

Adverse Outcomes in Clear Cell Renal Cell Carcinoma with Mutations of 3p21 Epigenetic Regulators *BAP1* and *SETD2*: A Report by MSKCC and the KIRC TCGA Research Network

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Abstract

Purpose: To investigate the impact of newly identified chromosome 3p21 epigenetic tumor suppressors *PBRM1*, *SETD2*, and *BAP1* on cancer-specific survival (CSS) of 609 patients with clear cell renal cell carcinoma (ccRCC) from 2 distinct cohorts.

Experimental Design: Select sequencing on 3p tumor suppressors of 188 patients who underwent resection of primary ccRCC at the Memorial Sloan-Kettering Cancer Center (MSKCC) was conducted to interrogate the genotype–phenotype associations. These findings were compared with analyses of the genomic and clinical dataset from our nonoverlapping The Cancer Genome Atlas (TCGA) cohort of 421 patients with primary ccRCC.

Results: 3p21 tumor suppressors are frequently mutated in both the MSKCC (*PBRM1*, 30.3%; *SETD2*, 7.4%; *BAP1*, 6.4%) and the TCGA (*PBRM1*, 33.5%; *SETD2*, 11.6%; *BAP1*, 9.7%) cohorts. *BAP1* mutations are associated with worse CSS in both cohorts [MSKCC, $P = 0.002$; HR 7.71; 95% confidence interval (CI) 2.08–28.6; TCGA, $P = 0.002$; HR 2.21; 95% CI 1.35–3.63]. *SETD2* are associated with worse CSS in the TCGA cohort ($P = 0.036$; HR 1.68; 95% CI 1.04–2.73). On the contrary, *PBRM1* mutations, the second most common gene mutations of ccRCC, have no impact on CSS.

Conclusion: The chromosome 3p21 locus harbors 3 frequently mutated ccRCC tumor suppressor genes. *BAP1* and *SETD2* mutations (6%–12%) are associated with worse CSS, suggesting their roles in disease progression. *PBRM1* mutations (30%–34%) do not impact CSS, implicating its principal role in the tumor initiation. Future efforts should focus on therapeutic interventions and further clinical, pathologic, and molecular interrogation of this novel class of tumor suppressors. *Clin Cancer Res*; 19(12); 3259–67. ©2013 AACR.

Introduction

Renal cell carcinoma (RCC) is the eighth leading cause of cancer in the United States (1), among which clear cell renal

cell carcinoma (ccRCC) represents the most common and aggressive form. To date, several adverse pathologic features of ccRCC have been used to successfully construct individual postoperative prognostic models that were subsequently validated to predict disease recurrence and cancer-specific survival (CSS; refs. 2–6). Increased size, advanced pathologic stage, higher Fuhrman nuclear grade, and the presence of necrosis, all have been shown to correlate with worse CSS on multivariate analysis (2, 3). On the contrary, similar progress is limited in the search for clinically relevant, correlative molecular biomarkers, although progress is now being made (7, 8). For example, *VHL*, the most commonly mutated, silenced gene in ccRCC, has not been proven to affect the overall clinical outlook of this disease, highlighting the need for identification and validation of novel molecular markers for prognostic and therapeutic purposes.

Recent large-scale targeted and whole-exome sequencing studies of ccRCC, including our TCGA (the Cancer Genome Atlas) Consortium, have discovered novel, prevalent genomic alterations (9–11), including frequent inactivation of

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

A full list of principal investigators and participating institutes for the ccRCC TCGA is listed in the Supplementary Appendix.

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Translational Relevance

Several recurrent mutations in 3p chromatin modulators/modifiers (*PBRM1*, *SETD2*, and *BAP1*) have been reported in clear cell renal cell carcinoma (ccRCC) in the past 2 years. We report the association of adverse cancer-specific outcomes with mutations of *BAP1* and *SETD2* in 2 large, distinct cohorts. Our results point to the need for therapeutic interventions and further clinical, pathologic, and molecular interrogation of this novel class of tumor suppressors, and suggest that they may be used for risk stratification.

several chromatin remodeling genes, *PBRM1* (41%; ref. 10), *SETD2* (3%–12%; ref. 11), and *BAP1* (8%–11%; refs. 12, 13). Remarkably, all 3 genes are located on chromosome 3p21 that is in close proximity to 3p25 where *VHL* resides. Emerging evidence indicates that these genes function as tumor suppressors in diverse malignancies, for example, *PBRM1* in pancreatic cancer (14), *BAP1* in uveal melanoma and mesothelioma (15, 16), and *SETD2* in breast cancer and leukemia (17, 18). Strikingly, these mutations in ccRCC take place in the setting of a near ubiquitous single copy loss of 3p (>90%; ref. 9). Hence, any single copy loss-of-function mutations of these 4 genes would result in respective, complete inactivation.

Our previous single-institutional sequencing effort on 185 patients with ccRCC focusing on pathologic correlations, not only consolidated recently reported mutation frequencies but also showed the association of *PBRM1*, *BAP1*, and/or *SETD2* mutations with higher tumor stage and, for *BAP1*, with higher tumor grade (19). Here, we focus on the impact of individual mutations of *PBRM1*, *BAP1*, and *SETD2* on CSS in an expanded version of our original cohort (3 additional patients and several more months of follow-up and events) and further show the association of *BAP1* and *SETD2* mutations with worse CSS (the TCGA cohort, $n = 421$), providing a molecular link between gene mutations and cancer-specific outcomes in ccRCC.

