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# Effects on survival of *BAP1* and *PBRM1* mutations in sporadic clear-cell renal cell carcinoma: a retrospective analysis with independent validation

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## SUMMARY

**Background—**Clear cell renal cell carcinoma (ccRCC) displays a variety of clinical behaviors. However, the molecular genetic events driving these behaviors are unknown. We discovered that *BAP1* is mutated in approximately 15% of ccRCC and that *BAP1* and *PBRM1* mutations are largely mutually exclusive. The aim of this study was to investigate the clinicopathological significance of these molecular subtypes and to determine whether patients with *BAP1*-mutant and *PBRM1*-mutant tumors had different overall survival.

**Methods—**In this retrospective analysis, we assessed 145 patients with primary clear-cell renal-cell carcinoma and defined *PBRM1* and *BAP1* mutation status from the University of Texas Southwestern Medical Center (UTSW), TX, USA, between 1998 and 2011. We classified patients into those with *BAP1*-mutant tumors and those with tumors exclusively mutated for *PBRM1* (*PBRM1*-mutant). We used a second independent cohort (n=327) from The Cancer Genome Atlas (TCGA) for validation. In both cohorts, more than 80% of patients had localized or locoregional disease at presentation. Overall both cohorts were similar, although the TCGA had more patients

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Contributors

PK reviewed histologic slides, analyzed the data, reviewed the literature, and wrote the manuscript. SPL collected SSDI information, managed and analyzed the data, performed statistical analyses and prepared the figures. LZ and APJ collected clinical data. AC performed statistical analyses under the supervision of XJX. WKR assisted with TCGA data access and analysis. JB conceived and supervised the study, analyzed the data, and wrote the manuscript.

Conflicts of interest

None

with metastatic and higher-grade disease, and more TCGA patients presented before molecularly targeted therapies became available.

**Findings—**The median overall survival in the UTSW cohort was significantly shorter for patients with *BAP1*-mutant tumors (4.6 years; 95% CI 2.1-7.2), than for patients with *PBRM1*-mutant tumors (10.6 years; 9.8-11.5), corresponding to a HR of 2.7 (95% CI 0.99-7.6, p=0.044). Median overall survival in the TCGA cohort was 1.9 years (95% CI 0.6-3.3) for patients with *BAP1*-mutant tumors and 5.4 years (4.0-6.8) for those with *PBRM1*-mutant tumors. A HR similar to the UTSW cohort was noted in the TCGA cohort (2.8; 95% CI 1.4-5.9; p=0.004). Patients with mutations in both *BAP1* and *PBRM1*, although a minority (three in UTSW cohort and four in TCGA cohort), had the worst overall survival (median 2.1 years, 95% CI 0.3-3.8, for the UTSW cohort, and 0.2 years, 0.0-1.2, for the TCGA cohort).

**Interpretation—**Our findings identify mutation-defined subtypes of ccRCC with distinct clinical outcomes, a high-risk *BAP1*-mutant group and a favorable *PBRM1*-mutant group. These data establish the basis for a molecular genetic classification of ccRCC that could influence treatment decisions in the future. The existence of different molecular subtypes with disparate outcomes should be considered in the design and evaluation of clinical studies.

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## Introduction

Over 60,000 new cases and 13,000 deaths from tumors of the kidney and renal pelvis were expected in the US in 2012.(1) Approximately 70% of renal cell carcinomas (RCC) present with localized disease and ~30% of patients who undergo surgery with curative intent may experience a recurrence.(2) Several variables have emerged that influence outcome including TNM stage, tumor size, Fuhrman grading and necrosis.(3-6) In the metastatic setting, patients can be stratified based on clinical and laboratory parameters.(7) More recently, Heng et al. stratified patients with metastatic RCC based on time from diagnosis to treatment, Karnofsky performance status, hemoglobin, corrected calcium, neutrophils and platelets into favorable (median OS, not reached), intermediate (median OS, 27 months), and poor (median OS, 8.8 months) risk groups.(8) However, what determinants in the tumor account for the different behaviors is poorly understood.

Different behaviors may be driven by different mutations. Clear cell renal cell carcinoma (ccRCC), which accounts for 70-80% of all RCC,(2) is characterized by inactivation of the von Hippel-Lindau gene (VHL).(9) In addition, truncating mutations in *PBRM1* (polybromo 1), a gene encoding a SWI/SNF chromatin-remodeling complex component, are found in 41% of ccRCC.(10) While mutated at a substantially lower frequency, other genes implicated in ccRCC are *SETD2, KDM6A* (UTX), and *KDM5C* (JARID1C).(10-12) However, whether these mutations affect outcomes is unknown.

Recently, we reported that the gene *BAP1* (BRCA1 associated protein-1) was mutated in approximately 15% of ccRCC.(13) Interestingly, *BAP1* and *PBRM1* mutations in tumors are largely mutually exclusive.(13) In addition, whereas tumors with *BAP1* mutations are typically of high grade, tumors exclusively mutated for *PBRM1* tend to be of lower grade.

