Clear Cell Renal Cell Carcinoma Subtypes Identified by BAP1 and PBRM1 Expression

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Abbreviations and Acronyms

BAP1 = BRCA associated protein 1
ccRCC = clear cell RCC
IHC = immunohistochemistry
PBRM1 = polybromo 1
RCC = renal cell carcinoma
RFS = relapse-free survival

Purpose: In clear cell renal cell carcinoma *BAP1* and *PBRM1* are 2 of the most commonly mutated genes (10% to 15% and 40% to 50%, respectively). We sought to determine the prognostic significance of PBRM1 and BAP1 expression in clear cell renal cell carcinoma.

Materials and Methods: We used immunohistochemistry to assess PBRM1 protein expression in 1,479 primary clear cell renal cell carcinoma tumors that were previously stained for BAP1. A centralized pathologist reviewed all cases and categorized tumors as positive or deficient for PBRM1 and BAP1. Kaplan-Meier and Cox regression models were used to evaluate association of PBRM1 and BAP1 expression with the risk of death from renal cell carcinoma and the risk of metastasis after adjustment for age and the Mayo Clinic SSIGN (stage, size, grade and necrosis) score.

Results: PBRM1 and BAP1 expression was PBRM1+ BAP1+ in 40.1% of tumors, PBRM1− BAP1+ in 48.6%, PBRM1+ BAP1− in 8.7% and PBRM1− BAP1− in 1.8%. The incidence of PBRM1 and BAP1 loss in the same tumor was significantly lower than expected (actual 1.8% vs expected 5.3%, $p < 0.0001$). Compared to patients with PBRM1+ BAP1+ tumors those with PBRM1− BAP1+ lesions were more likely to die of renal cell carcinoma (HR 1.39, $p = 0.035$), followed by those with PBRM1+ BAP1− and PBRM1− BAP1− tumors (HR 3.25 and 5.2, respectively, each $p < 0.001$). PBRM1 and BAP1 expression did not add independent prognostic information to the SSIGN score.

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Conclusions: PBRM1 and BAP1 expression identified 4 clinical subgroups of patients with clear cell renal cell carcinoma who had divergent clinical outcomes. The clinical value of these biomarkers will be fully realized when therapies targeting pathways downstream of PBRM1 and BAP1 are developed.

Key Words: kidney; carcinoma, renal cell; genes, tumor suppressor; biological markers; mortality

Patients with ccRCC show widely divergent clinical behavior, which is likely explained by genetic differences in the tumor. Genes implicated in ccRCC pathogenesis at a frequency of between 3% and 50% include *PBRM1, BAP1, SETD2, TCEB1* and *KDM5C*.[1–10] The impact of these mutations on ccRCC clinical outcomes remains unclear.

Two of the most commonly mutated genes in ccRCC are *PBRM1* (about 40% to 50%) and *BAP1* (10% to 15%). Both genes are located on chromosome 3p, which is the most commonly (about 90%) cytogenetically deleted region in ccRCC. Previous studies intimate that mutations in *PBRM1* and *BAP1* are largely mutually exclusive, suggesting a possible genetic interaction between these genes.[8,10] Previously we found that patients whose tumor harbors a *PBRM1* mutation had improved outcomes compared to those whose tumor harbors a *BAP1* mutation.[10] Separate investigators, including those using data from TCGA (The Cancer Genome Atlas), confirmed that mutations in *BAP1* are associated with a poor prognosis but mutations in *PBRM1* did not impact prognosis compared to wild-type *PBRM1*.[6]

Complementing the analysis of the clinical significance of mutations in *PBRM1* and *BAP1* in ccRCC, multiple groups, including ours, assessed the association of PBRM1 and BAP1 protein expression with clinical outcomes. We previously developed and validated IHC based assays with a high degree of reliability for detecting mutations in these genes (ie if the stain is negative, the gene is mutated).[8] Using IHC we noted that BAP1 loss was associated with a significant increase in the risk of RCC specific death.[11] In addition at least 2 studies demonstrate that loss of PBRM1 expression on IHC is associated with worse outcomes.[12,13]

Previous studies of the clinical significance of *PBRM1* and *BAP1* mutations in ccRCC are limited. 1) Relatively small sample sizes with short-term followup made it difficult to detect small differences in clinical outcomes or differences that might develop later. 2) Many prior groups analyzed the clinical significance of PBRM1 or BAP1 individually rather than both proteins simultaneously in the same cohort. In the current investigation we addressed the limitations of the prior studies by assessing PBRM1 and BAP1 in a large sample of greater than 1,400 cases with extended followup, demonstrating that PBRM1 and BAP1 are interrelated and identifying 4 subtypes of ccRCC.

MATERIALS AND METHODS

Patient Selection

After receiving institutional review board approval we identified 1,479 patients in the Mayo Clinic Rochester nephrectomy registry who presented with nonmetastatic disease and were treated with radical nephrectomy or nephron sparing surgery for unilateral, sporadic, noncystic ccRCC between 1990 and 2009. Followup data and clinicopathological covariates were abstracted from the registry. Briefly these data are routinely updated and maintained through a combination of active (mailed questionnaires) and passive (medical record and linkage to national databases) surveillance by experienced clinical coordinators. Pathological features were analyzed in standardized fashion by 1 urological pathologist (JCC) who centrally reviewed the microscopic hematoxylin and eosin slides from all specimens while blinded to patient outcome. As part of the review the pathologist determined components of the Mayo Clinic SSIGN score, an externally validated prognostic algorithm for ccRCC prognosis. A higher score implies poorer cancer specific survival.

Immunohistochemistry Assay Methodology

A representative formalin fixed, paraffin embedded tissue block with viable tumor was selected from each case. From each block serial 3 to 4 μm unstained sections were obtained and submitted for IHC staining. IHC was performed with the Benchmark XT™ automated stainer as previously described.[8,10,11] Briefly sections were deparaffinized, rehydrated and subjected to heat induced epitope retrieval. They were incubated with primary antibody against BAP1 (mouse monoclonal, clone C-4, Santa Cruz Biotechnology®) or PBRM1 (rabbit polyclonal, Bethyl Laboratories, Montgomery, Texas). After incubation slides were subjected to a DAB detection system (Ventana Medical Systems, Tucson, Arizona), counterstained with hematoxylin, dehydrated back to xylene and coverslipped. Three positive and negative ccRCCs with known mutation status served as controls for each immunostain run. Nuclear reactivity was considered a positive signal for BAP1 and/or PBRM1. In each tumor section lymphocytes, stromal fibroblasts and endothelial cells served as internal positive control cells.

Immunohistochemistry

Assay Validation. IHC assays for BAP1 and PBRM1 were validated using 176 genetically characterized ccRCC samples.[8] As previously reported, scoring was performed