Materials and Methods

MSKCC cohort and genomic DNA isolation

Tumor and adjacent normal kidney tissues from 189 consecutive, previously untreated patients who underwent either radical or partial nephrectomy for sporadic, resectable ccRCC from December 2001 to December 2011 were collected. All patients had previously consented to a tissue protocol and 1 patient was excluded due to insufficient DNA quality for mutation analysis. This study was preapproved by our Institutional Review Board and all patients signed informed consent. Tumor staging was based on the seventh American Joint Committee on Cancer/The International Union Against Cancer (AJCC/UICC) Tumor–Node–Metastasis (TNM) Edition and all samples were reviewed by a dedicated uropathologist. Paired fresh-frozen normal and primary tumor tissue blocks were identified, marked, and

macrodissected for maximal tumor density. DNA was extracted and quantified using the DNeasy Kit (Qiagen) and a Nanodrop spectrophotometer (Invitrogen).

Integrated mutational analysis

Mutation analysis of the entire coding regions of *VHL*, *PBRM1*, *SETD2*, and *BAP1* was conducted using PCR amplification and bidirectional Sanger sequencing on the first 183 tumor-normal pairs. The IMPACT assay (Integrated Mutation Profiling of Actionable Cancer Targets), a customized targeted-exome capture assay of 230 cancer-associated genes with an ultra-deep sequencing coverage (~500×) using either the Illumina HiSeq 2000 or the MiSeq platform (20), was conducted on additional 5 patients as part of a separate study. Details of mutation analysis can be seen in the Supplementary Methods.

TCGA cohort

Paired tumor-normal materials, genomic data, and clinical information were acquired by our ccRCC TCGA Consortium. This multi-institutional effort included clinical and pathologic information on 446 retrospectively identified patients who underwent either radical or partial nephrectomy for sporadic ccRCC from 1998 to 2010. Whole-exome sequencing data were available on 424 patients, which had 3 overlapping patients with our MSKCC cohort leaving a total of 421 patients for analysis. Full sequencing information is detailed in the main text and Supplementary information of the companion ccRCC TCGA biomarker paper. Mutation data were acquired from the MAF file "KIRC-BCM-BI-gapfill-v1.1.mafplus," and clinical information was obtained from the file entitled "KIRC+Clinical+Data+Jul-31-2012" encompassing the most recent follow-up information ("Max Followup"). Partial mutation validation (~70%) had been conducted by additional orthogonal platform (454 or Ion Torrent) at the time of analysis. Vital status was determined from the field "Composite Vital Status." Nonsilent, coding mutations were considered for both cohorts, with truncating mutations defined as nonsense, frameshift, or essential splice site (within first 2 base pairs of coding region).

Statistical analysis

Associations between binary variables and between binary and continuous variables were assessed using Fisher exact and Wilcoxon exact 2-tailed tests, respectively. Kaplan–Meier method was used to estimate the survival probabilities. CSS was analyzed using the competing risk method, using the "cmprsk" R package. Cox proportional hazard regression was used for analysis of time to recurrence (21). Multivariate competing risk models were also fitted in the TCGA cohort to adjust for clinical covariates (AJCC stage I and II vs. III vs. IV and grade 1 and 2 vs. 3 and 4). No adjustment was done for the MSKCC cohort due to shorter follow up and thus fewer events. Tumor sizes were dichotomized as a categorical variable (≥ 5 vs. < 5 cm) in Table 2.

In the TCGA cohort, deceased patients were considered dead from renal cancer-related causes if the field

"Composite Tumor Status" = "WITH TUMOR" at the time of death, or they had metastatic disease at presentation (M1), or, if "Composite Tumor Status" was unavailable ($n = 5$), they had lymph node disease (N1) or died within 2 years of surgery. Time to recurrence for patients who are N0/NX and M0 is defined as time between surgery and recurrence or day of last follow up. Patients with multiple kidney tumors at surgery were excluded from this analysis. In the TCGA cohort, patients without recurrence date but whose "Composite Tumor Status" is "WITH TUMOR" were considered as recurrences at the last day of follow-up or day of death.

To assess mutation distributions in regions of heterozygous deletion, we used discretized copy number alterations

predicted by Genomic Identification of Significant Targets in Cancer (GISTIC; ref. 22) and used in the parallel article submitted by the TCGA consortium. Number of mutations in regions of heterozygous deletions was analyzed using Poisson regression.

Results

Clinical and histologic data

To evaluate the impact of individual mutations on clinical outcomes, we assembled data sets encompassing 609 patients with ccRCC. The demographic, clinical, and pathologic characteristics of the MSKCC ($n = 188$) and TCGA ($n = 421$) cohorts are listed in Table 1. The majority of the patients are males (65%–70%) and Caucasians (90%–

Table 1. Demographic, pathologic, and clinical outcomes of discovery and validation cohorts

Cohort	Discovery—MSKCC ($n = 188$)	Validation—TCGA ($n = 421$)
Median age (quartiles)	61 (54,69)	61 (52,70)
Gender	Male—132 (70.2%) Female—56 (29.8%)	Male—275 (65.3%) Female—146 (34.7%)
Race	White—170 (90.4%) Black—7 (3.7%) Other—11 (5.9%)	White—394 (93.6%) Black—14 (3.3%) Other—13 (3.1%)
Median tumor size—cm (quartiles)	5.1 (3.4,8.5)	5.5 (4.0,8.5)
Primary tumor (T stage ^a)		
pT1	73 (38.8%)	204 (48.5%)
pT2	13 (6.9%)	50 (11.9%)
pT3	98 (52.1%)	162 (38.5%)
pT4	4 (2.1%)	5 (1.2%)
Regional lymph nodes (N stage ^a)		
pNx	103 (54.8%)	216 (51.3%)
pN0	78 (41.5%)	12 (2.9%)
pN1	7 (3.7%)	193 (45.8%)
Distant metastases (M stage ^a)		
pM0	165 (87.7%)	353 (83.8%)
pM1	23 (12.3%)	68 (16.2%)
AJCC Stage		
I	71 (37.8%)	199 (47.3%)
II	11 (5.9%)	41 (9.7%)
III	82 (43.6%)	111 (26.4%)
IV	24 (12.8%)	70 (16.7%)
Fuhrman nuclear grade		
G ₁	2 (1.1%)	7 (1.6%)
G ₂	75 (39.9%)	173 (41.1%)
G ₃	88 (46.8%)	174 (41.3%)
G ₄	23 (12.2%)	66 (15.7%)
Unknown	NA	1 (<1%)
Median follow-up for survivors (mo)	35	46
Overall 5-year survival	84.3%	61.1%
Median survival (95% CI)	NA (82.3, NA)	76.8 (69, NA)
Number of deaths	21	142
Number of deaths from RCC	13	102

NOTE: NA in median survival refers to not estimable.

