline mutations in *BAP1* have been implicated in causing an autosomal dominant tumor predisposition syndrome (6) (OMIM #6143237) that is associated with predisposition to uveal melanomas, melanocytic tumors and mesotheliomas

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(7–10). Since the original description of the *BAP1* predisposition syndrome additional cancers such as renal cell carcinoma, cutaneous melanoma and basal cell carcinoma, have been linked with germline mutations of *BAP1* (11–14). In the present study we report a novel germline *BAP1* mutation, c.1777C>T, identified in a patient with multiple cancers and a family history remarkable for autosomal dominant, early age melanocytic tumors and cutaneous melanomas. The c.1777C>T mutation introduces a premature stop codon into the *BAP1* open reading frame with resultant expression of a truncated protein. Immunohistochemical (IHC) assessment of the melanocytic tumors from this family documented loss of BAP1 protein expression within neoplastic cells. Additionally, IHC analysis of a thyroid papillary carcinoma and a thyroid adenoma arising in the proband carrying the c.1777C>T mutation germline mutation demonstrated loss of BAP1 protein expression in these tumors. The results of our investigation suggest that loss of *BAP1* tumor suppression contributes to the development of thyroid cancers, expands the clinical phenotype of the *BAP1*-associated tumor predisposition syndrome and underscores the increasing importance of recognizing this syndrome in the evaluation of suspected hereditary cancers. Finally, the current investigation provides further insight in the molecular pathobiology associated with loss of *BAP1* expression.

Materials and methods

Clinical genetic testing

Clinical germline testing of *CDKN2A* was performed in a CLIA-certified laboratory (Myriad genetics, Salt Lake City, Utah).

BAP1 sequencing and mutation analysis

Genomic DNA was extracted from 5 ml whole blood by protein salting out and nucleic acid precipitation (15). For patients who were deceased at the time of the study (patients III.3 and III.9) genomic DNA was obtained from the normal tissue portions of formalin-fixed, paraffin-embedded (FFPE) tumor blocks using the RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Life Technologies, Grand Island, NY). Exons 1–17 of the *BAP1* gene (NCBI Reference Sequence: NM_004656.3) were amplified employing a PCR protocol adapted from Wiesner et al. (9); Sanger sequencing of amplified fragments in forward and reverse directions was performed by Genewiz (La Jolla, California). Electropherogram results were analyzed and mutations identified using Mutation Surveyor 2.61.

Immunohistochemistry

Archived, formalin-fixed, paraffin-embedded tumor blocks of the thyroid cancer were employed for IHC studies. H&E staining was carried out using standard methodology. Staining for BAP1 protein was accomplished employing BAP1 antibody (C-4, catalog number: sc-28283) obtained from Santa Cruz Biotechnology (Dallas, Texas). In brief, 4 μm sections were cut and baked at 60 degrees for 1 hour. The slides were then processed through the Leica BOND-III automated IHC and ISH system (Buffalo Grove, Illinois). Post processing, the IHC slides were dehydrated and cover slipped mounted with Permount adhesive (ThermoFisher Scientific; Waltham, Massachusetts). BAP1 protein IHC staining of melanoma samples was previously performed at an outside institution as part of patient's

prior clinical assessment. Melanoma and melanocytic nevi IHC results described in the present study are based upon clinical reports provided by the patient.

Results

Patient cancer and family history

The proband is a 54 year old woman who was referred to the cancer genetics program at the University of Southern California Norris Comprehensive Cancer Center for evaluation of a personal history of multiple cancers including papillary thyroid cancer, basal cell carcinoma, cutaneous melanoma and B-cell lymphoma. A four generation cancer-focused pedigree was generated which demonstrated a multigenerational history of melanoma as well as additional cancers including bladder carcinoma, basal cell carcinoma, hepatocellular carcinoma and pancreatic cancer (Figure 1A). Physical examination of the patient documented greater than 100 moles on the chest and abdomen, but was otherwise unremarkable. Given the personal and family histories, which included melanoma and pancreatic cancer, a concern for familial atypical multiple mole melanoma (FAMM) syndrome arose. Clinical genetic testing for *CDKN2A* found no pathogenic mutations (Myriad Genetics, Salt Lake City, UT) (16).

