

Can Mutations in the BAP1 Gene be Detected by Immunohistochemistry in Hereditary Kidney Cancers?

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Abstract: *Background:* Hereditary renal cell carcinoma (RCC) constitutes about 5% of all RCCs. The most common and well studied syndromes include, VHL, HLRCC, BHD, Familial Oncocytoma, RCC Papillary Type 1, TSC, RCC associated with Succinate dehydrogenase B (SHDB) mutations and others. Several genes, including *VHL*, *MET*, *FLCN*, *FH* and genes encoding the succinate dehydrogenase (SDH) subunits B/C/D have been identified as causative. However, the genetic basis of a significant percentage of familial RCC, some with clear cell morphology remain unknown. *BAP1* (BRCA1 associated protein-1), a tumor suppressor gene that encodes a nuclear deubiquitinase, is inactivated in 15% of sporadic clear cell RCCs and its loss was associated with high tumor grade and poor prognosis. In this study, we investigated the possible role of this gene in the spectrum of RCC part of hereditary syndromes.

Materials and Methods: To elucidate the role of BAP1 in all the spectrum of hereditary RCC, we studied by IHC a panel of RCCs which covers the spectrum of kidney cancers and included 10 VHL tumors, 6 HLRCCs, 8 chromophobe, 5 Hereditary Papillary Type 1, 6 Oncocytomas, 3 BHD (hybrid), and 24 sporadic clear cell RCCs. To analyze the BAP1 expression in these tumors, formalin fixed paraffin embedded (FFPE) tissues were immunostained with mouse monoclonal anti-human BAP1 antibody (Clone C-4, Santa Cruz).

Results: We found that all the tumors except two showed positive nuclear staining for BAP1. The two negative cases that were negative for BAP1 were Clear cell type and belonged to two siblings. Molecular analysis in a prepublished study showed both patients harboring the p.L14H mutation.

Conclusion: Our study supports the hypothesis that *BAP1* mutations can play a role in hereditary syndromes predominantly in clear cell tumors. Staining for BAP1 should be done when there is no definite known mutation in a clear cell cancer but the patient gives history of familial kidney cancer. The two related patients who had similar mutations had aggressive, metastatic disease, which suggests that probably *BAP1* does play a role in hereditary RCC clear cell type.

Keywords: Hereditary kidney cancer, BAP1, mutation, immunohistochemistry.

INTRODUCTION

Hereditary kidney cancer accounts for more than 5% of the total number of RCC cases in the USA. Several genes have been already identified and the number continues to increase. These include the von Hippel Lindau (VHL), Birt Hogg Dube (BHD), Papillary type 1, Hereditary Leiomyomatosis and renal cell cancer (HLRCC), Succinate dehydrogenase-associated paraganglioma syndrome and Tuberous sclerosis complex.

VHL, is an autosomal dominant disease associated with lesions in the brain, spine, retina, pancreas, and kidneys. In the kidney, the tumors are bilateral, multifocal, have a clear cell morphology and are frequently associated with renal cysts. The gene encoding for this syndrome was found to be located in chromosome 3p 25-26 [1-4] and named as von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase.

BHD patients have an autosomal dominant syndrome characterized by skin fibrofolliculomas, lung

cysts and kidney cancer [5, 6]. The kidney cancers observed in BHD syndrome could be oncocytomas, hybrid tumors or clear cell RCC. The gene for BHD, a tumor suppressor gene, maps to 17p12q11.2 and was identified and named Folliculin (*FLCN*) [7]. Hereditary RCC papillary Type I, is an autosomal dominant syndrome characterized by multifocal, bilateral papillary type tumors [8, 9]. Mutations of the *MET* gene on 7q31 have been associated with this condition. HLRCC, is an autosomal dominant disease where patients develop cutaneous and uterine leiomyomas and kidney cancer [10, 11]. The kidney tumors show a spectrum of papillary, tubulo-papillary, tubular and solid or mixed. The most important histologic feature of these neoplasms, which we believe to be the hallmark of the HLRCC tumors, is the presence of a characteristic large nucleus with a very prominent inclusion like orangiophilic or eosinophilic nucleolus, surrounded by a clear halo [12]. The gene in the HLRCC syndrome is linked to *FH* (fumarate hydratase), an enzyme that converts fumarate to malate in the Krebs cycle [13, 14]. The SHDB associated renal tumors cell, show a characteristic appearance that mimics oncocytomas [15-17]. Tuberous sclerosis (TSC) syndrome complex is autosomal dominant characterized by hamartoma like lesions in multiple organs including brain, kidney,