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Table 2. Mutations associations with adverse tumor features

		Size >5 cm	Higher T stage	Higher Nuclear grade	Necrosis (>5%)	LN mets (N1)	Presence of Mets on presentation (M1)
MSKCC							
P value OR (95% CI)	<i>VHL</i>	1.000	0.107	0.458	0.748	1.000	0.825
	<i>PBRM1</i>	0.635	0.011	0.523	0.494	0.435	0.153
			2.34 (1.23–4.58)				
	<i>SETD2</i>	1.000	0.580	1.000	0.548	0.424	0.683
	<i>BAP1</i>	0.143	0.041	0.029	0.492	0.375	1.000
			4.57 (1.16–30.26)	8.17 (1.54–150.94)			
TCGA							
P value OR (CI)	<i>VHL</i>	0.067	0.230	0.921	0.065	0.771	0.424
	<i>PBRM1</i>	0.670	0.752	1.000	0.645	0.546	0.575
	<i>SETD2</i>	0.059	0.089	0.761	0.403	1.000	0.100
	<i>BAP1</i>	0.002	0.004	0.018	0.071	0.329	0.012
		3.8 (1.68–10.25)	2.62 (1.36–5.16)	2.43 (1.19–5.36)			2.74 (1.3–5.53)

NOTE: Bold, $P < 0.05$.

94%). The pathologic stages were similarly distributed in the 2 cohorts, whereas the TCGA one enlists patients with longer follow-up (46 vs. 35 months).

Mutation profiles

The individual mutation frequencies of both cohorts are illustrated in Fig. 1. Of the MSKCC cohort, *VHL* was mutated in 51.1% of tumors; *PBRM1* in 30.3%; *SETD2* in 7.4%; and *BAP1* in 6.4%. *VHL* and *PBRM1* were frequently comutated [$P = 0.007$; OR 2.5; 95% confidence interval (CI) 1.3–4.9], as were *VHL* and *SETD2* ($P = 0.01$; OR 6.4; 95% CI 1.7–42.1) and *PBRM1* and *SETD2* mutations trended toward cooccurrence ($P = 0.13$). In comparison, analysis of the TCGA cohort showed similar mutation frequencies: *VHL* at 56.4%, *PBRM1* at 33.5%, *SETD2* at 11.6%, and *BAP1* at 9.7%. Similar cooccurrences of *VHL* and *PBRM1* mutations ($P = 0.0002$; OR 2.2; 95% CI 1.4–3.4) as well as *PBRM1* and *SETD2* mutations ($P = 0.01$; OR 2.3, 95% CI 1.3–4.2) were seen.

Mutations and pathologic features

The associations between these mutations and known pathologic features that correlate with adverse outcome are outlined in Table 2. In the MSKCC cohort, tumors with mutations of either *PBRM1* or *BAP1* were more likely to present with higher tumor stage III–IV ($P = 0.01$; OR 2.34; 95% CI 1.23–4.58) and IV ($P = 0.04$; OR 4.57; 95% CI 1.16–30.26), respectively. *BAP1* mutations were associated with Fuhrman nuclear grade 3–4 tumors ($P = 0.03$, OR 8.17, 95% CI: 1.54–150.94), whereas mutations of the remaining genes were not associated with grade. In our TCGA cohort, *BAP1* mutations were significantly associated with multiple adverse tumor features including higher T stages ($P = 0.004$; OR 2.62; 95% CI 1.36–5.16), higher nuclear grades ($P = 0.02$; OR 2.43; 95% CI 1.19–5.36),

larger tumor sizes ($P = 0.002$; OR 3.8; 95% CI 1.68–10.25), and the presence of metastasis at presentation ($P = 0.01$; OR 2.74; 95% CI 1.3–5.53).

Mutations and survival

Survival analysis of the MSKCC cohort was limited to univariate association due to the relatively small number of events and was presented in Fig. 2. *BAP1* mutations are significantly associated with worse CSS in the competing risk model ($P = 0.002$; HR 7.71; 95% CI 2.08–28.6 relative to patients without *BAP1* mutations). This association was also observed in the TCGA cohort ($P = 0.002$; HR 2.21; 95% CI 1.35–3.63; Fig. 3). Interestingly, mutations of *PBRM1* as well as *VHL* had no survival impact in both cohorts (Figs. 2 and 3). Median overall survival for *BAP1* mutants in TCGA cohort is 31.2 months (95% CI 23.2, NA) versus 78.2 months (95% CI 70.3, NA) in wild-type patients; median is not reached in the MSKCC cohort.

In the TCGA cohort, mutations in *SETD2* were also associated with CSS ($P = 0.036$; HR 1.68; 95% CI 1.04–2.73). Such association was not detected in our MSKCC cohort, possibly reflecting the inherent lower statistical power associated with smaller number of events and shorter follow-up. Median overall survival for *SETD2* mutant in TCGA cohort is 62.7 months (95% CI 43.8, NA) versus 78.2 months (95% CI 71.8, NA) in wild-type patients; in the MSKCC cohort, median is 80.1 months (95% CI not estimable) for mutants and not reached for wild-type.

We further assessed the impact of mutation type on CSS. In the MSKCC cohort, 50% of the 12 *BAP1* mutations were truncating mutations and had a substantially worse prognosis on CSS (HR = 17.83; 95% CI 5.1–62.3, relative to patients without *BAP1* mutation) compared with missense mutations (HR = 3.63; 95% CI 0.4–32.6). There were too few patients with *SETD2* mutation to estimate the HRs.