(13) These results led us to hypothesize that *BAP1*-mutated tumors may be associated with worse outcomes than those of patients with *PBRM1*-mutated tumors.

## Methods

### Study Population

We did a retrospective analysis with an initial study cohort that included 176 patients who underwent resection of a ccRCC at the University of Texas Southwestern Medical Center (UTSW) between 1998 and 2011 and whose tumors were genotyped for *BAP1* and *PBRM1*. (13) In order for patients to be included in the mutation analyses, ≥70% ccRCC cellularity on sections flanking the specimen to be evaluated for genetic analyses was required.(13) For this study, patients were excluded if samples were derived from metastases (4 cases) or follow-up information was lacking (27 cases). The remaining 145 patients comprise the UTSW cohort. These studies were conducted under a protocol approved by the UTSW Institutional Review Board and in accordance to the Health Insurance Portability and Accountability Act guidelines.

### Data Collection

Clinical and pathological data were collected retrospectively from medical records as well as an electronic database and were entered into a standardized database. The social security death index (SSDI) was surveyed for dates of death (http://www.genealogybank.com/gbnk/ssdi).

Archived hematoxylin/eosin stained slides were centrally reviewed by a pathologist (PK) blinded to the mutation status and pathology reports (123 cases). For the remaining 22 cases, data was from pathology reports. Tumor histology and grade were determined according to 2004 World Health Organization criteria (14) and the Fuhrman grading system.(15) Presence of any tumor spindle cells reminiscent of sarcoma was sufficient to consider the tumor as exhibiting sarcomatoid dedifferentiation. Rhabdoid histology was assigned if there were foci of high-grade malignant cells with abundant eosinophilic cytoplasm, globular eosinophilic paranuclear inclusion bodies, large eccentric vesicular nuclei, and prominent nucleoli occupying at-least one field (10x objective). Tumor necrosis was defined as microscopic coagulative necrosis. Cases were staged using radiographic reports and postoperative pathological data and re-assigned according to the American Joint Committee on Cancer 2010 TNM classification.(16) In general, patients were followed postoperatively with physical examination, laboratory studies, chest imaging and abdominal/pelvic computerized tomography (CT) semiannually for the first two years and annually thereafter for five years.

Findings from the UTSW cohort were compared to a publically available, open-access, dataset of ccRCC from The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/tcga/) (July 16$^{th}$, 2012 update). In the TCGA, tumor necrosis was ascertained based on sections flanking a small specimen to be used for molecular studies. Greater than 50% of tumor nuclei in histological slides was required to qualify. In four instances, the staging did

not correspond to the TNM information provided and this information was excluded from analyses.

### Immunohistochemistry

The canonical mammalian target of rapamycin complex 1 (mTORC1) markers, phospho-S6 ribosomal protein (S235/236), and phospho-4E-BP1 (T37/46), were evaluated by immunohistochemistry (IHC) to asses for mTORC1 activation.(13) Results were scored by a pathologist (PK) blinded to clinicopathological variables and mutation status.

### Gene expression analyses

RNA-Seq information was obtained from the TCGA Data Portal and was available for 308 ccRCC annotated with mutations. RNA-Seq data was aligned using MapSplice and quantified and normalized using the reads per kilobase of exon model per million mapped reads [RPKM] method by the TCGA. Gene expression signatures were derived by comparing tumors with mutations in *BAP1* ($n$=20) (or *PBRM1* [$n$=66]) to the rest using unpaired $t$ tests adjusted for the group variances and a Benjamini & Hochberg false discovery rate (FDR) correction.(17) Nineteen genes out of 20,532 failed to provide valid $p$ values and were eliminated from the analyses. The significance of the gene expression signatures was assessed by comparing the number of genes identified to the number found in groups made up of random tumors of the same size ($n$=20 or 66). Pathways were analyzed using Ingenuity Pathway Analysis software.

### Statistical analysis

Unless otherwise indicated, tumors were classified into *BAP1*-mutant tumors and tumors exclusively mutated for *PBRM1* based on previously reported somatic (non-silent) mutations.(13) Associations between a mutation group and patient or tumor characteristics were determined using a Fisher's exact test (for categorical variables) or Student's $t$ test (for continuous variables). The significance of the gene expression signatures was evaluated using a one-sample $t$ test. Patients without residual disease after surgery were evaluated for metastases (either in regional lymph nodes or distant sites). Follow-up was considered from the time of nephrectomy to the date of death or last contact. Overall survival (OS) was computed from the date of surgery to the date of death from any cause. Patients alive at the end of the study period were censored at the date of last follow-up or the last date the patient was known to be alive, whichever was longer. OS was assessed using Kaplan-Meier estimates and comparisons were performed using the log-rank test. Hazard ratios (HR) were obtained from Cox regression analyses. Time-to-event results are reported with a HR, 95% confidence interval (CI) for the HR, and the log-rank $p$-value. Unless indicated, $p$-values are two-sided without adjusting for multiple comparisons. To assess whether mutation was independently associated with outcome, variables that were associated with OS at the 0.20 level were included in multivariate Cox regression models after a backwards conditional method, in which the variable with the highest $p$-value was removed one at a time until all variables left in the model were significant at the 0.05 level. pN, which had missing data for half of the patients was excluded from the model in both cohorts. Grade 2 was used as the reference since there were only two grade 1 patients in each cohort. All variables were

treated as categorical except for age. Statistical analyses were conducted using SAS 9.2 for the multivariate Cox regression analyses and SPSS Statistics 17.0 for the rest.