Dermatologic assessment unveils a BAP1-associated familial cancer syndrome

The patient's son (IV.1, Figure 1A) with a previous history of an early age dysplastic nevus detected at age 17 was subsequently diagnosed with another dysplastic nevus at age 26. Dermatologic evaluation with biopsy demonstrated compound Spitz proliferation of melanocytes; these histologic features raised concern for loss of expression of the *BAP1*gene (9). IHC staining of the dysplastic nevi confirmed an absence of BAP1 protein. Within this same time period, the proband's other son (IV.2, Figure 1A) developed early age melanoma on his knee for which IHC assessment also revealed absent BAP1 protein expression. Dysplastic nevi and melanoma are less common among individuals during the first three decades of life compared with older age cohorts (17,18). The early age of these dermatologic diagnoses together with lack of BAP1 protein expression in the tumor specimens created suspicion for an inherited germline mutation of *BAP1*; moreover, the constellation of cancer diagnoses within the family raised the possibility of a novel manifestation of the *BAP1* tumor predisposition syndrome.

Germline genomic sequencing of the BAP1 exome identifies a novel truncating mutation

Sequencing of the complete *BAP1* exome (17 exons) was carried out from germline DNA derived from the proband. A novel point mutation was identified in exon 14, c.1777C>T (p.Q593X), of the *BAP1* gene (Figure 1B, C) resulting in the introduction of a truncating, premature stop codon. Additional family members were tested for the presence of this germline mutation and the mutation was identified in family members III.6 (pancreatic cancer positive), III.9 (melanoma positive), IV.1 (dysplastic nevi positive) and IV.2 (melanoma positive). The mutation was absent from patient II.3, the father of the proband. DNA from the proband's mother was not available; however, the familial patterning of the *BAP1* mutation is consistent with obligate transmission via the maternal lineage. Patients IV. 3 and III.8, the daughter and sister of the proband, respectively, demonstrated wildtype *BAP1* genotypes; notably, both patients IV.3 and III.8 were without a cancer phenotype.

Immunohistochemical analysis implicates deficient BAP1 tumor suppression in the development of thyroid cancer

The tumor spectrum defining BAP1 predisposition syndrome includes, prominently, the constellation of uveal melanoma, cutaneous melanoma, melanocytic tumors, mesothelioma renal cell carcinoma and basal cell carcinomas (8,10,13,19,20). Although thyroid cancers have been identified in families of known *BAP1* mutation carrier (21), to date, loss of BAP1 protein expression or bi-allelic *BAP1* gene deletion has yet to be documented for these specific tumors. In the current family, the proband developed both a thyroid adenoma and thyroid papillary cancer. Demonstration of loss of BAP1 protein expression in these tumors would provide evidence for expanding the spectrum of cancers arising from absence of BAP1 tumor suppression. We observed intact BAP1 expression in normal tissue derived from the thyroid specimen (Figure 2A–C) and absence of nuclear expression in both the follicular adenoma (Figure 2A, D) and the papillary thyroid carcinoma (Figure 2B, E). These results are consistent with loss of BAP1 tumor suppression driving the formation of the thyroid adenoma and papillary carcinoma.

Discussion

In the current study we identify a novel germline *BAP1* mutation, c.1777C>T, resulting in a truncated protein, in a family with multiple cancers. These findings further define the evolving tumor spectrum characterizing the BAP1-related tumor predisposition syndrome and provide insight into the pathobiology of disrupted *BAP1* tumor suppression.