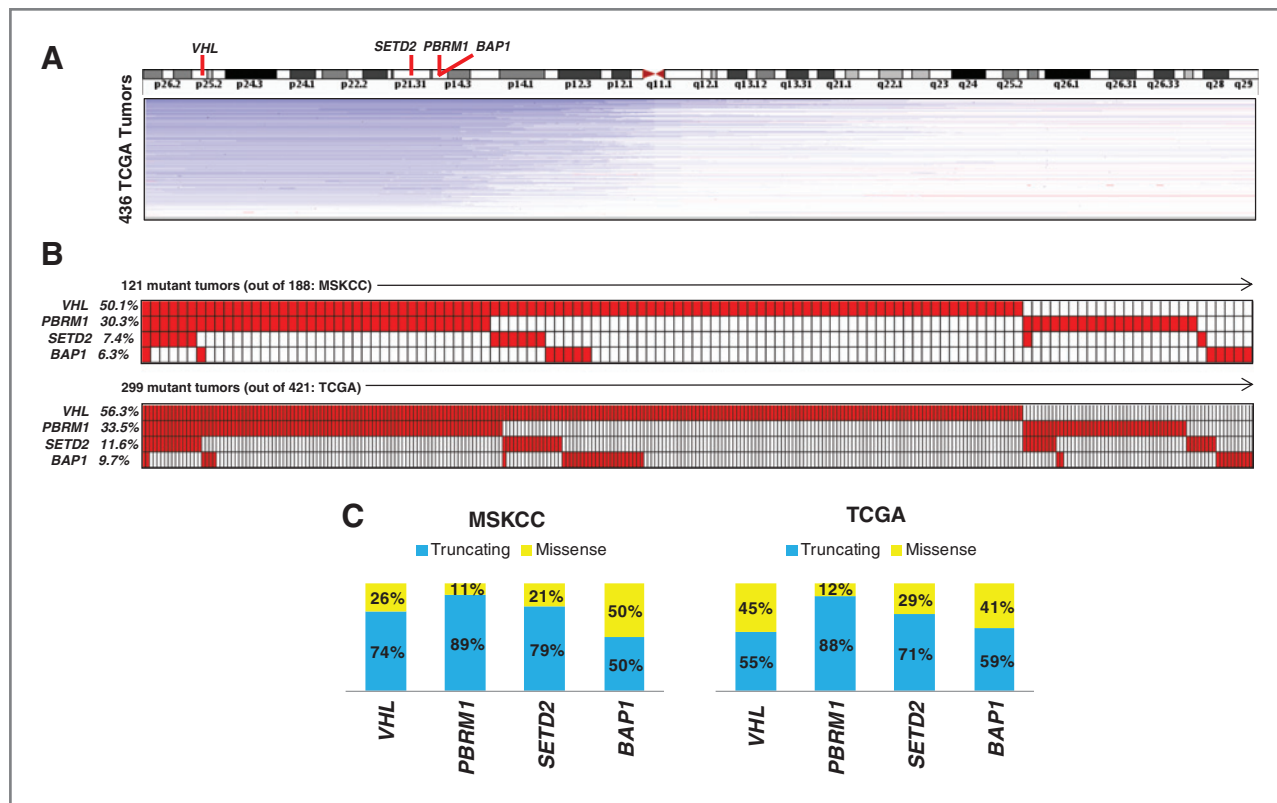


Figure 1. A, chromosome 3 map showing geographic proximity of the frequently mutated 3p tumor suppressors and GISTIC copy number plot showing heterozygous loss of a large segment of 3p in approximately 90% of the TCGA cohort. Blue color denotes copy number loss, and red denotes copy number gain. B, mutation heatmap of MSKCC and TCGA cohorts highlights similar mutation frequencies and cooccurrences of the 3p tumor suppressors in both cohorts. C, mutation percentages by type shows similar patterns in both cohorts (truncating = frameshift, nonsense, or essential splice site).

In the TCGA cohort, 59% of mutations in *BAP1* were truncating and they had similar effect on prognosis (HR = 2.33; 95% CI 1.2–4.5) as missense mutations (HR = 2.06; 95% CI 1.05–4.04) in the CSS model. Among *SETD2* mutations, 71% were truncating and they also had similar effect on prognosis as missense mutations (HR = 1.85; 95% CI 0.89–3.84 and HR = 1.59; 95% CI 0.87–2.9, respectively; Supplementary Fig. S1 for all curves).

Time to recurrence analysis within the TCGA data on 336 patients (72 recurrences) identified *SETD2* mutations as a univariate predictor ($P = 0.002$; HR 2.5; 95% CI 1.38–4.5). No other 3p mutations were associated with disease recurrence (Supplementary Fig. S2 for time to recurrence curves for *SETD2*). This finding was not seen in the MSKCC cohort, which may be related to small number of events ($n = 14$ among 162 patients included in TTR analysis), and warrants independent confirmation. None of the other mutations were significantly associated with TTR in MSKCC cohort.

We further analyzed the impact of mutation combinations on CSS in the TCGA cohort even though there might be limited power due to small sample size and low frequency of cooccurrences. For example, *BAP1* and *SETD2* mutations cooccurred in only 3 patients (Fig. 1). There was no significant interaction between mutations affecting CSS with the exception of *BAP1* and *PBRM1* mutations ($P = 0.035$). Having a *BAP1* mutation alone leads to increased

risk of cancer death with HR = 1.78 (95% CI 0.99–3.23), having *PBRM1* alone has HR of 0.86 (95% CI 0.54–1.37), but having both *BAP1* and *PBRM1* has a HR of 4.18 (95% CI 2.17–8.05), consistent with a recent report (ref. 8; Supplementary Figs. S3 and S4).

