Title:

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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All other authors – none.
Statement of 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 two years. We report the association of adverse cancer specific outcomes with mutations of BAP1 and SETD2 in two 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.
ABSTRACT

Purpose

To investigate the impact of newly identified chromosome 3p21 epigenetic tumor suppressors \textit{PBRM1}, \textit{SETD2}, and \textit{BAP1} on cancer specific survival (CSS) of 609 clear cell renal cell carcinoma (ccRCC) patients from two distinct cohorts.

Patients and Methods

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

Results

3p21 tumor suppressors are frequently mutated in both the MSKCC (\textit{PBRM1}, 30.3%; \textit{SETD2}, 7.4%; \textit{BAP1}, 6.4%) and the TCGA (\textit{PBRM1}, 33.5%; \textit{SETD2}, 11.6%; \textit{BAP1}, 9.7%) cohorts. \textit{BAP1} mutations are associated with worse CSS in both cohorts (MSKCC, \textit{p}=0.002, HR 7.71 (2.08-28.6); TCGA, \textit{p}=0.002, HR 2.21 (1.35-3.63)). \textit{SETD2} are associated with worse CSS in the TCGA cohort (\textit{p}=0.036, HR 1.68 (1.04-2.73)). On the contrary, \textit{PBRM1} mutations, the second most common gene mutations of ccRCC, have no impact on CSS.

Conclusion

The chromosome 3p21 locus harbors three frequently mutated ccRCC tumor suppressor genes. \textit{BAP1} and \textit{SETD2} mutations (6-12%) are associated with worse CSS, suggesting their roles in disease progression. \textit{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.
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 utilized to successfully construct individual postoperative prognostic models that were subsequently validated to predict disease recurrence and cancer specific survival (CSS) (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 (Submitted, Nature, Copy for Reference) (9, 10), including frequent inactivation of several chromatin remodeling genes, PBRM1 (41%) (9), SETD2 (3-12%) (10), and BAP1 (8-11%) (11, 12). Remarkably, all three 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, e.g., PBRM1 in pancreatic cancer (13), BAP1 in uveal melanoma and mesothelioma (14, 15), and SETD2 in breast cancer and leukemia(16, 17). Strikingly, these mutations in ccRCC take place in the setting of a near ubiquitous single copy loss of 3p (>90%) (revised in Nature). Hence, any single copy loss-of-function mutations of these four genes would result in respective, complete inactivation.

Our previous single-institutional sequencing effort on 185 ccRCC patients focusing on pathologic correlations, not only consolidated recently reported mutation frequencies but also demonstrated the association of PBRM1, BAP1, and/or SETD2 mutations with higher tumor stage and, for BAP1, with higher tumor grade (18). Here, we focus on the impact of individual mutations of PBRM1, BAP1, and SETD2 on cancer specific survival in an expanded version of our original cohort (3 additional patients and several more months of followup and events) and further demonstrate the association of BAP1 and SETD2 mutations with worse cancer specific
survival (the TCGA cohort n=421), providing a molecular link between gene mutations and cancer specific outcomes in ccRCC.

PATIENTS 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 one patient was excluded due to insufficient DNA quality for mutation analysis. This study was pre-approved by our Institutional Review Board and all patients have signed informed consent. Tumor staging was based on the 7th AJCC/UICC TNM Edition and all samples were reviewed by a dedicated uropathologist. Paired fresh frozen normal and primary tumor tissue blocks were identified, marked, and macro-dissected 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 performed 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 (~500x) using either the Illumina HiSeq 2000 or the MiSeq platform (19), was performed on an additional 5 patients as part of a separate study. Details of mutation analysis can be seen in the Supplemental 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 1998-2010. Whole exome sequencing data was 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 supplemental information of the companion ccRCC TCGA biomarker paper. Mutation data was 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.” Non-silent, 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’s Exact and Wilcoxon Exact two tailed tests, respectively. Kaplan-Meier method was used to estimate the survival probabilities. Cancer specific survival (CSS) was analyzed using the competing risk method, utilizing the “cmprsk” R package. Cox proportional hazard regression was used for analysis of time to recurrence (20). Multivariate competing risk models were also fitted in the TCGA cohort in order to adjust for clinical covariates (AJCC stage 1-2 vs. 3 vs. 4 and Grade 1-2 vs. 3-4). 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 who’s “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 GISTIC (21) and used in the parallel paper 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 ccRCC patients. 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 (90-94%). The pathologic stages were similarly distributed in the two 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 Figure 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 co-mutated (p=0.007, OR 2.5, 95% 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 towards co-occurrence (p=0.13). In comparison, analysis of the TCGA cohort demonstrated similar mutation frequencies: VHL at 56.4%, PBRM1 at 33.5%, SETD2 at 11.6% and BAP1 at 9.7%. Similar co-occurrences 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 as well.

