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Impact of Recurrent Copy Number Alterations and Cancer Gene Mutations on the Predictive Accuracy of Prognostic Models in Clear Cell Renal Cell Carcinoma

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Abstract

Purpose—Several recently reported recurrent genomic alterations in clear cell renal cell carcinoma (ccRCC) are linked to pathological and clinical outcomes. We determined if any of the recurrent cancer gene mutations or copy number alterations identified in the Cancer Genome Atlas (TCGA) ccRCC dataset could add to the predictive accuracy of the current prognostic models.

Materials and Methods—413 patients who underwent nephrectomy/partial nephrectomy with whole exome, copy number array analyses, and clinical variables were interrogated. Sixty-five recurrent genomic alterations were identified based on prevalence and combined into 35 alterations including 12 cancer gene mutations. The genomic markers were modeled using the elastic-net algorithm with preoperative variables (tumor size + age) and in the postoperative setting using the externally validated Mayo Clinic stage, size, grade, and necrosis (SSIGN) prognostic scoring system. These models were subjected to internal validation using bootstrap.

Results—The median follow up for survivors was 45 months. Several markers correlated with adverse cancer-specific survival (CSS) and time to recurrence (TTR) on univariate analysis. However, most lost significance when controlling for tumor size +/- age in the preoperative models or SSIGN score in the postoperative setting. The addition of multiple genomic markers selected by the elastic-net algorithm failed to substantially add to the predictive accuracy of any of the preoperative or postoperative models for CSS or TTR.

Conclusions—While recurrent copy number alterations and cancer gene mutations are biologically significant, they do not appear to improve the predictive accuracy of existing clinical CSS or TTR models in ccRCC.

Keywords

Renal cell carcinoma; mutations; copy number alterations; prognostic models

1. Introduction

Several externally validated prognostic models based on histological and clinical factors are widely used to predict patient outcome in ccRCC, the most common and aggressive subtype of renal cell carcinoma¹⁻⁴. Multiple studies have suggested that recurrent chromosomal losses and gains in ccRCC may influence survival, although the improvements in predictive accuracy were small⁵⁻⁷. Many of these studies suffered from small sample size, lack of multivariate modeling, lack of validation, and older, less precise genetic techniques. Finally, these studies did not integrate recently reported recurrent mutations in their models.

Many of the recurrent mutations found in ccRCC have since been elucidated through large-scale, whole-exome sequencing studies⁸⁻¹⁰, and more recently through the TCGA project. The clinical and pathological associations of several recurrently mutated genes have been reported¹¹⁻¹³. In the present study, we sought to determine if any of the recurrent mutations or CNAs identified in the TCGA analysis could improve the predictive accuracy of clinical prognostic models, using a rigorous statistical approach.

2. Materials and Methods

2.1 TCGA data set

Paired tumor-normal samples, genomic data, clinical and pathologic information were acquired from the multi-institutional ccRCC TCGA consortium on 446 retrospectively identified patients who underwent radical or partial nephrectomy from 1998-2010 for sporadic ccRCC. All clinical and pathologic information were approved by the respective institutional review boards. Whole-exome sequencing data was available for 413 for analysis. Full sequencing and copy number information is detailed in the ccRCC TCGA biomarker paper¹⁴. For “low-level” events (one copy loss or gain), a minimum frequency of occurrence threshold of 5% was used. For “high-level” events (homozygous deletions or 2 copy number gain), a threshold of occurrence of 2.5% was used. Methods for selected mutations and copy number events appear in Supplemental Methods.

2.2 Statistical analysis

Of the 62 genomic markers obtained, 12 were copy number gains (including 1 high-level amplification), 38 copy number losses (including 1 homozygous deletions), and 12 mutations (Supplementary Table S1). Since many of the CNAs on the same chromosomal arm were highly correlated, we combined all CNAs on the same arm into one variable (ie, the combined marker was considered present if any marker on the chromosome arm was present). This was performed separately for high- and low-level events, resulting in a total of 35 genomic markers, 12 of them mutations (herein referred to as “combined genomic markers”). Pairwise correlations between markers were assessed using Fisher's exact test. CSS and TTR calculations appear in Supplemental Methods. Adjustment for multiple comparisons was performed for combined markers only using the Benjamini-Hochberg method separately within each analysis (pairwise correlations between markers, CSS or TTR association after adjusting for SSIGN score or size and age).