To further explore the impact of *BAP1* and *SETD2* mutations on postoperative prognostic modeling of CSS, the effects of each of these mutations were estimated after adjusting for clinical stage (I and II vs. III vs. IV) and nuclear grade (1, 2 vs. 3,4) in the TCGA cohort. As expected, given the strong link of these mutations to adverse tumor features, both *BAP1* ($P = 0.73$; HR 1.09; 95% CI 0.67–1.76), and *SETD2* ($P = 0.73$; HR 1.1; 95% CI 0.64–1.9) mutations were no longer significant after inclusion of stage and grade.

3p tumor suppressors and kidney cancer progression

Given the near ubiquitous loss of 1 copy of 3p (>90%) and the prevalent concurrent mutations of *VHL*, *PBRM1*, *SETD2*, and *BAP1* in ccRCC, we exploited the wealth of genomic information available on the TCGA cohort to interrogate the contribution of individual 3p tumor suppressor mutations to disease progression. We first determined the number of potential tumor suppressor mutations (concurrent copy number loss and mutations) present through disease progression, according to size, nuclear grade, and clinical stage. There is a trend of a small,

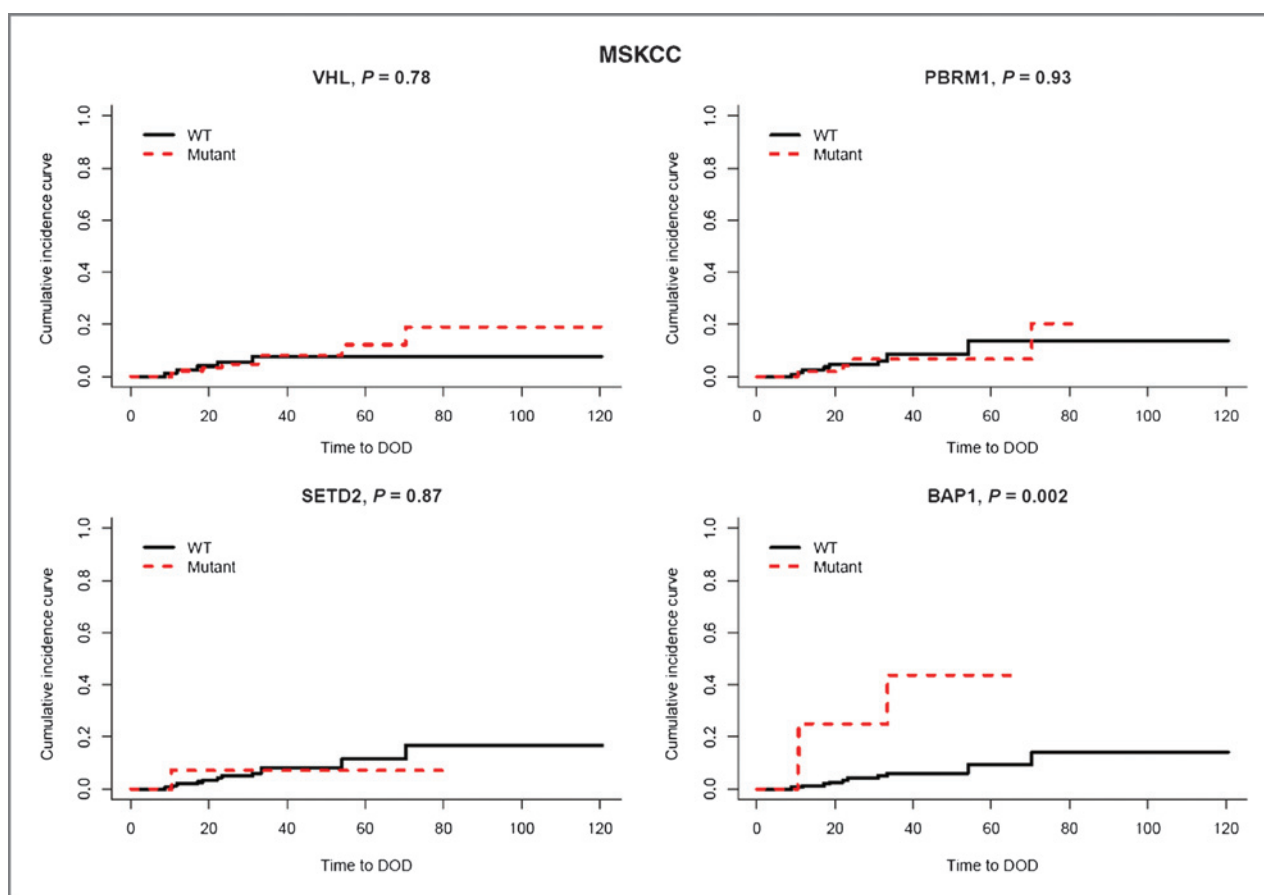


Figure 2. Competing risk cumulative incidence curves for the MSKCC cohort ($n = 188$). DOD, death of disease.

but statistically significant steady increase ($\sim 3\text{--}6$) of mutations in candidate tumor suppressor genes (TSG) with advancing disease [size (continuous) $P = 0.006$; grade, $P = 0.001$; stage, $P = 0.0002$; Fig. 4 top], highlighting the fact that loss of function in a small number of TSGs is likely critical in both disease initiation and progression of ccRCC.

We next examined the clinical and pathologic impact posed by the dysfunction of individual 3p TSGs. Importantly, the sum mutations of these four 3p TSGs makes up a very large portion of the total candidate TSG mutations discovered in ccRCC, further pinpointing the unique clustering of critical ccRCC TSGs on chromosome 3p21–25. Although relatively steady in frequency, predominant mutations of *VHL* and *PBRM1* through disease progression suggest their principal role in the initiation rather than the progression phase of the disease (Fig. 4, middle and bottom and Supplementary Fig. S5). Increased rates of *BAP1* mutations trended with increasing nuclear grades and clinical stages. Hence, *BAP1* was likely mutated during disease progression and thus associated with worse survival.