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## Results

The median age was 62 years (IQR=54, 70) and there was a male predominance (Table 1). The mean tumor size was 5.7 cm (IQR=4.1, 8.7). Fifty-two (76 of 145) percent of the tumors were of high grade (Fuhrman grade 3 and 4). Eleven percent (7 of 63) of patients who underwent regional lymph node dissection had nodal metastases and 9% (13 of 145) had distant metastases at the time of surgery.

We investigated a second, completely independent, cohort of ccRCC cases from TCGA. At the time of our analyses, mutation data was available for 327 patients, which represent the cohort analyzed. Demographics and other characteristics are summarized in table 1. The mean age was 61 years (IQR=52,71), with a male predominance. Fifty-eight percent (187 of 325) of the tumors were of high grade. Eight percent (12 of 159) of patients who underwent regional lymph node resection had nodal metastases and 16% (52 of 327) had distant metastases at the time of surgery. Overall, the TCGA and UTSW cohorts were similar, although more patients presented with higher grade and metastases in the TCGA cohort.

Among the patients in the UTSW cohort, there were 21 with *BAP1*-mutated tumors, including 3 with mutations in both *BAP1* and *PBRM1*. The number of patients with tumors exclusively mutated for *PBRM1* was 78. *VHL* mutations were present in 71% (15 of 21) of *BAP1*-mutant and 87% (68 of 78) of *PBRM1*-mutant tumors, respectively. A comparison of patients with *BAP1*- versus *PBRM1*-mutated tumors showed that patients with *BAP1*-mutated tumors were more likely to present with aggressive features including higher grade, sarcomatoid and rhabdoid histology, coagulative tumor necrosis, and mTORC1 activation ($p<0.05$ for all) (table 2).

In the TCGA cohort, there were 20 *BAP1*-mutant tumors (including 4 tumors with mutations in both *BAP1* and *PBRM1*) and 74 tumors with mutations exclusively in *PBRM1*. Consistent with the results in the UTSW cohort, *BAP1*-mutated tumors showed a trend towards higher grade ($p=0.095$) and *BAP1* mutation was associated with necrosis ($p=0.038$). In addition, *BAP1*-mutated tumors were more likely to have advanced pT and clinical stage ($p<0.05$ for both) (table 2). While the latter associations were not observed in the UTSW cohort, in every instance, *BAP1*-mutant tumors were associated with indicators of poor outcome.

In the UTSW cohort, patients with *BAP1*-mutant tumors had a median OS of 4.6 years (95%CI, 2.1-7.2 years), which was substantially shorter than that of patients with *PBRM1*-mutated tumors, whose median OS was 10.6 years (95%CI, 9.8-11.5 years). The differences in OS corresponded to a HR of 2.7 (95%CI, 0.99-7.6, $p=0.044$) (figure 1). As in the UTSW

cohort, in the TGCA cohort, patients with *BAP1*-mutated tumors had a significantly higher probability of death (HR, 2.8; 95%CI, 1.4-5.9; $p$=0.004) (figure 1). While the median OS values differed between the TCGA and UTSW cohorts, possibly reflecting differences in the patient population and the availability of targeted therapies at the time of presentation (supplementary figure 1), the HR in both cohorts were almost identical (2.7 vs. 2.8). These data show that *BAP1*-mutated tumors are associated with significantly worse OS than *PBRM1*-mutated tumors.

To assess how representative the cohorts of patients with *BAP1*- and *PBRM1*-mutated tumors were, we performed univariate Cox regression analyses. As expected, in both the UTSW and TCGA cohorts, pN, M, stage, grade and necrosis were all associated with OS (supplementary table 1). Race, on the other hand, was not associated with OS in either cohort (supplementary table 1).

Multivariate Cox regression analyses were performed with all variables that reached 0.20 significance in univariate analyses in each cohort respectively (supplementary table 1) except for pN, which had missing data in half of the patients. A backwards elimination process to identify the best fit model found that M and grade were independently associated with OS in the UTSW cohort (supplementary table 2). Other known predictors of outcome were not recovered, possibly owing to the small sample size. Interestingly, in addition to M and grade, mutations in *BAP1* and *PBRM1* were independently associated with OS in the TCGA cohort (HR, 2.3; 95% CI, 1.03-5.1; $p$=0.041) (table 3).