The *BAP1* gene lies on the reverse strand of chromosome 3 spanning 3p21.31-p21.2 (22). *BAP1* encodes a ubiquitin carboxy-terminal hydrolase which modulates cell proliferation and cell cycle progression acting as a tumor suppressor gene via association with and/or deubiquitination of a host of transcription-regulating proteins including among others *ASXL1, ASXL2, FOXK1, OGT* and *HCF-1* (2,23,24). The open reading frame of *BAP1* spans 2166 nucleotides with 17 exons comprising multiple functional domains, including ubiquitin carboxyl-terminal hydrolase (UCH)(exons 1–7), the host cell factor-1 binding domain (HCF1-BD)(exon 10) and the C-terminal domain (exons 13–17); the C-terminal domain contains the nuclear localization signals 1 and 2 and the UCH37-like domain (ULD) (6,22,25). Previously identified germline *BAP1* mutations are mostly nonsense or frameshift mutations; the c.1777C>T mutation described in the current study is a nonsense mutation. These mutations result in premature stop codons which give rise to truncated proteins and likely nonsense-mediated mRNA decay (26). The germline *BAP1* mutations described to date are evenly distributed across the open reading frame of the *BAP1* gene, although a disproportionate number of mutations are situated on exon 13 as it represents the largest exon. Approximately one half of reported germline *BAP1* mutations occur within a functional domain; and these mutations exhibit proportional prevalence across these domains (6).

Germline loss of *BAP1* expression has been documented to underlie a number of familial cancer occurrences (27). The tumors originally associated with mutated germline *BAP1* include melanocytic tumors (9), uveal melanomas (7) and mesotheliomas (8). At that same time, it was postulated that germline *BAP1* mutations likely additionally promote cutaneous

melanoma (9), and might predispose to a constellation of additional cancers (8). And since the initial reports of these cancers linked with germline mutated *BAP1*, the scope of associated cancers has, indeed, significantly expanded (28).

The strongest causative association between loss of *BAP1* expression and a specific cancer are those cancers for which bi-allelic mutations/deletions and/or disruption of BAP1 protein expression have been demonstrated in the tumor. These neoplasms include, in addition to the originally documented cancers, renal cell carcinoma, basal cell carcinoma, lung cancer, tumors of the CNS and sarcomas (8,10–14,20,21). The present study enlarges this collection also to encompass papillary thyroid cancer and thyroid adenoma.

The current observation that loss of *BAP1* tumor suppression drives progression of thyroid cancer additionally clarifies the spectrum of the *BAP1* tumor predisposition syndrome and also elucidates the pathobiology resulting from mutation of the gene. In the proband we observed that loss of BAP1 expression occurred in both a follicular adenoma as well as an adjacent papillary thyroid cancer. Follicular thyroid cancer may develop via an adenoma to carcinoma progression sequence (29). Both *RAS* gene mutations and *PAX8/PPARγ* gene fusions have previously been implicated as initiating events of this molecular sequence (30); the current study raises speculation that loss of *BAP1* expression may represent an alternate early event in this progression pathway. The question arises whether loss of *BAP1* expression may also drive similar early adenoma-initiating events in other tissues. The occurrence of the thyroid neoplasms in the proband is further remarkable; it is also unique for tumors of two different histologies to develop synchronously within the thyroid gland (31–33). This circumstance may belie singular molecular pathways which are fostered by the underlying germline *BAP1* mutation; further elucidation of other somatic mutations in these thyroid tumors may provide further insight into such pathways.

The proband, in addition to diagnoses of melanoma and thyroid cancer, also developed B-cell lymphoma. Relative to solid tumors, there exist few reports of hematopoietic cancers associated with germline mutations of *BAP1*. Previous associations are limited to histories of family members of patients carrying germline *BAP1* mutations having leukemia (34,35) and one unspecified hematologic cancer (21). Laboratory investigations, however, unambiguously document that loss of *BAP1* expression can disrupt normal bone marrow function, cause myeloid transformation and give rise to myeloid dysplasia (24). The present study broadens the list of hematopoietic cancers associated with individuals carrying germline *BAP1* mutations; our investigation suggests that loss of *BAP1* expression may disrupt not merely ontogeny of the myeloid cell lineage but may extend to lymphoid cell lineage causing B cell lymphoma, as well.