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 3-4 (p=0.01, OR 2.34, 95% CI 1.23, 4.58) and (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), while 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 Figure 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 (Fig. 2 & 3). Median overall survival for *BAP1* mutants in TCGA cohort is 31.2 months (95% CI 23.2, NA) vs 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) vs 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 cancer-specific survival (HR=17.83, 95% CI 5.1, 62.3, relative to patients without *BAP1* mutation) compared to missense mutations (HR=3.63, 95% CI 0.4, 32.6). There were too few patients with *SETD2* mutation to estimate the hazard ratios.

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) ((Supplemental Fig 1 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 (Supplemental Fig 2 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 co-occurrences. For example, BAP1 and SETD2 mutations co-occurred in only 3 patients (Figure 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 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 (8) (Supplemental Fig 3 & 4).

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 (1 and 2 vs 3 vs 4) 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 one 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, but statistically significant steady increase (~3 to ~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 panels), 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. The 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 panels and Supplemental Fig 5). 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**

Clear cell renal cell carcinoma, 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 (11, 22). However, it likely underestimates the actual loss of VHL since the VHL gene can be inactivated by promoter methylation and there is known inherent challenges in sequencing the GC rich regions of VHL. Additionally, we saw no correlation between the status of VHL mutations and clinical/pathologic features or CSS, which concurs with most prior reports (22). Previous studies have suggested that loss of the predominant tumor suppressor VHL alone is insufficient (23, 24) and additional genetic events are required for 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 (25). 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 (13). In line with the initial *PBRM1* mutation study in ccRCC (9), *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 demonstrated 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) suggests 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 (14, 15). 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 (26). Of note, these mesothelioma patients fared poorly irrespective of their *BAP1* status (median survival, 14.8 (mutant) vs. 15.3 (wild type) months). With respect to ccRCC, *BAP1* appears to be a critical gatekeeper from disease progression, a finding that was recently reported in ccRCC while our paper 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 cancer specific survival. In addition to *BAP1* mutations, we demonstrated a strong association between *SETD2* mutations and worse cancer specific survival 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.
Since 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 \textit{BAP1} mutations are highly correlative with the protein loss determined by immunohistochemistry (12), it might be beneficial to determine its mutation-expression status in smaller 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 four ccRCC tumor suppressors that locate in close proximity and possess critical pathological and clinical significance. Loss of function mutations of \textit{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. \textit{BAP1} and \textit{SETD2} mutations are associated with worse cancer specific survival and likely take place during the disease progression phase. Future efforts focus on these alterations may provide novel therapeutic opportunities.

\textbf{Acknowledgement:}

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References:


Table 1: Demographic, Pathologic and Clinical Outcomes of Discovery and Validation Cohorts