Our previous studies with a small number of samples suggested that *BAP1*- and *PBRM1*-mutated tumors have different gene expression signatures.(13) To evaluate this notion further, we analyzed gene expression signatures from RNASeq data of the TCGA (available for 308 of the 327 samples with mutation information). We asked whether *BAP1*-mutant tumors could be distinguished from the rest. A comparison of gene expression between *BAP1*-mutated tumors ($n$=20) and the rest identified 3,250 genes that were deregulated in the *BAP1*-mutant group after a false-discovery rate (FDR) correction ($q$<0.05) (table 4). To ascertain the significance of these deregulated genes, we asked how many genes would distinguish a group of the same size chosen randomly. When 20 tumors were chosen at random, only 115 genes distinguished this group from the rest. This was repeated twice, and the numbers that distinguished these arbitrary groups were 63 and 120 respectively (table 4). The differences in the number of genes identified between the *BAP1*-mutant group (3,250 genes) and the groups of random tumors was highly statistically significant ($p$=<0.0001). These data show that *BAP1*-mutated tumors are associated with a characteristic gene expression signature.

A comparison of *PBRM1*-mutant tumors ($n$=66) to the rest revealed 2,235 genes that distinguished these tumors at an FDR $q$<0.05 (table 4). When compared to three groups of 66 tumors selected at random, the difference in the number of distinguishing genes (2,235 vs. 0, 0, and 3) was highly significant ($p$=<0.0001) (table 4). Thus, *PBRM1* mutated tumors are also associated with a characteristic gene expression signature.

The number of genes in common between the *BAP1* and *PBRM1* signatures was 369 (figure 2). However, the overlap expected at random was 381. Thus, the signatures were non-overlapping and this reflected aberrations in different pathways (figure 2). Supplementary figure 2 lists genes in the *BAP1* and *PBRM1* signatures that most clearly distinguished these groups. *BAP1*-mutant tumors were characterized by changes in the expression of genes implicated in growth factor signaling, whereas *PBRM1*-mutant tumors exhibited expression changes in genes implicated in cytoskeleton and tissue architecture.

Finally, while the number of double mutant tumors was very small (3 and 4 in the UTSW and TCGA cohorts respectively), pathological studies of the UTSW cohort had suggested that these tumors were associated with rhabdoid features and may be particularly aggressive.(13) In keeping with these results, Kaplan-Meier estimates of the UTSW cohort showed that double mutant tumors were associated with the worst outcomes (HR 5.3; 95% CI, 1.2-22.9; $p$=0.012) (supplementary figure 3A). This was also the case for the TCGA cohort (HR, 10; 95%CI, 3.2-33.6, $p$=<0.0001) (figure 3). We also included in our analyses tumors for which mutations in *BAP1* or *PBRM1* were not identified (figure 3 and supplementary figure 3B). However, this group is likely to be heterogeneous and made up by more than one molecular genetic subtype.

## Discussion

Our findings show that ccRCC can be subclassified into at least two biologically and clinically distinct entities: *BAP1*-mutant and *PBRM1*-mutant tumors. These tumors are associated with distinct gene expression signatures, and therefore different biology, and *BAP1*-mutant tumors exhibited pathological features suggestive of aggressive disease. *BAP1*-mutant tumors are associated with significantly worse overall survival than *PBRM1*-mutant tumors (median OS in UTSW of 4.6 vs. 10.6 years; $p$=0.044). This difference corresponded to a HR of 2.7 (95%CI, 0.99-7.6) and an almost identical HR was observed in the TCGA cohort (HR, 2.8; 95%CI, 1.4-5.9; $p$=0.004). Thus, this study establishes the foundation for the first molecular genetic classification of sporadic ccRCC.

Why *BAP1*-mutant tumors are associated with worse survival is not understood. However, in both cohorts, when compared to *PBRM1*-mutated tumors, *BAP1* mutation in tumors was associated with coagulative necrosis, an independent predictor of outcome.(18) In addition, *BAP1*-mutated tumors exhibited higher Fuhrman grade ($p$=<0.0001 and $p$=0.095 in UTSW and TCGA, respectively). The main determinant of Fuhrman grading in everyday practice is nucleolar prominence,(19) which by itself is associated with survival.(20) The nucleolus is the site within the cell where ribosomes are synthesized. Ribosomes are necessary for mRNA translation and both ribosome biogenesis as well as mRNA translation are regulated by mTORC1.(21) Furthermore, mTORC1 activity has been linked to nucleolar size.(22, 23) In RCC, a correlation was found between Fuhrman grading and S6 phosphorylation,(24) a marker of mTORC1 activation. Interestingly, *BAP1* mutation is linked to mTORC1 activation (although this association is likely to be indirect)(13) and this may contribute to explain the connection between *BAP1* mutation high Fuhrman grade and outcome. Interestingly, in uveal melanoma, where *BAP1* is also found to be somatically mutated,