Pancreatic cancer has been reported previously in family members of patient's having deleterious *BAP1* mutations (8,21). This association suggests the possibility that loss of *BAP1* tumor suppression may also drive the progression of pancreatic cancer. In the current study we document the occurrence of a *BAP1* mutation in a patient (patient III.6, Figure 1) with pancreatic cancer which further strengthens this association. Unfortunately, insufficient pancreatic cancer tissue from this patient was available to perform IHC analysis to assess

loss of BAP1 protein expression. We speculate that future studies may unambiguously implicate loss of *BAP1* tumor suppression in the development of pancreatic cancer.

The father of the proband (Figure 1, patient II.3) did not carry a *BAP1* mutation, suggesting a high probability that this mutation originated in the mother unless the BAP1 mutation was de novo (Figure 1, patient II.4). We note that the mother developed hepatocellular carcinoma. This clinical presentation raises speculation that loss of BAP1 tumor suppression may also play a role in the oncogenesis of hepatocellular carcinoma; unfortunately, no tissue specimens were available from the mother to test this hypothesis.

In summary, the burgeoning spectrum of tumors associated with germline mutations of BAP1 together with the observation that BAP1 is ubiquitously expressed among tissues (36), suggest that the gene may function as a generalized tumor suppressor gene. That there manifests some diversity with regard to what tumors develop within and among families with germline mutations of *BAP1* may also imply that the gene exhibits variable penetrance and/or exerts a modifier effect within the context of a landscape of additional familial genetic variation (37,38). Therefore, it is reasonable to consider germline *BAP1* mutations as a possible causative mechanism in families exhibiting a familial cancer syndrome but whose genetic workup identifies no typical genetic abnormality. It may be anticipated that as whole exome sequencing becomes more widely adopted as a part of medical genetic and, more specifically, cancer genetic workups, the identification of additional germline BAP1 mutations and clarification of the molecular mechanisms contributing to the development of familial cancer syndrome will be forthcoming (39–41).