Prognostic models with clinical variables alone were fitted using Cox proportional hazards regression. To incorporate genomic markers, we used the elastic-net Cox model in the “glmnet” R package v. 1.7.3¹⁵. We chose Elastic Net algorithm since it is known to work well for multiple correlated predictors and it is not prone to overfitting, i.e. the estimated model is frequently generalizable to other cohorts. Prognostic accuracy was measured using Harrell's concordance probability, C-index (a generalization of the area under the curve [AUC]), and was subjected to internal validation using bootstrap.

Our goal was to build prognostic models based on risk factors known pre- or post-surgery in combination with genomic markers. For post-surgical models we used the Mayo Clinic SSIGN score². We chose this model over other validated models^{3, 4161718} because SSIGN

score has performance characteristics similar to the other models¹⁹ and certain clinical variables necessary for the calculations of the other models were unavailable. For presurgical models, tumor size and age at diagnosis were used as clinical variables because gender was not a significant factor in preoperative models. SSIGN score, tumor size, and age at diagnosis were used as continuous variables. Statistical analyses were performed using R v. 2.13.1 (www.r-project.org) and package `glmnet_v. 1.7.3`.

3. Results

Clinical, copy number, and mutation data were available for 413 patients. Clinical characteristics of the cohort are shown in Table 1. CSS and TTR curves are given in the Supplementary Fig. 1.

3.1 Associations between genomic markers

Frequencies of genomic markers ranged between 2% and 92% with about 80% having a frequency above 5% (Supplementary Table S2). Markers located on the same chromosome were highly correlated, making a case for combining such markers. Supplementary Table S3 shows *q*-values (false discovery rate adjusted *p* values) and odds ratios (OR) for associations between combined markers. Many combined CNAs were highly correlated even when located on different chromosomes. A partial explanation is that some patients have many CNAs, while others have few. Thus, a CNA on one chromosome often co-occurs with CNAs on other chromosomes. This is illustrated in Supplementary Fig. 2 and supports our choice of the elastic-net method for modeling.

The number of combined CNA markers per patient ranged between 0 and 17, and number of CNAs significantly associated with CSS (full cohort: hazard ratio 1.09 per additional CNA, 95% CI 1.03, 1.15, *p*=0.001). This effect, however, was not significant after adjusting for SSIGN score (*p*=0.9). Similarly, TTR was not significant after adjusting for SSIGN score.

3.2 Analysis of individual genomic markers

The univariate associations between CSS in both the complete and combined sets of genomic markers are presented in Supplemental Tables S4 and S5, respectively. Several previously described markers were associated with worse CSS univariately such as loss and homozygous deletions of chromosome 9p (*CDKN2A*), gain at 8q (*MYC*) and mutations in *BAP1* and *TP53*. However, when controlling for SSIGN score, the majority of the markers became non-significant with the exception of heterozygous loss of 11q and mutations in *KDM5C*, and all were non-significant when controlling for multiple testing (supplemental table S5). With respect to TTR, markers analysis demonstrates similar univariate findings in terms of copy number alterations and shorter TTR, as well as mutations in the tumor suppressors *SETD2* and *PTEN* (Supplemental Table S4-S5 for complete and combined marker list). When controlling for SSIGN score, heterozygous loss at chromosome 10q (which includes *PTEN*) and gain of 15q (including *IDH2*) remained significant in the combined markers, but this effect was again lost when controlling for multiple testing (Table S5). Supplemental Table S4 and S5 also demonstrate univariate associations with genomic markers and CSS and TTR in the preoperative setting (using size and age alone). Only

associations between CSS and *TP53* mutations and Chromosome 9 homozygous deletions adjusted for size and age remained significant after correcting for multiple comparisons, but the frequency of these events were low (3% and 2% respectively) (Supplemental Table S5).

3.3 Prognostic models

Consistent with previous reports ^{2, 19, 20}, the prognostic model for CSS that included SSIGN score alone had an 84.9% bootstrap-adjusted C-index. The addition of either the complete or combined set of genomic markers failed to substantially improve prediction accuracy (Table 2). Elastic net selected only five combined genomic markers for this model, setting coefficients for the other 30 markers at zero (Table 3). Fitted coefficients for all markers and models are in Supplementary Table S6.