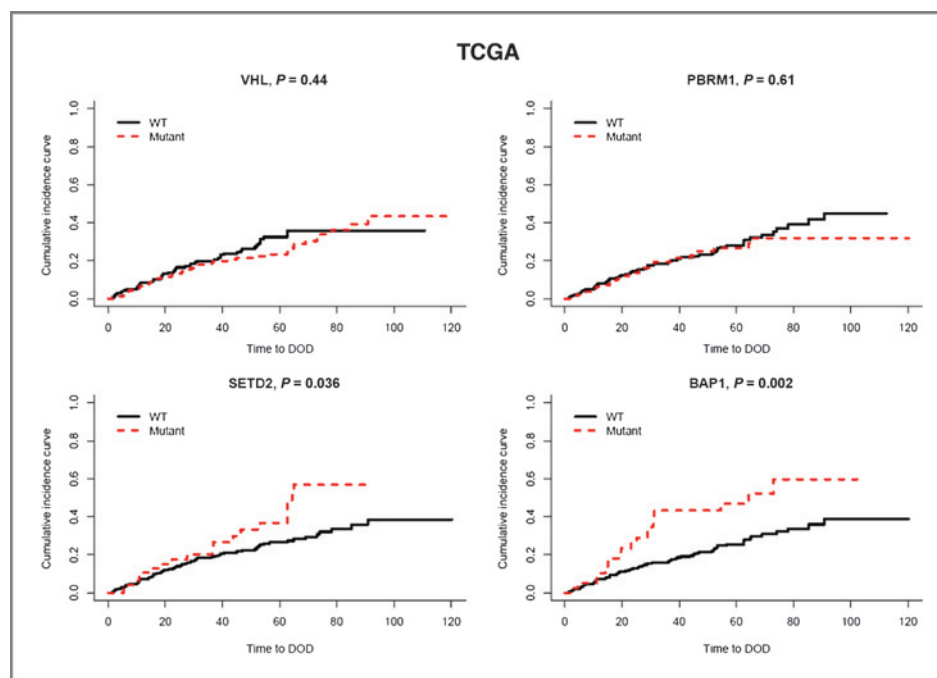
Discussion

ccRCC, the most common and aggressive subtype of RCC, is a lethal disease once it has progressed to the

metastatic state. Several models have been developed and externally validated to predict the development of metastatic disease following surgical resection, which primarily rely on micro- and macroscopic features such as size, pathologic or clinical stage, and nuclear grade (2–6). While these morphologic risk criteria are fairly straightforward and reproducible, they offer little to no molecular and therapeutic insight concerning targeted cancer therapy. Current analysis of our MSKCC and TCGA cohorts represents the largest scale attempt to address the issues of fundamental genetic events leading to tumor initiation and cancer-specific outcomes.

Both cohorts identified *VHL* mutations in more than half of the 609 tumors examined. Such prevalence is in agreement with reported mutation frequencies (12, 23). However, it likely underestimates the actual loss of *VHL*, as the *VHL* gene can be inactivated by promoter methylation and there are known inherent challenges in sequencing the guanine-cytosine-rich regions of *VHL*. In addition, we saw no correlation between the status of *VHL* mutations and clinical/pathologic features or CSS, which concurs with most prior reports (23). Previous studies have suggested that loss of the predominant tumor suppressor *VHL* alone is insufficient (24, 25) and additional genetic events are required for

Figure 3. Competing risk cumulative incidence curves for the TCGA cohort ($n = 421$). DOD, death of disease.



developing clinically relevant clear cell kidney cancer. Prior loss of heterozygosity studies have implicated the chromosome 3p21 locus as a candidate region harboring additional tumor suppressors (26). Indeed, recent large-scale high-throughput sequencing studies have reported several recurrently mutated genes in ccRCC. Remarkably, many of these frequently mutated genes such as *PBRM1*, *SETD2*, and *BAP1* function in the epigenetic regulation of gene expression and are located in the frequently lost 3p21 locus (Fig. 1).

Recent genomic data also implicated *PBRM1* mutations in the evolution of pancreatic cancer (14). In line with the initial *PBRM1* mutation study in ccRCC (10), *PBRM1* was mutated at 32.5% (198/609) in our patients and nearly all of which were truncating mutants. Intriguingly, analysis of our MSKCC cohort showed a significant association of *PBRM1* mutations with higher T stages, which was at odds with the data obtained from the TCGA cohort. This might be explained by the discrepancy in determining pathologic stages for smaller tumors (<4cm) between our institution and others. Of note, 30% of small tumors (<4cm) in our MSKCC cohort were classified as pT3a diseases and such pathologic upstaging strongly associated with *PBRM1* mutations, whereas only 7% of small tumors in the TCGA cohort were at pT3a. Although *PBRM1* mutations may be associated with earlier invasion in smaller tumors, both our cohorts did not find any other clinical associations. These data coupled with the high, stable mutation frequency of *PBRM1* across tumor size, nuclear grade, and clinical stage (Fig. 4) suggest that inactivation of *PBRM1* likely constitute an early, essential event in kidney tumorigenesis.

Importantly, both cohorts identified *BAP1* mutation as a poor prognostic factor. *BAP1* mutations were also

identified in uveal melanoma and mesothelioma (15, 16). In uveal melanoma, *BAP1* loss alone defines an aggressive subgroup. In contrast, *BAP1* mutations in mesothelioma did not associate with worse clinical outcomes in a recent report (27). Of note, these patients with mesothelioma fared poorly irrespective of their *BAP1* status [median survival, 14.8 (mutant) vs. 15.3 (wild-type) months]. With respect to ccRCC, *BAP1* seems to be a critical gatekeeper from disease progression, a finding that was recently reported in ccRCC while our article was under consideration (8). Our study interconnected *BAP1* mutations with essentially all known, validated poor prognostic factors, including higher tumor stage, higher nuclear grade, larger size, more necrosis, and the presence of metastatic disease at presentation (Table 2), which further supports the strong association between *BAP1* mutations and CSS. In addition to *BAP1* mutations, we showed a strong association between *SETD2* mutations and worse CSS in the TCGA cohort. Intriguingly, mutations of *SETD2* but not other 3p tumor suppressors were associated with increased likelihood of developing recurrent and/or metastatic diseases. This finding was only seen in one cohort (albeit with the limitations of the MSKCC cohort) and requires independent validation.