Acknowledgments

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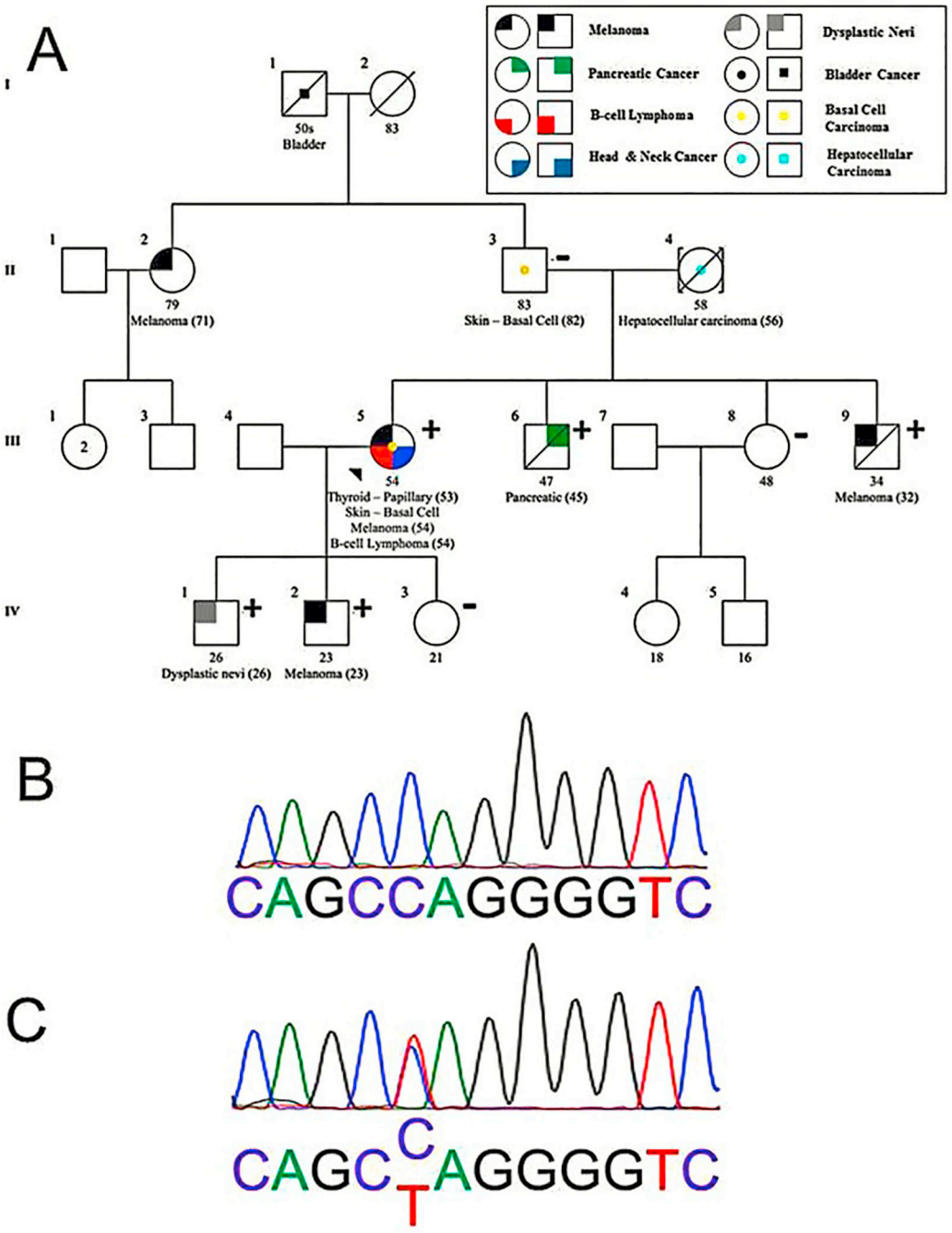

Figure 1.

(A) Four generation pedigree of family with novel *BAP1* mutation, g.1777C>T, documents co-segregation of *BAP1* mutation and multiple cancers. Individuals carrying the familial mutation *BAP1* g.1777C>T are designated with a "+" and tested individuals proven not to carry the *BAP1* g.1777C>T mutation are shown by "−". The proband, patient III-5, is indicated with an arrow. Her mother, patient II-4, is assumed to be an obligate carrier given the negative test result in her father and absence of available DNA for testing in her deceased mother. Further information for the maternal family to the proband is unavailable because

patient II-4 was adopted into the family. (B) Electropherogram depicting portion of germline DNA exon 14 of *BAP1* gene from unaffected family member II-3 documenting wildtype *BAP1* sequence. (C) Electropherogram of germline DNA obtained from the proband (III-5) illustrating novel *BAP1* mutation g.1777C>T, resulting in truncated BAP1 protein. Genotyping of other unaffected family members (III-8 and IV-3) revealed wildtype germline *BAP1*; genotyping of affected family members (III-6, III-9, IV-1 and IV-2) revealed the novel *BAP1* mutation, g.1777C>T.

