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Loss of PBRM1 and BAP1 Expression Is Less Common in Non–Clear Cell Renal Cell Carcinoma Than in Clear Cell Renal Cell Carcinoma

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Abstract

Background—Recurrent mutations in *polybromo-1* (*PBRM1*, ~40%) and *BRCA1-associated protein-1* (*BAP1*, ~10%) occur in clear cell renal cell carcinoma (ccRCC), but their prevalence in non clear cell renal cell carcinoma (non-ccRCC) or renal oncocyoma (RO) is unknown. We

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evaluated loss of PBRM1 and BAP1 staining in ccRCC, papillary RCC (pRCC), chromophobe RCC (chRCC) and RO tumors using an immunohistochemistry (IHC) assay for which negative staining associates with loss-of-function mutations.

Methods—We identified 458 patients treated surgically for ccRCC, pRCC, chRCC and RO between 2004 and 2012. We performed IHC assays to evaluate PBRM1 and BAP1 protein expression to classify tumors as PBRM1- or BAP1-negative. We compared loss of staining of these 2 proteins in ccRCC and non-ccRCC using Fisher's exact test.

Results—For the total cohort of 458 patients, we successfully stained both PBRM1 and BAP1 in 408 tumor samples. Consistent with the mutation rate, loss of PBRM1 and BAP1 staining occurred in 43% (80/187) and 10% (18/187) of ccRCC respectively. However, loss of PBRM1 staining occurred in only 3% (2/59), 6% (1/17) and 0% (0/34) of pRCC, chRCC and RO tumors, respectively ($P<0.0001$). BAP1 loss was not observed in any of the pRCC ($N=61$), chRCC ($N=17$) or RO ($N=34$) tumors ($P=.00021$).

Conclusion—Our data suggest that biallelic inactivation of *PBRM1* or *BAP1* is less common in non-ccRCC when compared to ccRCC tumors. These findings suggest that loss of PBRM1 or BAP1 are key events in ccRCC, whereas other pathways may support tumorigenesis in non-ccRCC subtypes.

Keywords

BRCA1-associated protein-1; renal cell carcinoma; polybromo-1; papillary

1. Introduction

Renal cell carcinoma (RCC) is the most common form of cancer of the human kidney (>85%) and represents a histologically diverse set of tumors that all originate from the renal parenchyma. While RCCs were historically classified based on cell type and growth pattern, this classification has been recently updated to incorporate a wider variety of features including morphology, growth pattern, cell of origin as well as immunohistochemical staining and other molecular testing.¹ Related to this, there are currently several individual RCC subtypes and of these the most common are clear cell (ccRCC, 75-85%), papillary (pRCC, 10-15%), chromophobe (chRCC, 5-10%) and oncocytic (3-7%) RCC. In addition, there are a variety of rare subtypes (e.g., collecting duct, medullary, multilocular cystic, translocation, etc.) that each make up less than 1% of all RCCs diagnosed each year. Of note, while technically not a cancerous lesion, renal oncocytomas are benign tumors believed to arise from the intercalated cells of the collecting duct and represent roughly 5-15% of all renal neoplasms.

Given the unique features and clinical outcomes associated with the various RCC subtypes, investigators have shown considerable interest in exploring the molecular underpinnings of RCC subtypes and in particular, those molecular events that distinguish ccRCC from the remaining non-ccRCC subtypes. Specifically, several investigators have reported that loss-of-function mutations in chromatin-modifying enzymes, such as polybromo-1 (*PBRM1* [~40%])^{2, 3}, BRCA1-associated protein-1 (*BAP1* [~10%])^{3, 4}, and SET domain containing protein-2 (*SETD2* [~12%]) are common events in ccRCC tumors.^{2, 3, 5} Of interest, while

cytogenetic analyses have revealed different patterns of chromosomal aberrations between ccRCC and non-ccRCC subtypes, the actual prevalence of chromatin-modifying enzyme mutations in the key non-ccRCC subtypes remains unknown. Motivated by this gap in knowledge, we employed our own immunohistochemistry (IHC)-based assays for detection of PBRM1 and BAP1 protein products in archival formalin-fixed paraffin-embedded tissue to evaluate loss of PBRM1 and BAP1 expression in ccRCCs, pRCC, chRCC and renal oncocytomas.