As expected, the prognostic model for CSS that includes only tumor size and age at diagnosis is less accurate than the post-surgical SSIGN model (73.4% bootstrap adjusted C-index). The model with size and 21 selected additional combined genomic markers also failed to improve prediction accuracy (Table 2 and Supplemental Table S6).

The model predicting TTR that included SSIGN score alone had a 74.2% bootstrap adjusted C-index (Table 2). The addition of 12 combined genomic markers (Table 4) did not lead to an improvement in predictive accuracy (bootstrap-adjusted C-index 74.3% for SSIGN + combined genomic markers). The model predicting TTR that included tumor size and age at diagnosis alone had a 71.6% bootstrap adjusted C-index, and including 13 additional combined genomic markers raised the C-index to 72.8% (Tables 3 and 5). Models that were fitted using 62 complete genomic markers had similar results (Supplemental Table S6).

4. Discussion

Using the genomic and clinical data from the TCGA ccRCC data set, we critically interrogated the additional prognostic value of recurrent CNAs and cancer gene mutations compared to the validated SSIGN scoring system. Our models benefitted from the use of next-generation whole-exome sequencing and high throughput, SNP arrays. We set frequency thresholds for recurrent CNAs because less prevalent biomarkers have little practical clinical relevance. The top 12 recurrent cancer gene mutations were incorporated into the models. Rigorous statistical analysis was needed to address the prognostic value of these alterations.

Inclusion of several correlated variables increases the risk of overfitting and makes commonly used procedures prone to yield spurious results. To address this we first utilized internal validation. Second, we utilized elastic net, a novel statistical method that can handle correlated predictors while minimizing overfitting.

While several markers (some previously published) were identified that predicted worse cancer-specific outcomes on univariate analysis, most markers were not significant when corrected for SSIGN (Supplemental Tables S4 and S5) and none was significant after controlling for multiple testing. Additionally, we saw no benefit to the additional genomic

markers in any of the TTR models using SSIGN or size and age (Supplemental Tables S4 and S5).

Several previous reports evaluated the effect of cytogenetic alterations on the recurrence-free, disease-specific, and overall survival of patients with ccRCC^{5-7, 21-30}. These studies used a variety of techniques to identify copy number changes including genome-wide assays such as G-banding^{5-7, 23}, comparative genomic hybridization^{21, 25, 29}, and SNP arrays^{26, 30} as well as targeted analyses such as FISH^{6, 21, 22, 27, 29} and microsatellite LOH analysis^{24, 28}. Overall, higher numbers of CNAs led to a decrease in RFS and OS^{21, 25}. We found similar associations with CSS and TTR, but this effect was not significant after adjusting for SSIGN score.

The associations between recurrent, specific CNAs and clinical outcomes are found in multiple previous studies and presented in Table 5 along with their univariate validation in the TCGA dataset. 3p loss, either alone or combined with a gain at 5q, was associated with improved CSS^{7, 27}. Multiple studies have shown that loss of 9p (*CDKN2A*) is associated with decreased CSS^{6, 7, 22, 25, 29}. Loss of 14q^{7, 24, 26} and gain of 8q (*MYC*) have also been associated with decreased CSS and OS^{5, 26}. While we validated many of these findings, the alterations lost significance when controlled for SSIGN. Additionally, while several recurrent gains or losses were selected for our final models, we were unable to show any additional benefit in predictive accuracy compared to tumor size +/- age alone in the presurgical models, or using the SSIGN score in the post-surgical setting (Table 2). While the UCLA group has found that deletions of 9p and gain of 8q are associated with worse CSS even in multivariate models^{5, 6}, we could not validate this after adjusting for SSIGN (Table 5). However, their studies did not use regularized regression methods (like elastic net) that minimize overfitting, and in one case internal validation; thus, their findings required verification on independent datasets. Further, while statistically significant in the cohort where their models were developed, the improvement in predictive accuracy of survival models was very small (~0.5%).

Our analysis of small tumors (< 4 cm) was limited by both number of events (cancer-specific death and recurrences) and frequency of genomic alterations (Supplementary Table S7). We cannot rule out the possibility that some of the alterations may be associated with worse disease-specific outcomes. The lower incidence of mutations and recurrent copy number events in this group overall is noteworthy, and should impact future trial designs to better risk-stratify small renal masses. Future studies focusing on smaller masses are certainly warranted.