*BAP1* mutations were present in the majority of metastasizing but in only a minority of non-metastasizing tumors.(25)

ccRCC is characterized by *VHL* mutations, but *VHL* inactivation alone is insufficient for tumor initiation.(26, 27) Both *BAP1* and *PBRM1* are two-hit tumor suppressor genes and they are located on chromosome 3p (where *VHL* is found), in a region that is deleted in the vast majority of sporadic ccRCC.(14, 28) We speculate that, in many instances, ccRCC development is initiated by a focal mutation in *VHL*, followed by a 3p deletion. 3p loss would leave cells without *VHL* gene function and with just one copy of *BAP1* and *PBRM1*. Mutation of the remaining *BAP1* or *PBRM1* allele may initiate tumorigenesis resulting in tumors, depending upon which gene is mutated, of different aggressiveness. Thus, tumor aggressiveness may be established early on during the process of tumorigenesis.

We hypothesize that *BAP1* and *PBRM1*, when mutated, represent truncal events and that the pathways deregulated by their loss are ideal targets for drug development. Likely accounting for macro- and microscopic differences within tumors, significant genetic heterogeneity has been reported in primary ccRCC.(29) According to their prevalence, mutations can be classified into ubiquitous, shared and private. Ubiquitous mutations are shared across all tumor cells and include initiating events. We postulate that mutations in *BAP1* and *PBRM1* (and *VHL*) represent ubiquitous, truncal, drivers of tumor development. Thus, the discovery of *BAP1* and *PBRM1* mutations in ccRCC may pave the way for the next generation of targeted therapies. Given the particularly poor outcomes of *BAP1*-mutant tumors, identifying vulnerabilities resulting from BAP1 loss is particularly important. Once candidate drugs are found, their evaluation may be facilitated by the availability of tumorgraft models reproducing the molecular genetics and treatment responsiveness of RCC in humans.(30)

How mutations in *BAP1* and *PBRM1* drive renal carcinogenesis is not understood. We convincingly show that *BAP1*- and *PBRM1*-mutant tumors exhibit highly specific gene expression signatures that distinguish these tumors from the rest. The BAP1 and PBRM1 gene expression signatures are quite distinct and this is in keeping with differences in pathological features and patient outcomes. Interestingly, most genes that make up the *BAP1* signature were downregulated in *BAP1*-mutated tumors. These data raise the possibility that BAP1, which is a nuclear deubiquitinase, may act by deubiquitinating transcription factors, which in the absence of BAP1 are ubiquitinated and targeted for proteosomal-mediated degradation. Support for such a model is provided by a recent report.(31) Similarly, most genes that made up the PBRM1 signature were downregulated in *PBRM1*-mutant tumors. PBRM1 is the chromatin targeting subunit of a nucleosome remodeling complex and we speculate that when *PBRM1* is mutated, increased levels of closed chromatin impair transcription reducing thereby gene expression.

Other genes have been implicated in ccRCC development including *SETD2*, *KDM5C* and genes of the MLL family.(10-12, 32) How mutations in these genes relate to mutations in *BAP1* and *PBRM1* remains to be explored. Similarly, it is presently unknown whether these mutations define other molecular subtypes with different biology and outcomes.

There are several limitations to this study. First, our study evaluated mostly Caucasian patients and the distribution of mutations in different patient populations remains to be determined. Second, the sample size and follow-up are modest. However, for a study assessing the molecular genetics of renal cancer, two completely independent cohorts of 145 and 327 patients respectively is not insubstantial, and in the TCGA, median follow-up was 35 months. In addition, mutation analyses are less susceptible to subjective calls than the more conventional IHC studies. More importantly, these features did not preclude the identification of meaningful and statistically significant differences in OS associated with *BAP1*- and *PBRM1*-mutated tumors and the HR were almost identical in both cohorts (2.7 and 2.8). The nearly identical HR and statistically significant *p* values in two representative and independent patient cohorts strongly supports the conclusion that *BAP1*- and *PBRM1*-mutated tumors are associated with distinct survival outcomes. Third, the median OS for patients with either *BAP1* or *PBRM1* mutant tumors was shorter in the TCGA cohort. However, regardless of mutation status, the OS for all patients was shorter for the TCGA than the UTSW cohort (10.3 vs 5.4 years). Factors that may explain this difference include differences in the patient population with higher rates of patients with metastatic disease [16% (52 of 327) vs 9% (13 of 145)] and high grade [58% (187 of 327) vs 52% (76 of 145)] in the TCGA. Another factor is the availability of targeted therapies. In fact, 37% (43 of 115) of patients in the TCGA cohort died prior to 2006 (when molecularly targeted therapies became available) and only 11% (4 of 35) in the UTSW cohort (see supplementary figure 1).