2. Material and Methods

2.1 Patient Selection and Data Abstraction

After approval from the Mayo Clinic Institutional Review Board, we utilized the Mayo Clinic Florida Renal Mass Registry database to identify 458 patients treated surgically (radical nephrectomy or nephron-sparing surgery) for clinically-localized ccRCC, pRCC, chRCC and RO between 2004 and 2012. As part of this Registry effort, an experienced urologic pathologist (K.J.W., J.C.C.) centrally reviews hematoxylin-eosin slides for all patient tumors in order to confirm histological classification and systematically record standard pathologic features. Using the Registry database, our study coordinator abstracted demographic, clinical and pathologic data on all 458 patients identified for this investigation.

2.2 PBRM1 and BAP1 Protein Expression by IHC

We previously validated IHC assays to evaluate PBRM1 and BAP1 protein expression in which negative staining associates with *PBRM1* and *BAP1* mutant genotypes.^{4, 6} Sanger sequencing confirmed mutations in 90% of PBRM1 IHC negative and 95% of BAP1 IHC negative tumors. The following antibodies and dilutions were used: PBRM1 (Bethyl Laboratories, catalog A301-591A; 1:250) and BAP1 (Santa Cruz, catalog sc-28383; 1:50). PBRM1 and BAP1 are chromatin-modifying enzymes; positivity was evaluated as tumor nuclei staining with intensity equal to or stronger than that in the surrounding stromal cells and lymphocytes. A centralized pathologist (P.K.) reviewed all IHC slides and categorized tumors as positive, negative, weak positive or focal negative. Tumors were categorized as PBRM1 or BAP1 negative when tumor cells showed diffuse absence of nuclear PBRM1 or BAP1 staining. A minority of tumors had heterogeneous staining for PBRM1 and BAP1, with some cells staining positive and some cells staining negative. Additionally, a small number of tumors had uniform but diffusely weak staining of PBRM1 and BAP1.

2.3 Statistical Methods

To compare clinical and pathologic data as well as PBRM1 and BAP1 status (+/-) across ccRCC and the non-ccRCC subtypes, we employed Fisher's exact and Kruskal-Wallis tests as appropriate. In secondary analysis, we employed a similar strategy to evaluate the combined PBRM1 and BAP1 status (i.e. four pairwise groups) across the various subtypes as well. For all our analysis we considered a *P value* < 0.05 as evidence of statistical significance. Version 3.02 of the R programming language was used for statistics.

3. Results

3.1 Comparison of Clinicopathologic Features

For the total cohort of 458 patients, we successfully stained PBRM1 in 419 (91.5%) and BAP1 in 447 tumor samples (97.6%), reducing our sample size to N=408 (Figure 1, Table 1). We previously validated IHC assays with Sanger sequencing to evaluate PBRM1 and BAP1 protein expression in which negative nuclear staining associates with *PBRM1* and *BAP1* mutations.^{4, 6} We used background stroma and lymphocyte nuclei as a positive control, and therefore, we excluded from our analysis samples that lacked stroma/lymphocyte nuclear staining, focal negative or weak positive staining in these cells (supplemental material). In the current study, after the aforementioned exclusions, we were left with a final sample size of 299 tumors (187 ccRCC, 61 pRCC, 17 chRCC and 34 RO) that had both PBRM1 and BAP1 staining available for analysis. In Table 1 we provide a comparison of demographic, pathologic and clinical features across the ccRCC, pRCC, chRCC, and RO tumors. Across the four histologies, tumors were collected from more male (71%) than female (29%) patients. Most tumors were early stage (TNM Stage I; 75%) with chRCC comprising the largest mean size of tumors (6.9 cm). Overall pRCC tumors exhibited the highest observed necrosis (36%) compared to ccRCC, chRCC and ROs. Since ROs are considered benign entities, no necrosis was observed and tumor grade was not reported.