As macro- and microscopic prognostic surrogates can only be defined postoperatively, clinical implications of mutations in these chromatin modulators/modifiers could impact preoperative risk stratification given their strong association with known adverse tumor features. As *BAP1* mutations are highly correlative with the protein loss determined by immunohistochemistry (13), it might be beneficial to determine its mutation/expression status in smaller

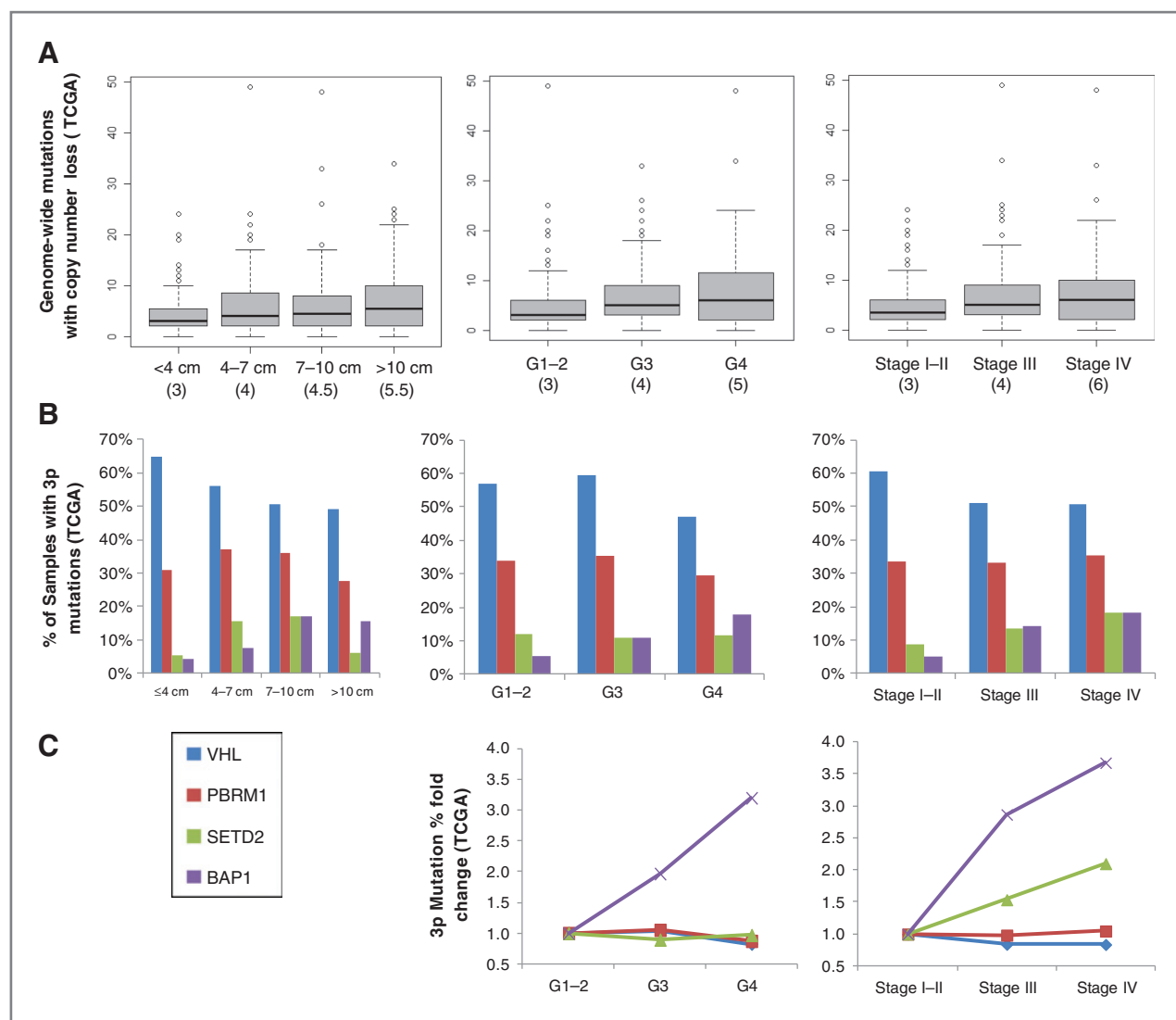


Figure 4. A, box plots show the statistically significant incremental increase of purported TSGs as defined by mutations occurring in the setting of copy number loss across the ccRCC genome. Values in parentheses represent median TSG mutation counts. B, percentage of TCGA tumors with 3p TSGs mutated as defined by increasing tumor size, nuclear grade, and AJCC stage. C, fold change in mutation (%) frequency for 3p TSGs. Note the marked increase in *BAP1* mutations with both advanced tumor grade and stage, and the trend in *SETD2* mutations for advanced stage.

renal masses, which could guide treatment planning, i.e., partial or radical nephrectomy or close observation only. Of course, such an idea must be prospectively validated in lieu of tumor heterogeneities.

In conclusion, the chromosome 3p locus harbors 4 ccRCC tumor suppressors that are located in close proximity and possess critical pathologic and clinical significance. Loss-of-function mutations of *PBRM1* (30%–34%) constitute the second most common genetic event in ccRCC but do not impact clinical outcome, implicating its principal role in tumor initiation. *BAP1* and *SETD2* mutations are associated with worse CSS and likely take place during the disease progression phase. Future efforts focusing on these alterations may provide novel therapeutic opportunities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed by the authors.