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skin and lung. Patients with TSC2 mutations are more severely affected with kidney tumors.

BAP1 (BRCA1 ASSOCIATED PROTEIN 1 gene) is a tumor suppressor gene that encodes a nuclear deubiquitinase [18-20]. It functions as the classic two-hit tumor suppressor gene and is somatically mutated in uveal melanoma and mesothelioma [21, 22]. Somatic mutations of BAP1 were identified through whole genome sequencing studies [23] in sporadic renal cell carcinomas, clear cell type. BAP1 was found to be inactivated in 15% of the sporadic tumors and BAP1 mutations were found to be associated with higher Fuhrman nuclear Grade and aggressive behavior. Germline mutations were identified in 1 of 83 families studied by Farley *et al.* with a novel missense mutation in *BAP1* described [26]. In another study, of 32 unrelated individuals with familial RCC, no BAP1 mutations were found. In the same study, however, in 60 unrelated individuals, who were predisposed to uveal melanoma, cutaneous melanoma or mesothelioma identified 11 probands with deleterious BAP1 germline mutations. In 6 of 11 families, RCC was present, suggesting that BAP1 predisposes to RCC [24]. A multi-institutional study confirmed that RCC, clear cell type that stained positive by immunohistochemical stains for BAP1 correlated with the mutation and also with higher Fuhrman nuclear grade, pT stage, tumor necrosis, sarcomatoid differentiation as well as poor oncologic outcomes and adverse clinical features in clear cell RCC [25].

We researched the BAP1 status in the spectrum of hereditary RCCs to investigate the possibility of identification of patients with this mutation.

MATERIAL AND METHODS

Patient Population

Patients were accepted for enrollment in an IRB approved protocol of the UOB, NCI. This study included tissue samples from 62 patients, who underwent partial or radical nephrectomy in the last 10 years. All H&E slides were obtained and reviewed. Tumors from all the different varieties of hereditary RCC, were studied including 10 VHL tumors, 6 HLRCCs, 8 chromophobe, 5 Hereditary Papillary Type 1, 6 Oncocytomas, 3 BHD (hybrid), and 24 sporadic clear cell RCCs.

Immunohistochemistry

Five micron formalin-fixed paraffin-embedded sections were deparaffinized and blocked with

methanol-30% H₂O₂. After antigen retrieval by boiling in citrate buffer, slides were incubated with monoclonal anti-BAP1 antibody (C-4; Santa Cruz Biotechnology Inc, Dallas, Texas) diluted 1/150. Then, slides were immunostained with avidin–biotin–peroxidase complex and developed with diaminobenzidine. Harris hematoxylin was used to counterstain the slides. Nonimmune mouse immunoglobulin was used as a negative control. Expression was evaluated as positive or negative. Staining was considered positive when more than 10% of the nuclei showed immunoreaction. Results were scored by a pathologist blinded to the clinical data.

RESULTS

Clinical and Histological Characteristics

The distribution of tumor characteristics were as shown in Table 1. A group of 24 sporadic RCC, clear cell type were chosen to compare to the hereditary group. Median age in this group was 50 years. Most of these were clear cell type with Fuhrman nuclear Grade 2 (50%) while a few were Grade 3 and 4 and presented with unilateral tumors. Among the clear cell RCC in VHL patients (median age 54) most had multiple, bilateral tumors and had undergone several partial nephrectomies or tumorectomies, most were Fuhrman nuclear Grade 2, some were Grade 3. Patients with oncocytomas, had a median age of 52, tumors were both unilateral and bilateral. Patients with papillary type 1 tumors, presented around age 56 and the tumors were mostly bilateral. For patients with chromophobe tumors, median age was 48, and 2 of 8 tumors had sarcomatoid differentiation.