3.2 Comparison of Immunohistochemical Staining for PBRM1 and BAP1

In Table 2 we also provide a comparison of the PBRM1 and BAP1 staining across the four RCC subtypes. The estimated mutation rate of *PBRM1* is 40% and *BAP1* is 10%²⁻⁴; consistent with the mutation rate, the loss of protein staining for PBRM1 and BAP1 in our ccRCC cohort was 43% and 10%, respectively (Figure 2). We noted loss of PBRM1 staining in 43% (80/187) of the ccRCC tumors, but we observed only 3%, 6% and 0% loss of PBRM1 staining in pRCC, chRCC, and RO, respectively ($P<0.0001$). Similarly, we observed loss of BAP1 staining in 10% (18/187) of the ccRCC tumors compared to none of the pRCC, chRCC or RO tumors ($P=0.00021$). The frequency of loss of staining for both PBRM1 and BAP1 in our ccRCC cohort was 2% (3/187), whereas loss of both markers was not observed in pRCC, chRCC or RO.

4. Discussion

Although recurrent mutations are observed in chromatin-modifying enzymes in ccRCC, there are no data regarding expression in pRCC, chRCC or RO. We evaluated loss of PBRM1 and BAP1 staining in ccRCC, pRCC, chRCC and RO tumors using an IHC assay for which negative staining associates with loss-of-function mutations. Our data are the first to suggest that biallelic inactivation of *PBRM1* and *BAP1* are less common in pRCC, chRCC and RO tumors than in ccRCC tumors.

Phylogenetic trees generated by multiregion sequencing of ccRCC tumors identified intratumor heterogeneity for *PBRM1* and *BAP1* mutations.⁷ We identified ccRCC tumors with heterogeneous PBRM1 and BAP1 staining; we further excluded samples with focal negative or weak positive staining of PBRM1 (n=44) or BAP1 (n=65) for the analysis.

Related to this, in our previous work, we focused on BAP1 expression and ccRCC outcomes⁶, we noted an increased risk of cancer-specific death for those patients with weak positive BAP1 staining versus positive BAP1 staining (HR=2.00; 95% CI 1.09 – 3.67). Of note, when we excluded the weak positive BAP1 tumors from our overall analysis, it did not alter the association of loss of BAP1 expression with increased risk of cancer-specific death. To date, we have not evaluated the association of weak positive PBRM1 staining with risk of cancer specific death in any RCC subtypes.

The World Health Organization classification of renal tumors incorporates morphological, molecular, IHC, and clinical information to define risk groups and refine histological subtypes.¹ ROs and chRCCs both arise from the collecting duct and share common histomorphologic and chromosomal abnormalities (loss of chromosomes 1 and 17).⁸ Diagnostic challenges exist in differentiating between the two subtypes and chRCC have a more aggressive clinical course with increased risk for metastases and sarcomatoid transformation. In our series, we did not observe any RO with loss of PBRM1 or BAP1 staining whereas we did observe a small proportion of loss of PBRM1 staining in chRCC. Further study may be warranted to determine if loss of PBRM1 staining would aid in differentiation between RO and chRCC in a setting of other equivocal histological and immunophenotypic markers. Similarly, our IHC data suggest that loss of BAP1 staining could potentially discriminate ccRCC tumors from pRCC or chRCC tumors. We did not observe any loss of BAP1 staining or BAP1 focal negatives in pRCC, chRCC or RO tumors.