It remains to be determined whether *BAP1* and *PBRM1* are independent predictors of outcome. Multivariate analysis of the TCGA cohort showed that mutations in *BAP1* and *PBRM1* predicted outcome independently of other variables. While this analysis found other known predictors of outcome (grade and M status) several established predictors failed to surface. This is likely due to the small sample size. Consistent with this, prognostic factors in existing nomograms were identified in significantly larger patient cohorts.(3-6) This may also explain why mutation status was not found as an independent predictor in the UTSW cohort. Our efforts continue in the development of robust immunohistochemistry assays that accurately report on BAP1 and PBRM1 and which could facilitate their definitive evaluation as independent predictors of patient survival in larger patient cohorts.

Importantly, however, this is not simply a biomarker study. Biomarkers refer to indicators of disease state that provide prognostic or predictive information. Biomarkers do not necessarily inform on the biology of the tumor and their value is typically predicated upon how much information they add onto existing nomograms. Furthermore, biomarkers may represent epiphenomenological variables with poorly understood links to tumor biology. In contrast, we linked two genetic drivers of ccRCC to disparate outcomes. The classification we propose is based on mutations in driver genes which are associated with distinct gene expression patterns and a different biology. The study is also not simply a classification based on gene expression, which has been reported previously.(33) Rather, the novelty of the study is in establishing the foundation for the first molecular genetic classification of sporadic renal cancer based on distinct biological subtypes that are associated with different outcomes. Furthermore, these different subtypes may have different responses to treatment and only with their recognition can drug effectiveness be properly assessed. For example, a

drug could be very active against BAP1-deficient tumors, but since these tumors account for just 15% of all ccRCC, the effect would be masked in an unselected population.

Finally, while the number of patients with *BAP1* and *PBRM1* double mutant tumors was very small in both cohorts and these tumors are infrequent (1-2% [3 of 145 and 4 of 327] of all sporadic ccRCC), they tend to be associated with both pathological and outcome measures suggestive of greatest aggressiveness.

In conclusion, our results provide the basis for a biologically meaningful and clinically relevant molecular genetic classification of ccRCC that may influence strategies for improved targeted therapies and personalized care.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Research in context

No systematic review was performed. This work stems from discoveries made in our laboratory.(13) Through a combination of genome and exome sequencing followed by Sanger sequencing of candidate genes in a large number of ccRCC, we discovered that the *BAP1* gene was mutated in approximately 15% of ccRCC. We found that *BAP1* and *PBRM1* mutations in tumors were largely mutually exclusive and that BAP1 loss, but not PBRM1 loss, was associated with high tumor grade. These data suggested that the *BAP1* and *PBRM1* genes defined different subtypes of ccRCC that could be associated with different outcomes. In this manuscript, we show that *BAP1*- and *PBRM1*-mutated tumors are associated with distinct gene expression patterns and consequently different biology. Most importantly we show that *BAP1*- and *PBRM1*-mutated tumors are associated with disparate patient overall survival.

## Interpretation

While the number of patients with *BAP1*- and *PBRM1*-mutant tumors in each cohort is relatively small, the HR are almost identical in both the UTSW and TCGA cohorts and the log-rank p values are significant at the 0.044 and 0.004 level. These data indicate that *BAP1*- and *PBRM1*-mutated tumors are associated with distinct overall survival. *BAP1*- and *PBRM1*-mutant tumors exhibited different gene expression signatures reflecting different biology and pathogenesis. This study sets the foundation for the first molecular genetic classification of sporadic ccRCC and paves the way for therapies tailored to the different molecular subtypes.