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<thead>
<tr>
<th>Cohort</th>
<th>Discovery –MSK (n=188)</th>
<th>Validation -TCGA (n=421)</th>
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<tr>
<td>Median age (quartiles)</td>
<td>61 (54,69)</td>
<td>61 (52,70)</td>
</tr>
<tr>
<td>Gender</td>
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</tr>
<tr>
<td>Male</td>
<td>132 (70.2%)</td>
<td>Male – 275 (65.3%)</td>
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<tr>
<td>Female</td>
<td>56 (29.8%)</td>
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<td>Race</td>
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<tr>
<td>White</td>
<td>170 (90.4%)</td>
<td>White – 394 (93.6%)</td>
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<td>Black</td>
<td>7 (3.7%)</td>
<td>Black – 14 (3.3%)</td>
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<tr>
<td>Other</td>
<td>11 (5.9%)</td>
<td>Other – 13 (3.1%)</td>
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<tr>
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<td>pT1</td>
<td>73 (38.8%)</td>
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<td>pT2</td>
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<td>pT3</td>
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<td>Regional Lymph Nodes (N Stage*)</td>
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<tr>
<td>pNx</td>
<td>103 (54.8%)</td>
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<td>pN0</td>
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<td>I</td>
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<tr>
<td>III</td>
<td>82 (43.6%)</td>
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<tr>
<th>Fuhrman Nuclear Grade</th>
<th>G1 (1.1%)</th>
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<tr>
<td></td>
<td>G2 (39.9%)</td>
<td>173 (41.1%)</td>
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<td>G3 (46.8%)</td>
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<td>G4 (12.2%)</td>
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<tr>
<th>Median Followup for Survivors (months)</th>
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<th>46</th>
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<tr>
<td>Overall 5 Year Survival</td>
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<tr>
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<td>NA (82.3, NA)</td>
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<tr>
<td>Number of deaths</td>
<td>21</td>
<td>142</td>
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<tr>
<td>Number of deaths from RCC</td>
<td>13</td>
<td>102</td>
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*TNM 7th Edition

NA in median survival refers to not estimable
Table 2: Mutations associations with adverse tumor features

<table>
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<tr>
<th>Mutations</th>
<th>Size &gt;5cm</th>
<th>Higher T Stage</th>
<th>Higher Nuclear Grade</th>
<th>Necrosis (&gt;5%)</th>
<th>LN mets (N1)</th>
<th>Presence of Mets on Presentation (M1)</th>
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<tr>
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<td>(1.23,4.58)</td>
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<td>4.57</td>
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<td>(1.16,30.26)</td>
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Figure Legends:

Figure 1:

A: Chromosome 3 map showing geographic proximity of the frequently mutated 3p tumor suppressors, and GISTIC copy number plot demonstrating heterozygous loss of a large segment of 3p in ~90% of the TCGA cohort. Blue color denotes copy number loss, and red – denotes copy number gain.

B: Mutation heat map of MSKCC and TCGA cohorts highlights similar mutation frequencies and co-occurrences of the 3p tumors suppressors in both cohorts.

C: Mutation percentages by type shows similar patterns in both cohorts (truncating = frameshift, nonsense, or essential splice site).

Figure 2:

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

Figure 3:

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

Figure 4:

A: Box plots demonstrate the statistically significant incremental increase of purported tumor suppressor genes (TSG) 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 TSG’s 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.
Figure 1

A. 436 TCGA Tumors

B. 121 mutant tumors (out of 188: MSKCC)

VHL 50.1%
PBRM1 30.3%
SETD2 7.4%
BAP1 6.3%

299 mutant tumors (out of 421: TCGA)

VHL 56.3%
PBRM1 33.5%
SETD2 11.6%
BAP1 9.7%

C. 26% 11% 21% 50%

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<tr>
<td></td>
<td>PBRM1</td>
<td>89%</td>
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<td>79%</td>
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<td>50%</td>
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<tr>
<td>TCGA</td>
<td>VHL</td>
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<td>BAP1</td>
<td>59%</td>
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Figure 2

**MSKCC**

**VIIL, p=0.70**

- WT
- Mutant

Cumulative Incidence Curve

Time to DOD

**PDRM1, p=0.93**

- WT
- Mutant

Cumulative Incidence Curve

Time to DOD

**SETD2, p=0.87**

- WT
- Mutant

Cumulative Incidence Curve

Time to DOD

**BAP1, p=0.002**

- WT
- Mutant

Cumulative Incidence Curve

Time to DOD
Figure 3

TCGA

VIIL, p=0.44

PDRM1, p=0.61

SETD2, p=0.036

BAP1, p=0.002

Cumulative Incidence Curve vs. Time to DOD

WT
Mutant

Cumulative Incidence Curve vs. Time to DOD

WT
Mutant

Cumulative Incidence Curve vs. Time to DOD

WT
Mutant

Cumulative Incidence Curve vs. Time to DOD

WT
Mutant
Figure 4
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

A Ari Hakimi, Irina Ostrovnaya, Boris A Reva, et al.

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