Prior studies of pRCC, chRCC and RO tumors have focused on the analysis of tissue microarrays to assess biomarkers.^{9, 10} However tissue microarrays may not accurately represent biomarker heterogeneity or overall expression.¹¹ In our study, we used whole-tissue sections with stroma as an internal staining control to assess the overall PBRM1 and BAP1 staining as a surrogate of biallelic inactivation. Even within an individual ccRCC tumor, there is molecular heterogeneity with subclonal populations sharing chromosome 3p loss and *VHL* aberrations.^{7, 12} Loss of PBRM1 or BAP1 expression by IHC is associated with a worse RCC-specific survival in ccRCC supporting a role for distinguishing molecular subtypes of ccRCC.^{6, 13-17} The frequency of loss of IHC staining in our ccRCC cohort was consistent with the reported mutational frequency by sequencing for *PBRM1* and *BAP1*. *PBRM1* is the second most commonly mutated gene (~40%) in ccRCC, and yet, we observed only 3%, 6% and 0% loss of PBRM1 staining in pRCC, chRCC, and RO respectively.^{2, 3} Similarly, *BAP1* mutations have been detected in 10% of ccRCC tumors,^{3, 4} whereas we did not observe loss of BAP1 staining in our pRCC, chRCC, or RO tumors.

The 4 most common mutations (*VHL*, *PBRM1*, *SETD2*, and *BAP1*) in ccRCC are located on a region of chromosome 3p that is deleted in more than 90% of ccRCCs, supporting a model that leads to biallelic inactivation (concurrent copy number loss and mutation) of these genes.³ Associated with somatic mutations in the *MET* gene on chromosome 7 or *MET* kinase activation, pRCC is divided into 2 subtypes of pRCC, types 1 and 2, on the basis of their morphologic characteristics.^{1, 18, 19} In contrast to ccRCC, pRCC lacks 3p alterations but exhibits trisomy 7, leading to multiple copies of the *MET* allele.²⁰ A more heterogeneous cytogenetic profile is found in chRCC, with reported chromosomal losses of 1, 2, 6, 10, 13, 17, 21, and Y.²¹ No recurrent somatic mutations have been characterized in chRCC. ROs

have a normal chromosomal complement (40-50%) or loss of chromosome 1 (40%). Most of the tumors in our study were Stage I (75%), and may underestimate the loss of *PBRM1* or *BAP1* staining in more advanced stage tumors or clonal evolution in distant metastases. Although our IHC assay evaluates protein expression and may not detect monoallelic mutations or copy number loss at *PBRM1* and *BAP1* loci, the different patterns of chromosomal aberrations from ccRCC suggest that biallelic inactivation of *BAP1* and *PBRM1* is less common in pRCC, chRCC and RO tumors. In addition to mutations, there may be other pathways that downregulate *PBRM1* and *BAP1* expression. If these findings are independently confirmed, they would suggest that while loss of *PBRM1* and *BAP1* are key events in ccRCC, disruption of other pathways may support tumorigenesis in pRCC, chRCC and RO tumors. Consistent with our findings, whole-exome sequencing of chRCC identified recurrent mutations in *TP53* (32%) and *PTEN* (9%).²² Deep sequencing of sporadic pRCC by The Cancer Genome Atlas Program may provide further insight into lower prevalence or other secondary mutations. Prognostic algorithms that incorporate *PBRM1* or *BAP1* mutations and/or loss of expression may not be applicable to pRCC and chRCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ccRCC	clear cell renal cell carcinoma
chRCC	chromophobe renal cell carcinoma
IHC	immunohistochemistry
pRCC	papillary renal cell carcinoma
RCC	renal cell carcinoma
RO	renal oncocytoma
VHL	von Hippel-Lindau

Figure 1.

PBRM1 and BAP1 Immunohistochemistry Assay in Renal Cell Carcinoma. A, Flow diagram of sample cohort used for analysis. The IHC assay for PBRM1 and BAP1 successfully stained tumors 91.5% and 97.6%, respectively. After samples with focal negative and weak positive expression were excluded (n=109), 299 samples were available for final analysis. The IHC assay was validated with Sanger sequencing of *PBRM1* and *BAP1*.

Figure 2.