BAP1 Associations

BAP1 IHC was positive in all hereditary tumor categories (Figure 1) cases except 2 (Figure 2). One patient, negative for BAP1 by immunohistochemistry, had recurrent kidney cancers, was surgically operated several times and diagnosed as clear cell type renal cell carcinoma, Fuhrman nuclear grade 2 and at times, Grade 3. In the same family, a sibling was diagnosed with kidney cancer too. One of the BAP1 negative tumors shown in Figure 2, is from one of the siblings. These cases were previously published and found to harbor the p.L14H missense mutation as reported before [26]. It is possible that this point mutation (Leucine to Histidine), has changed the protein conformation or protein folding in which case the antibody could not detect the protein.

Table 1:

Histological Type	Number of Cases	Median Age	Range of Size Of tumors	Grade if applicable	Laterality	Sarcomatoid differentiation	BAP 1 expression	
							POS	NEG
Oncocytoma	6	52	3.5-6.5cm	NA	Bilateral and unilateral	0	6	
VHL	10	54	0.5-5.4cm	Mostly G2, some 3	Multiple, bilateral	0	10	
Clear Cell RCC	24	50	0.3-6.5cm	16 cases G2 3 cases G3 3 cases mixed 2 and 3 2 cases G4	Mostly unilateral, 2 bilateral.	1	22	2
Papillary Type 1	5	53	1.2-6.0cm	NA	2 bilateral 1 unilateral	0	5	0
Chromophobe	8	48	1.2-15 cm	NA	2 bilateral	2	8	0
HLRCC	6	52	0.4-2.0cm	High	2 unilateral	0	6	
BHD (Hybrid)	3	50	0.6-5.3cm	low	bilateral	0	3	0

