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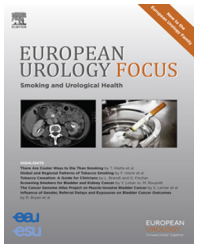

Platinum Priority – Kidney Cancer
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Integration of Recurrent Somatic Mutations with Clinical Outcomes: A Pooled Analysis of 1049 Patients with Clear Cell Renal Cell Carcinoma

Brandon John Manley[a], *Emily C. Zabor*[b], *Jozefina Casuscelli*[c], *Daniel M. Tennenbaum*[c], *Almedina Redzematovic*[d], *Maria F. Becerra*[c], *Nicole Benfante*[a], *Yusuke Sato*[e,f], *Teppei Morikawa*[g], *Haruki Kume*[e], *Masashi Fukayama*[g], *Yukio Homma*[e], *Seishi Ogawa*[f], *Maria E. Arcila*[h], *Martin H. Voss*[d], *Darren R. Feldman*[d], *Jonathan A. Coleman*[a], *Victor E. Reuter*[h], *Robert J. Motzer*[d], *Paul Russo*[a], *James J. Hsieh*[d], *A. Ari Hakimi*[a,*]

[a] *Urology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA;* [b] *Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA;* [c] *Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA;* [d] *Genitourinary Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA;* [e] *Department of Urology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;* [f] *Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan;* [g] *Department of Pathology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;* [h] *Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

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Abstract

Background: Analyses of associations between clinicopathologic outcomes and recurrent somatic mutations in clear cell renal cell carcinoma (ccRCC) have been limited to individual cohorts.
Objective: To define clinicopathologic associations between specific mutations and ccRCC disease characteristics.
Design, setting, and participants: DNA sequencing data were pooled from three collaborative genomic cohorts (n = 754) and our institutional database (n = 295). All patients had clinical data and identification of somatic mutations from their primary tumors.
Outcome measurements and statistical analysis: Analysis of gene mutations for associations with maximal tumor size (linear regression) and pathologic stage (logistic regression). Cancer-specific survival (CSS) and recurrence-free survival (RFS) were calculated using competing risks methods. Analyses were adjusted for cohort site, and results were adjusted for multiple testing (q value). Relevant genes were used in multivariable models that included confounding variables and the validated Mayo Clinic Stage, Size, Grade, and Necrosis (SSIGN) score.
Results and limitations: Association with tumor size was found for mutations in *BAP1* (q = 0.013). No mutations were found to be associated with stage after adjusted analysis. Mutations in *BAP1* (q = 0.004) and *TP53* (q = 0.001) were associated with decreased CSS in a multivariable model; only *TP53* (q = 0.005) remained significant when SSIGN score was included. *SETD2* mutations (q = 0.047) were associated with decreased RFS in multivariable models, including models with SSIGN score.

* Corresponding author. Sidney Kimmel Center for Prostate and Urologic Cancers, Urology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, 353 E 68th St, New York, NY 10065, USA. Tel. +1 646 422 4497; Fax: +1 212 988 0760.
E-mail address: hakimia@mskcc.org (A. Ari Hakimi).

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Conclusions: In >1000 patients with ccRCC, pooled analysis and multivariable modeling demonstrated that three mutated genes have statistically significant associations with poor clinical outcomes. This included the more commonly mutated *BAP1* and *SETD2* and the less frequently mutated *TP53*. After adjustment for clinical confounders, mutations of *TP53* and *SETD2* were associated with decreased CSS and RFS, respectively.
Patient summary: Using rigorous statistical methods, this study affirmed that certain mutations in clear cell renal cell carcinoma may portend inferior survival and an increased risk of recurrence.

1. Introduction

Despite recent advances in surgical and systemic treatments, renal cell carcinoma (RCC) remains the most lethal urologic malignancy, accounting for about 3% of all human cancers. Clear cell RCC (ccRCC) is the most common and aggressive histologic subtype [1]. The advent of next-generation sequencing technology has resulted in a fundamental shift in the understanding and potential treatment of ccRCC. Large-scale efforts by cooperative groups like The Cancer Genome Atlas [2], the International Cancer Genome Consortium [3], and the University of Tokyo [4] helped define the genomic landscape of ccRCC [5] and identified several recurrently mutated genes [6–8].