Representative photomicrographs of A, PBRM1 and B, BAP1 illustrating clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (pRCC), chromophobe renal cell carcinoma (chRCC), and renal oncocyoma (RO). Positivity was evaluated as tumor nuclei staining with intensity equal to or stronger than that of the surrounding stromal cells and lymphocytes (positive controls). Tumors with PBRM1 or BAP1 focal negativity or weak staining were removed from the primary analysis. Scale bar 100 μM. (Original magnification 100×; inset 400×.)

Table 1
Demographics and Clinicopathological Features of 299 Patients With Renal Cell Carcinoma Treated by Nephrectomy

	ccRCC (N=187)	pRCC (N=61)	chrRCC (N=17)	RO (N=34)	Total (N=299)	P value*
Sex						0.0252
F	63 (33.7%)	9 (14.8%)	4 (23.5%)	12 (35.3%)	88 (29.4%)	
M	124 (66.3%)	52 (85.2%)	13 (76.5%)	22 (64.7%)	211 (70.6%)	
Age at Surgery (years)						0.0205
Mean	63.1	66.8	66.1	67.8	64.6	
Median	64.5	68.5	72.3	68.3	66.4	
Range	(27.2-92.1)	(42.9-83.8)	(33.3-86.3)	(42.4-84.3)	(27.2-92.1)	
Tumor Size (cm)						0.0055
Mean	4.9	4.4	6.9	4.1	4.8	
Median	4.0	3.8	5.5	3.3	4.0	
Range	(0.3-13.6)	(1.0-15.0)	(2.5-15.0)	(1.2-13.0)	(0.3-15.0)	
TNM Stage						0.3696
Missing	1	0	0	34	35	
1	138 (74.2%)	48 (78.7%)	11 (64.7%)	0 (0.0%)	197 (74.6%)	
2	18 (9.7%)	5 (8.2%)	5 (29.4%)	0 (0.0%)	28 (10.6%)	
3	26 (14.0%)	7 (11.5%)	1 (5.9%)	0 (0.0%)	34 (12.9%)	
4	4 (2.2%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	5 (1.9%)	
Grade						0.0803
Missing	1	0	0	34	35	
1	17 (9.1%)	2 (3.3%)	0 (0.0%)	0 (0.0%)	19 (7.2%)	
2	118 (63.4%)	48 (78.7%)	9 (52.9%)	0 (0.0%)	175 (66.3%)	
3	42 (22.6%)	10 (16.4%)	8 (47.1%)	0 (0.0%)	60 (22.7%)	
4	9 (4.8%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	10 (3.8%)	
Necrosis						0.0001
Missing	1	0	0	0	1	
No	153 (82.3%)	39 (63.9%)	15 (88.2%)	34 (100.0%)	241 (80.9%)	
Yes	33 (17.7%)	22 (36.1%)	2 (11.8%)	0 (0.0%)	57 (19.1%)	

Abbreviations: ccRCC, clear cell renal cell carcinoma; chrRCC, chromophobe renal cell carcinoma; N/A, not applicable; pRCC, papillary renal cell carcinoma; RO, renal oncocytoma.

* All *P*-values result from Fisher's exact test, except for Age at Surgery and Tumor Size, for which the Kruskal-Wallis test was employed.

Table 2

Immunohistochemical Staining of Both BAP1 and PBRM1 Protein in Clear Cell Renal Cell Carcinoma, Papillary Renal Cell Carcinoma, Chromophobe Renal Cell Carcinoma, and Renal Oncocytoma

	ccRCC	pRCC	chRCC	RO
Combined	N=187	N=61	N=17	N=34
BAP1 Negative PBRM1 Negative	3	0	0	0
BAP1 Negative PBRM1 Positive	15	0	0	0
BAP1 Positive PBRM1 Negative	77	2	1	0
BAP1 Positive PBRM1 Positive	92	59	16	34

Abbreviations: ccRCC, clear cell renal cell carcinoma; chRCC, chromophobe renal cell carcinoma; pRCC, papillary renal cell carcinoma; RO, renal oncocytoma.