Authors' Contributions

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References

- Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012;62:10–29.
- Sorbellini M, Kattan MW, Snyder ME, Reuter V, Motzer R, Goetzl M, et al. A postoperative prognostic nomogram predicting recurrence for patients with conventional clear cell renal cell carcinoma. *J Urol* 2005;173:48–51.
- Frank I, Blute ML, Cheville JC, Lohse CM, Weaver AL, Zincke H. An outcome prediction model for patients with clear cell renal cell carcinoma treated with radical nephrectomy based on tumor stage, size, grade and necrosis: the SSIGN score. *J Urol* 2002;168:2395–400.
- Thompson RH, Leibovich BC, Lohse CM, Cheville JC, Zincke H, Blute ML, et al. Dynamic outcome prediction in patients with clear cell renal cell carcinoma treated with radical nephrectomy: the D-SSIGN score. *J Urol* 2007;177:477–80.
- Karakiewicz PI, Briganti A, Chun FK, Trinh QD, Perrotte P, Ficarra V, et al. Multi-institutional validation of a new renal cancer-specific survival nomogram. *J Clin Oncol* 2007;25:1316–22.
- Tan MH, Li H, Choong CV, Chia KS, Toh CK, Tang T, et al. The Karakiewicz nomogram is the most useful clinical predictor for survival outcomes in patients with localized renal cell carcinoma. *Cancer* 2011;117:5314–24.
- Schutz FA, Pomerantz MM, Gray KP, Atkins MB, Rosenberg JE, Hirsch MS, et al. Single nucleotide polymorphisms and risk of recurrence of renal-cell carcinoma: a cohort study. *Lancet Oncol* 2013;14:81–7.
- Kapur P, Pena-Llopis S, Christie A, Zhrebker L, Pavia-Jimenez A, Rathmell WK, et al. Effects on survival of *BAP1* and *PBRM1* mutations in sporadic clear-cell renal-cell carcinoma: a retrospective analysis with independent validation. *Lancet Oncol* 2013;14:159–67.
- The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature*. In press.
- Varela I, Tarpey P, Raine K, Huang D, Ong CK, Stephens P, et al. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene *PBRM1* in renal carcinoma. *Nature* 2011;469:539–42.
- Dalgliesh GL, Furge K, Greenman C, Chen L, Bignell G, Butler A, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature* 2010;463:360–3.
- Guo G, Gui Y, Gao S, Tang A, Hu X, Huang Y, et al. Frequent mutations of genes encoding ubiquitin-mediated proteolysis pathway components in clear cell renal cell carcinoma. *Nat Genet* 2012;44:17–9.
- Pena-Llopis S, Vega-Rubin-de-Celis S, Liao A, Leng N, Pavia-Jimenez A, Wang S, et al. *BAP1* loss defines a new class of renal cell carcinoma. *Nat Genet* 2012;44:751–9.
- Shain AH, Giacomini CP, Matsukuma K, Karikari CA, Bashyam MD, Hidalgo M, et al. Convergent structural alterations define SWI/
- Sucrose Non-Fermentable (SWI/SNF) chromatin remodeler as a central tumor suppressive complex in pancreatic cancer. *Proc Natl Acad Sci U S A* 2012;109:E252–9.
- Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, et al. The nuclear deubiquitinase *BAP1* is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat Genet* 2011;43:668–72.
- Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, Worley LA, et al. Frequent mutation of *BAP1* in metastasizing uveal melanomas. *Science* 2010;330:1410–3.
- Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* 2012;481:157–63.
- Al Sarakbi W, Sasi W, Jiang WG, Roberts T, Newbold RF, Mokbel K. The mRNA expression of *SETD2* in human breast cancer: correlation with clinico-pathological parameters. *BMC Cancer* 2009;9:290.
- Hakimi AA, Chen YB, Wren J, Gonen M, Abdel-Wahab O, Heguy A, et al. Clinical and pathologic impact of select chromatin-modulating tumor suppressors in clear cell renal cell carcinoma. *Eur Urol* 2013;63:848–54.
- Iyer G, Hanrahan AJ, Milowsky MI, Al-Ahmadie H, Scott SN, Janakiraman M, et al. Genome sequencing identifies a basis for everolimus sensitivity. *Science* 2012;338:221.
- Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999;94:496–509.
- Beroukhim R, Getz G, Nghiemphu L, Barretina J, Hsueh T, Linhart D, et al. Assessing the significance of chromosomal aberrations in cancer: methodology and application to glioma. *Proc Natl Acad Sci U S A* 2007;104:20007–12.
- Gossage L, Eisen T. Alterations in *VHL* as potential biomarkers in renal-cell carcinoma. *Nat Rev Clin Oncol* 2010;7:277–88.
- Mandriota SJ, Turner KJ, Davies DR, Murray PG, Morgan NV, Sowter HM, et al. HIF activation identifies early lesions in *VHL* kidneys: evidence for site-specific tumor suppressor function in the nephron. *Cancer Cell* 2002;1:459–68.
- Rankin EB, Tomaszewski JE, Haase VH. Renal cyst development in mice with conditional inactivation of the von Hippel-Lindau tumor suppressor. *Cancer Res* 2006;66:2576–83.
- Clifford SC, Prowse AH, Affara NA, Buys CH, Maher ER. Inactivation of the von Hippel-Lindau (*VHL*) tumour suppressor gene and allelic losses at chromosome arm 3p in primary renal cell carcinoma: evidence for a *VHL*-independent pathway in clear cell renal tumourigenesis. *Genes Chromosomes Cancer* 1998;22:200–9.
- Zauderer MG, McMillan R, et al. Clinical characteristics of patients with malignant pleural mesothelioma (MPM) harboring somatic *BAP1* mutations. *J Clin Oncol* 30, 2012 (suppl; abstr 1541).

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