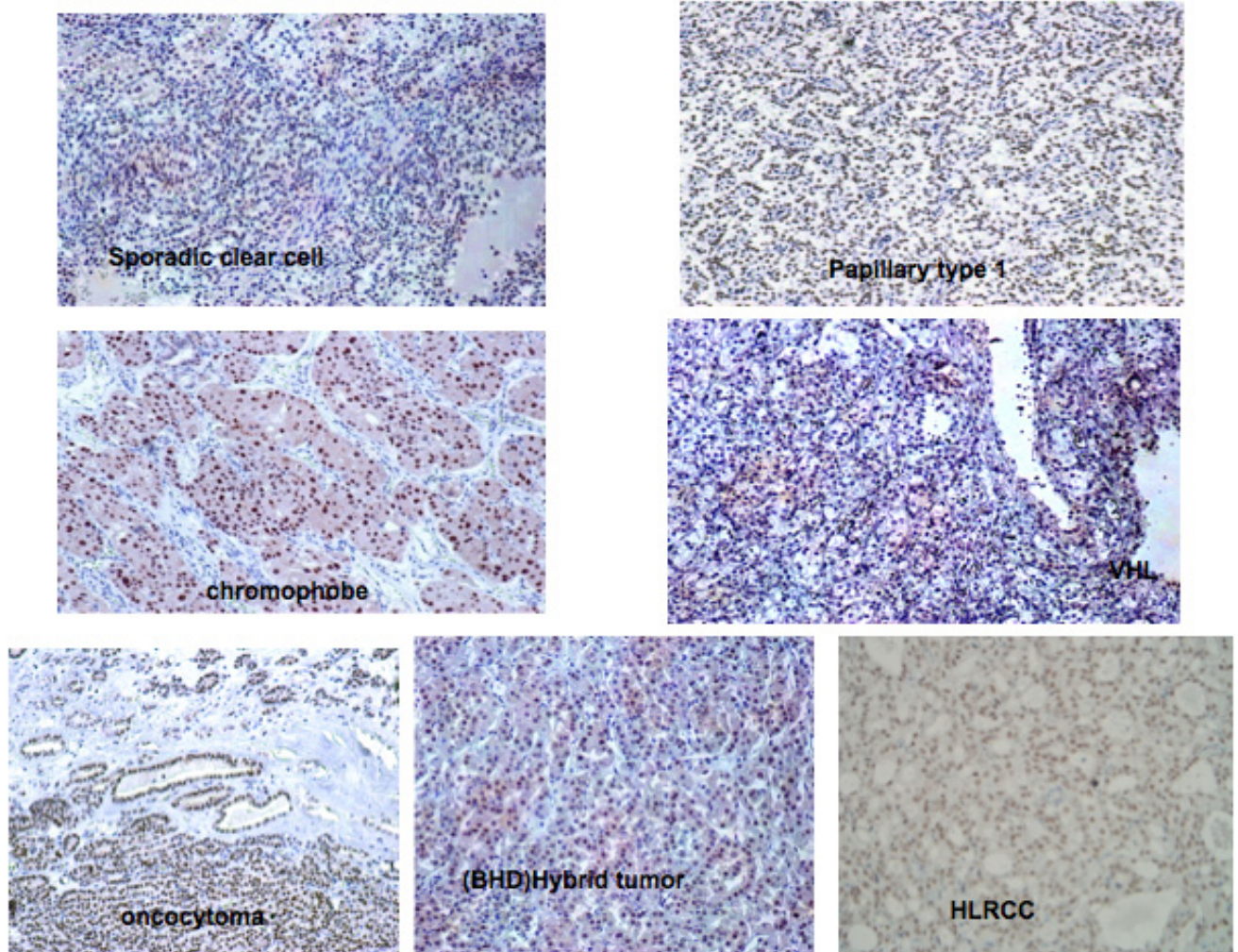


Figure 1: Immunohistochemical stains for BAP1 in all the different types of familial kidney cancer patients reviewed. The figure shows that BAP1 nuclear stain was positive in all the different tumor types.

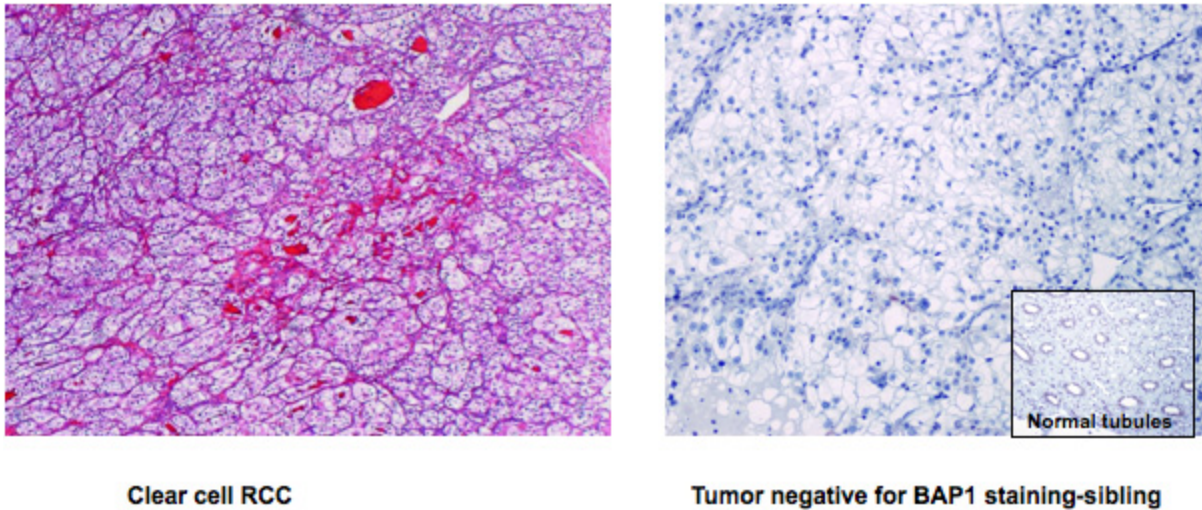


Figure 2: A H&E and corresponding BAP1 immunostain in a kidney tumor where the siblings had a familial syndrome and both stained negative for BAP1 and harbored the mutation.