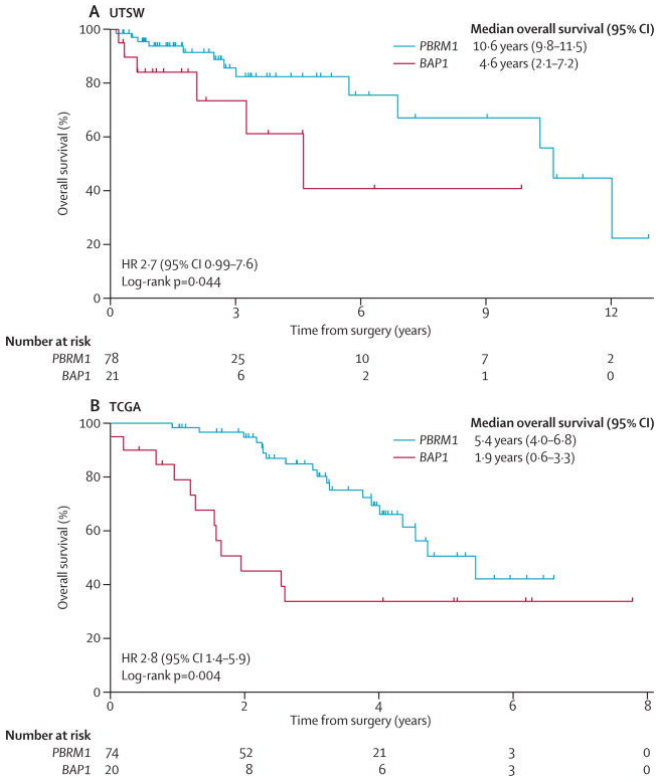


**Figure 1.**
Kaplan-Meier curves of overall survival for UTSW (A) and TCGA (B).

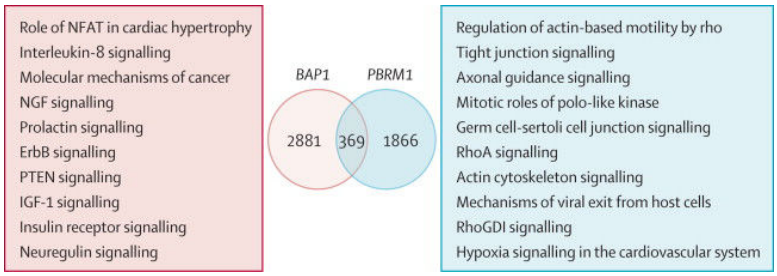


**Figure 2.**

Pathway analysis for the BAP1 and PBRM1 signatures. Venn diagrams with number of genes that characterize *BAP1*- and *PBRM1*-mutant tumors, as well as overlap, and selected deregulated pathways in each group.

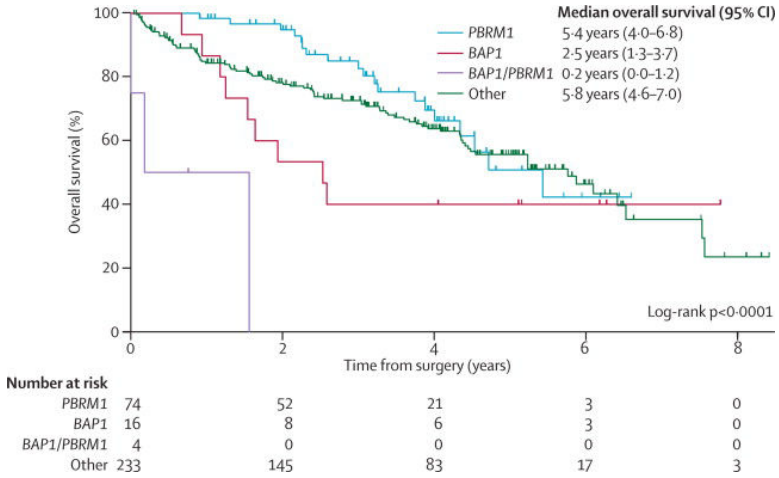


**Figure 3.**

Kaplan-Meier curves of overall survival for the indicated groups from TCGA.

## Table 1

Patient and tumor characteristics

| | UTSW (*n*=145) | TCGA (*n*=327) |
|---|---|---|
| **Age** | | |
| Median | 62 (54-70) | 61 (52-71) |
| **Race** | | |
| White | 103/132 (78%) | 288/322 (89%) |
| Hispanic | 16/132 (12%) | 15/322 (5%) |
| African American | 9/132 (7%) | 11/322 (3%) |
| Indian | 3/132 (2%) | 0/322 (0%) |
| Asian | 0/132 (0%) | 8/322 (2%) |
| Native American | 1/132 (<1%) | 0/322 (0%) |
| **Sex** | | |
| Female | 65/145 (45%) | 118/327 (36%) |
| Male | 80/145 (55%) | 209/327 (64%) |
| **Fuhrman grade** | | |
| 1 | 2/145 (1%) | 5/325 (2%) |
| 2 | 67/145 (46%) | 133/325 (41%) |
| 3 | 54/145 (37%) | 136/325 (42%) |
| 4 | 22/145 (15%) | 51/325 (16%) |
| **pT** | | |
| T1 | 67/145 (46%) | 149/327 (46%) |
| T2 | 22/145 (15%) | 41/327 (12%) |
| T3 | 50/145 (34%) | 131/327 (40%) |
| T4 | 6/145 (4%) | 6/327 (2%) |
| **pN** | | |
| 0 | 56/63 (89%) | 147/159 (92%) |
| 1 | 7/63 (11%) | 12/159 (8%) |
| **M** | | |
| 0 | 132/145 (91%) | 275/327 (84%) |
| 1 | 13/145 (9%) | 52/327 (16%) |
| **Stage (clinical)** | | |
| I | 65/145 (45%) | 145/323 (45%) |
| II | 18/145 (12%) | 31/323 (10%) |
| III | 45/145 (31%) | 93/323 (29%) |
| IV | 17/145 (12%) | 54/323 (17%) |

Data are number (%) unless otherwise stated. Percentages exclude missing samples. UTSW=University of Texas Southwestern Medical Center. TCGA=The Cancer Genome Atlas.