There are important limitations to our analysis. While we used state-of-the-art statistical modeling, it is possible that in our analysis we missed some interactions between genomic markers that might improve the prognostic models, since the elastic-net method does not incorporate interaction effects. However, due to the sample size and low prevalence of many markers, the power to detect and validate such interactions would be slight and the addition to predictive accuracy modest. Further, we focused our analysis on DNA alterations because of their causal relationship as biologic drivers of cancer behavior. We cannot exclude the possibility that changes in gene mRNA or miRNA expression might improve predictive

accuracy. However, any efforts to incorporate these expressions should include the current best predictive models, not simply a multivariate model including pathologic stage and grade. Additionally, TCGA and other large sequencing efforts are limited by the challenges of intratumoral heterogeneity and we cannot exclude the possibility that obtaining multiple tumor biopsies may give more accurate biomarker information. However, the clinical feasibility and cost of this approach remains quite challenging.

Finally, while many recurrent mutations and chromosomal losses and gains do not improve predictive models, their associations with disease behavior make them critical targets for drug development, and their impact on response to targeted therapies requires further investigation.

5. Conclusions

Our analysis, evaluating whether recurrent cancer gene mutations or CNAs could add to the predictive accuracy of current prognostic models, suggests that while these predict treatment outcome, they do not improve the accuracy of current clinical CSS or TTR models in ccRCC. Further studies regarding the role of these alterations in predicting response to systemic treatment are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix 1

Final combined genomic markers in the cancer-specific survival models

CSS~SSIGN+combined genomic markers		
Markers in model with SSIGN	Well characterized cancer genes in region	Effect on CSS
TP53 mutation	NA	Worse
KDM5C mutation	NA	Worse
9p21 deletion	CDKN2A	Worse
11q loss	-	Worse
2q gain	-	Better

NA = not applicable

Appendix 2

Final combined genomic markers in the time-to-recurrence models

Markers in model with SSIGN	Well-characterized cancer genes in region	Effect on TTR
SETD2 Mutation	NA	Worse
KDM5C Mutation	NA	Worse
PTEN mutation	NA	Worse
PIK3CA mutation	NA	Better
1q loss	-	Worse
2q loss	-	Better
3q gain	PIK3CA	Worse
4q loss	TET2	Worse
5q gain		Better
9p21 deletion	CDKN2A	Worse
10q loss	PTEN	Worse
15q gain	IDH2	Worse

NA = not applicable

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Table 1
Demographic, pathologic and clinical outcomes of cohort

Cohort (n=413)	
Median age (quartiles)	61 (52,70)
Gender	Male – 268 (65%) Female – 145 (35%)
Race	White – 386 (93%) Black – 14 (3%) Other – 13 (4%)
Median tumor size – cm (quartiles)	5.5 (4,8.7)
Nephrectomy Type	Partial – 85 (21%) Radical – 328 (79%)
AJCC Stage	194 (47%) I 40 (10%) II 110 (27%) III 69 (17%) IV
Fuhrman nuclear grade	7 (2%) G1 170 (41%) G2 168 (41%) G3 67 (16%) G4 1 Unknown
Lymph Node Status	N0 – 188 (46%) N1 – 12 (3%) Nx – 213 (51%)
Median follow-up for survivors (mo)	45
Overall 5-yr survival	60.9%
Number of deaths	140
Number of deaths from RCC	101
Number eligible for TTR analysis	329
Number of recurrences	71

AJCC = American Joint Committee on Cancer

Table 2
Accuracy of prognostic models

	Number of variables in the model	C-index	Bootstrap-corrected C-index
CSS~SSIGN	1	0.8489	0.8489
CSS~SSIGN +complete genomic markers	11	0.8588	0.8257
CSS~SSIGN +combined genomic markers	6	0.8560	0.8317
CSS~size+age	2	0.7375	0.7343
CSS~ size + age+complete genomic markers	29	0.7905	0.7303
CSS~ size +age+combine d genomic markers	23	0.7777	0.7313
TTR~SSIGN	1	0.7431	0.7417
TTR ~SSIGN+ complete genomic markers	15	0.7916	0.7258
TTR ~SSIGN +combined genomic markers	13	0.7903	0.7426
TTR~ size+age	2	0.7214	0.7164
TTR~ size+age + complete genomic markers	15	0.7823	0.7152
TTR~ size+age +combined genomic markers	15	0.7771	0.7280