DISCUSSION

Here we provide a detailed description of the expression of BAP1 in the spectrum of hereditary renal cell carcinomas seen in our institution. Our results indicate that *BAP1* may not play a significant role in the hereditary kidney tumors with morphologies other than clear cell cancer but supports the theory that *BAP1* mutation and loss of expression may define a new class of renal cell cancer syndrome [23].

BAP1 mutations have been reported to be associated with disease progression and aggressive clinicopathological features in sporadic renal cell carcinoma [25]. In another multi-institutional study, which was based on immunohistochemistry, BAP1 negative tumors significantly correlated with adverse pathologic features and worse outcomes in clear cell renal cell carcinoma [27]. In addition, a novel variant (c.41T>A; p.L14H) of *BAP1* mutation has previously been identified in a family with clear cell renal cell carcinoma, with high Fuhrman nuclear grade and thus suggested *BAP1* as a predisposing gene in a familial setting [26].

In our study, BAP1 expression was positive in all the different types of hereditary kidney cancer types we studied, which included VHL, BHD (hybrid), chromophobe, Papillary Type I, and HLRCC tumors. As a comparison, 24 sporadic RCC, clear cell type tumors were tested as control.

Two tumors stained negatively for BAP1, positive staining in the adjacent normal nuclei served as internal control. These 2 patients by mutation analysis,

had the p.L14H missense mutation as reported before [26]. This point mutation (Leucine to Histidine), possibly changes the protein conformation or protein folding in which case the antibody may not detect the protein. Also if this mutation made a truncated protein, the antibody may still not recognize the protein. It is also possible that this protein may be degraded or inactivated much faster so there is no detectable protein in the fixed tissue sample. We need to validate this by more protein biochemistry experiments which are currently being done in the laboratory.

Germline *BAP1* mutations have been reported to predispose to several additional cancers including uveal and cutaneous melanoma and mesothelioma, lung cancers, meningiomas and mesotheliomas [28-31]. However, in the one *BAP1* kidney cancer family that was identified here, there was no evidence of other malignancies.

Recent studies have reported several mutations in chromosome 3p locus, including BAP1, SETD2 and PBRM1. As the TCGA network suggested that PBRM1 mutations (30-34%) most probably play an important role in tumor initiation, while BAP1 and SETD2 mutations (6-12%) are associated with worse cancer specific survival [32]. In a different study, BAP1 and PBRM1 mutations anti correlated in tumors and combined loss of BAP1 and PBRM1 in a few tumors was associated with rhabdoid features [23].

In conclusion, BAP1 does not seem to play a role in the well known/characterized familial kidney cancer syndromes. However, BAP1 may constitute a new genre of hereditary kidney cancer syndrome which

have a clear cell morphology distinct of VHL and similar to sporadic clear cell, but that can occur in families. From our first family identified here at our institution, the BAP1 cancers are bilateral, multifocal, with multiple renal cysts; they lack the germline VHL mutations, and other VHL findings such as hemangioblastomas and cysts in other organs such as pancreatic neuroendocrine tumors and pheochromocytomas [2]. There are still families with malignant tumors that do not have the classical mutations of known hereditary kidney cancer syndromes and they may harbor the BAP1 mutation. While right now, the clinical care is resection/partial nephrectomy, however, BAP1 has gained recognition as to predispose to a familial tumor. This necessitates the timely screening of family members and this we feel is of great significance. Staining for BAP1 followed by further confirmation with other tests may help recognize and classify patients better not only for clinical trials but for establishment of proper therapies.

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Received on 29-04-2014

Accepted on 10-06-2014

Published on 12-08-2014

<http://dx.doi.org/10.6000/1927-7229.2014.03.03.3>© 2014 Ghosh *et al.*; Licensee Lifescience Global.

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