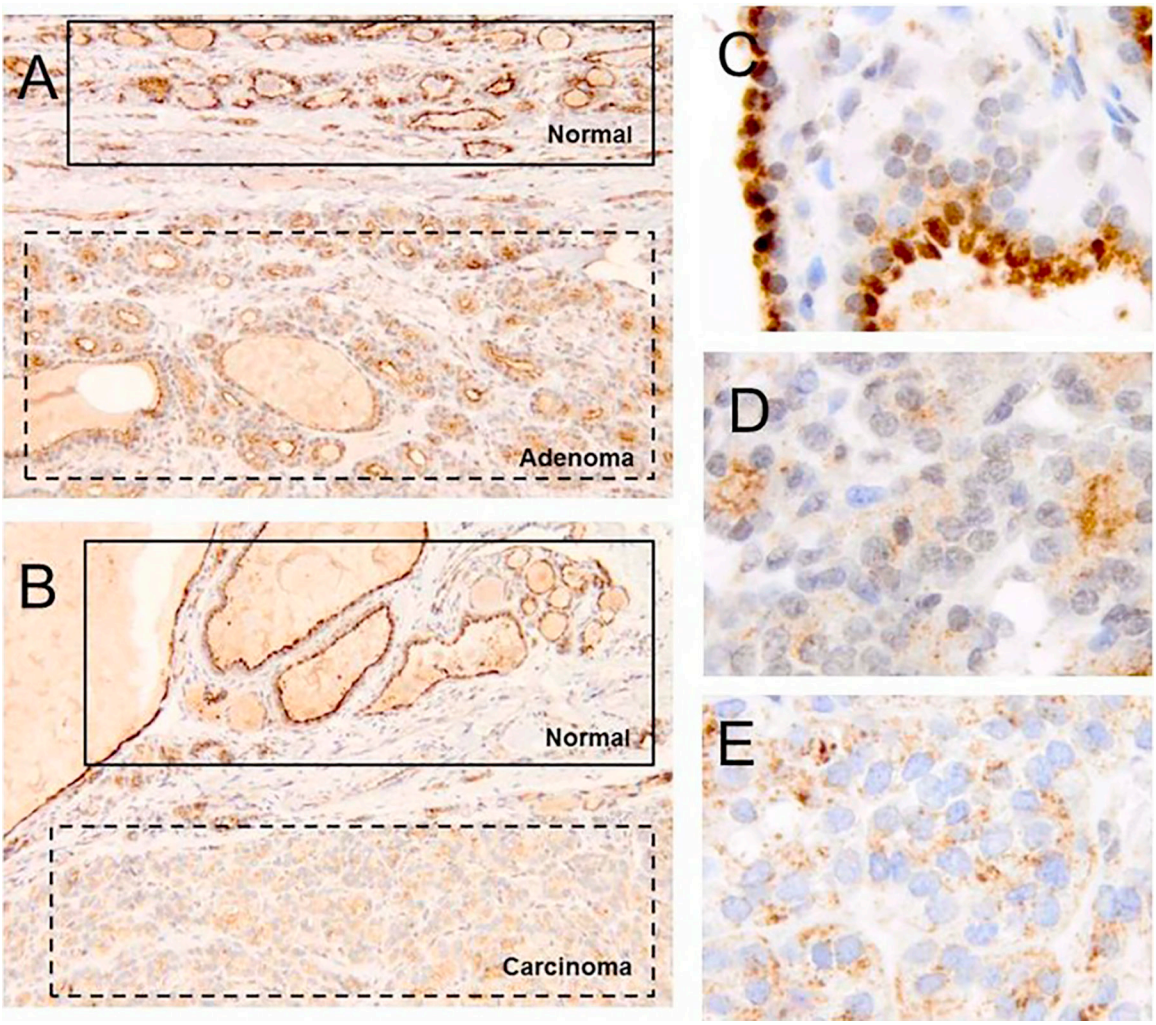

Figure 2.

(A) BAP1 IHC staining (100x) of normal thyroid tissue together with thyroid adenoma originating from proband patient (III-5) reveals strong nuclear staining of BAP1 in normal tissue compared with absence of staining in adenoma. (B) BAP1 IHC staining (100x) of normal thyroid tissue together with papillary thyroid carcinoma originating from proband patient (III-5) demonstrates strong nuclear staining of BAP1 in normal tissue compared with absence of staining in carcinoma. (C) Higher power image (630x) of normal thyroid tissue documents strong nuclear staining of BAP1. (D) Higher power image (630x) of thyroid adenoma demonstrates absence of BAP1 nuclear staining. (E) Higher power image (630x) of papillary thyroid carcinoma reveals absence of BAP1 nuclear staining.