## Table 2

Phenotypic and pathologic variables of patients with BAP1-mutant tumors and tumors exclusively mutated for *PBRM1* in the UTSW and TCGA

| | UTSW | | | TCGA | | |
|---|---|---|---|---|---|---|
| | ***PBRM1* (*n*=78)** | ***BAP1* (*n*=21)** | **p value** | ***PBRM1* (*n*=74)** | ***BAP1* (*n*=20)** | **p value** |
| pT | | | 0·28 | | | 0·011 |
| 1 | 33/78 (42%) | 7/21 (33%) | | 40/74 (54%) | 4/20 (20%) | |
| 2 | 10/78 (13%) | 6/21 (29%) | | 11/74 (15%) | 3/20 (15%) | |
| 3 | 33/78 (42%) | 7/21 (33%) | | 23/74 (31%) | 13/20 (65%) | |
| 4 | 2/78 (3%) | 1/21 (5%) | | 0/74 (0%) | 0/20 (0%) | |
| pN | | | 0·33 | | | 0·59 |
| 0 | 33/36 (92%) | 11/14 (79%) | | 29/31 (94%) | 13/15 (87%) | |
| 1 | 3/36 (8%) | 3/14 (21%) | | 2/31 (6%) | 2/15 (13%) | |
| M | | | 1·00 | | | 0·081 |
| 0 | 70/78 (90%) | 19/21 (90%) | | 65/74 (88%) | 14/20 (70%) | |
| 1 | 8/78 (10%) | 2/21 (10%) | | 9/74 (12%) | 6/20 (30%) | |
| Stage (clinical) | | | 0·76 | | | 0·003 |
| I | 32/78 (41%) | 7/21 (33%) | | 40/73 (55%) | 3/20 (15%) | |
| II | 10/78 (13%) | 4/21 (19%) | | 9/73 (12%) | 2/20 (10%) | |
| III | 28/78 (36%) | 7/21 (33%) | | 16/73 (22%) | 9/20 (45%) | |
| IV | 8/78 (10%) | 3/21 (14%) | | 8/73 (11%) | 6/20 (30%) | |
| Fuhrman grade | | | <0.0001 | | | 0·095 |
| 1 | 2/78 (3%) | 0/21 (0%) | | 2/74 (3%) | 0/19 (0%) | |
| 2 | 41/78 (53%) | 3/21 (14%) | | 32/74 (43%) | 4/19 (21%) | |
| 3 | 31/78 (40%) | 9/21 (43%) | | 32/74 (43%) | 9/19 (47%) | |
| 4 | 4/78 (5%) | 9/21 (43%) | | 8/74 (11%) | 6/19 (32%) | |
| Necrosis | | | 0·029 | | | 0·038 |
| No | 47/64 (73%) | 9/20 (45%) | | 65/74 (88%) | 13/20 (65%) | |
| Yes | 17/64 (27%) | 11/20 (55%) | | 9/74 (12%) | 7/20 (35%) | |
| Sarcomatoid | | | 0·0010 | | | -- |
| No | 76/78 (97%) | 15/21 (71%) | | -- | -- | |
| Yes | 2/78 (3%) | 6/21 (29%) | | -- | -- | |
| Rhabdoid | | | 0.00034 | | | -- |
| No | 74/78 (95%) | 13/21 (62%) | | -- | -- | |
| Yes | 4/78 (5%) | 8/21 (38%) | | -- | -- | |
| Phospho-S6 | 1·2 (0·2) | 3·8 (0·6) | 0.0010 | -- | -- | -- |
| Phospho-4E-BP1 | 1·3 (0·2) | 2·2 (0·4) | 0·029 | -- | -- | -- |

Data are number (%) or mean (SE), for immunohistochemistry. UTSW=University of Texas Southwestern Medical Center. TCGA=The Cancer Genome Atlas.

**Table 3**

Multivariate associations of patient/tumor characteristics from the TCGA with time to death.

| | HR (95% CI) | p-value |
|---|---|---|
| Mutation Group | | 0·041 |
| *PBRM1* | 1·00 (reference) | |
| *BAP1* | 2·3 (1·03–5·1) | |
| M | | 0·023 |
| No | 1·00 (reference) | |
| Yes | 2·7 (1·1–6·2) | |
| Grade | | 0·018 |
| 1 | NA | |
| 2 | 1·00 (reference) | |
| 3 | 0·8 (0·3–2·0) | |
| 4 | 3·3 (1·3–8·6) | |

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**Table 4**

*BAP1*- and *PBRM1*-mutant tumors exhibit characteristic gene expression signatures.

| | ***BAP1*-mutated (n=20) *vs* the rest (n=288)** | **p value** | ***PBRM1*-mutated (n=66) *vs* the rest (n=242)** | **p-value** |
|---|---|---|---|---|
| *BAP1* (n=20) | 3250 | <0·0001 | -- | -- |
| *PBRM1* (n=66) | -- | -- | 2235 | <0·0001 |
| Random 1 | 115 | -- | 0 | -- |
| Random 2 | 63 | -- | 0 | -- |
| Random 3 | 120 | -- | 3 | -- |

Number of genes that distinguish *BAP1*- and *PBRM1*-mutant groups when compared to the rest vs. control groups of the same size (n=20 or n=66) made up of random tumors. *P* values reflect comparisons between the mutated groups and the random groups.