Observations and interpretations of the genetic landscape of ccRCC have been limited by several constraints. Most of the reports in this arena are based on relatively small patient cohorts with similar pathologic stages [2–4,9,10]. This, coupled with the low frequency of some mutations, has resulted in multivariable analyses being underpowered.

In this study, we performed comprehensive analyses of pooled publicly available cohorts, along with our institutional cohort, to identify associations between relevant mutations in ccRCC and clinicopathologic outcomes while controlling for known prognostic variables.

2. Methods

2.1. Patient selection

All patients included in the study signed informed consent at their respective institutions allowing for genomic testing. On approval by the institutional review board at Memorial Sloan Kettering Cancer Center (MSKCC), we searched our institutional kidney cancer database and identified 348 patients with ccRCC with prospectively collected genomic and clinical data between 2001 and 2015 (MSKCC cohort). Patients were excluded from this study if sequencing had not been performed on their primary tumors ($n = 53$), leaving 295 patients available for analysis. This included patients ($n = 185$ [62.7%]) who had targeted sequencing of five genes and had been previously described [11] but now had >3 yr longer follow-up. Of these 185 patients, 54 (29.2%) had next-generation sequencing performed, which was used in place of their targeted sequencing results for this analysis. One hundred ten patients (37.3%) in the MSKCC cohort have not been previously described. A majority of patient samples ($n = 157$ [53.2%]) were analyzed using MSK-IMPACT (MSKCC Integrated Mutation Profiling of Actionable Cancer Targets), a hybridization-based exon capture assay of select introns and commonly altered oncogenes and tumor suppressor genes [12]. The panel of targeted genes is listed in Supplement 1. Details of our genomic pipeline have been previously published [12]. For 35 patients, sequencing data were obtained using MSK-IMPACT performed on dissociated cells from their primary kidney tumors after an average of 1.7 passages. Remaining samples were analyzed using Sanger ($n = 131$ [44.4%]) and whole-genome ($n = 7$ [2.4%]) sequencing.

The second cohort in this study (public cohort) consisted of three previously published cohorts of patients with ccRCC [2–4], with documentation of appropriate ethics and consent for all study participants. Details of these study populations may be found in Supplement 2.

2.2. Statistical analysis

Data from a total of 1049 patients were available for analysis. A panel of 14 genes was chosen for analysis based on previously published works focusing on the clinical relations of significantly mutated genes across all cohorts. All patients had data available on four mutated genes (*VHL*, *PBRM1*, *SETD2*, and *BAP1*). Depending on the original study parameters, the 10 remaining genes had varying levels of data missing. To account for differences in patient characteristics and mutation frequencies across cohorts, all analyses were adjusted for cohort by including cohort as a categorical variable in all regression models. Linear regression analysis was used to assess the association between maximum pathologic tumor diameter and genetic mutations. Logistic regression estimated the association between gene mutations and American Joint Committee on Cancer (AJCC) stage [13]. We examined two ways of categorizing AJCC stage: (1) dichotomized as stages I/II/III versus stage IV and (2) categorized as stages I/II versus stage III versus stage IV.

Follow-up time was calculated from date of nephrectomy. Recurrence was determined according to previously described methods among the patients from the public cohort [2–4]. Date of recurrence was defined as date of pathologic confirmation of diagnosis, that is, biopsy. For patients with no pathology-confirmed recurrence, we used the date of the radiologic examination at which recurrence was diagnosed. Patients with documented recurrence who were reported as deceased on last follow-up and those with stage IV disease who were deceased without a listed cause were considered to have died from their disease. We used competing risks methods to estimate recurrence-free survival (RFS) and cancer-specific survival (CSS), treating death without recurrence and death from other causes as competing events, respectively.

To control for potential confounding in multivariable models, two sets of covariates were determined a priori. The first set, the base set, included age at diagnosis, sex, AJCC stage, and Fuhrman nuclear grade. The second set, the Mayo Clinic Stage, Size, Grade, and Necrosis (SSIGN) set, included age at diagnosis, sex, and SSIGN score as a continuous variable. The SSIGN score [14] is a validated prognostic model that is widely used to control for confounding in analytic models for RCC prognosis; because a SSIGN score was not available for all patients under study, it was treated as a secondary analysis.

All q values reported in this paper are the p values adjusted for multiple testing using the Benjamini-Hochberg false discovery rate method. A q value < 0.05 was considered statistically significant. All statistical analyses were conducted using R software v.3.1.1

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