Genomic Copy Number Alterations in Renal Cell Carcinoma with Sarcomatoid Features

Timothy Ito, Jianming Pei, Essel Dulaimi, Craig Menges, Philip H. Abbosh, Marc C. Smaldone, David Y. T. Chen, Richard E. Greenberg, Alexander Kutikov, Rosalia Viterbo, Robert G. Uzzo and Joseph R. Testa*

From the Cancer Biology Program and Blood Cell Development and Function Program (ED), Fox Chase Cancer Center, Philadelphia, Pennsylvania

Abbreviations and Acronyms

ccRCC = clear cell RCC

chRCC = chromophobe RCC

CNA = copy number alteration

EMT = epithelial-mesenchymal transition

FBP1 = fructose-1,6-bisphosphatase

HIF = hypoxia inducible factor

pRCC = papillary RCC

RCC = renal cell carcinoma

SNP = single nucleotide polymorphism

sRCC = sarcomatoid RCC

Accepted for publication October 8, 2015.

No direct or indirect commercial incentive associated with publishing this article.

The corresponding author certifies that, when applicable, a statement(s) has been included in the manuscript documenting institutional review board, ethics committee or ethical review board study approval; principles of Helsinki Declaration were followed in lieu of formal ethics committee approval; institutional animal care and use committee approval; all human subjects provided written informed consent with guarantees of confidentiality; IRB approved protocol number; animal approved project number.

Supported by National Institutes of Health, National Cancer Institute Grant P30 CA006927 and Fox Chase Cancer Center via Kidney Cancer Keystone Program institutional support.

* Correspondence: Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, Pennsylvania 19111 (telephone: 215-728-2610; FAX: 215-214-1623; e-mail: Joseph.Testa@fccc.edu).

For another article on a related topic see page 1120.

Purpose: Sarcomatoid changes in renal cell carcinoma are associated with a poor prognosis. The identification of genetic alterations that drive this aggressive phenotype could aid in the development of more effective targeted therapies. In this study we aimed to pinpoint unique copy number alterations in sarcomatoid renal cell carcinoma compared to classical renal cell carcinoma subtypes.

Materials and Methods: Genomic copy number analysis was performed using single nucleotide polymorphism based microarrays on tissue extracted from the tumors of 81 patients who underwent renal mass excision, including 17 with sarcomatoid renal cell carcinoma.

Results: Sarcomatoid renal cell carcinoma showed a significantly higher number of copy number alterations than clear cell, papillary and chromophobe renal cell carcinoma (mean 18.0 vs 5.8, 6.5 and 7.2, respectively, $p < 0.0001$). Copy number losses of chromosome arms 9q, 15q, 18p/q and 22q, and gains of 1q and 8q occurred in a significantly higher proportion of sarcomatoid renal cell carcinomas than in the other 3 histologies. Patients with sarcomatoid renal cell carcinoma demonstrated significantly worse overall survival compared to those without that condition on Kaplan-Meier analysis ($p = 0.0001$). Patients with 9 or more copy number alterations also demonstrated significantly worse overall survival than those with fewer than 9 copy number alterations ($p = 0.004$).

Conclusions: Sarcomatoid changes in renal cell carcinoma are associated with a high rate of chromosomal imbalances with losses of 9q, 15q, 18p/q and 22q, and gains of 1q and 8q occurring at significantly higher frequencies in comparison to nonsarcomatoid renal cell carcinoma. Identifying candidate driver genes or tumor suppressor loci in these chromosomal regions may help identify targets for future therapies.

Key Words: kidney; carcinoma, renal cell; polymorphism, single nucleotide; chromosome aberrations; microarray analysis

SARCOMATOID changes, which occur in approximately 5% of RCCs, may arise from any histological subtype. Its presence portends an extremely poor prognosis even compared to that of other high grade RCCs with median overall survival ranging from 4 to 12 months.[1] These outcomes stem from the fact that the majority of patients with sRCC present with

0022-5347/16/1954-0852/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2015.10.180
Vol. 195, 852-858, April 2016
Printed in U.S.A.

metastatic disease and to date effective systemic therapy has not been identified.

Evidence suggests that sarcomatoid changes are the result of a divergent clone that demonstrates unique patterns of allelic loss compared to its root RCC histological subtype.[2] Better understanding of the genetic differences between sRCC and nonsRCC may point to those changes essential for the development of this aggressive phenotype and ultimately lead to the identification of new therapeutic targets. In this study we aimed to identify unique CNAs in sRCC compared to nonsRCC using SNP based microarrays.

MATERIALS AND METHODS

Study Population

In 89 patients undergoing renal mass excision for RCC between November 2010 and July 2014 at our institution SNP array analysis was done on portions of the tumors. A single pathologist (ED) reviewed and confirmed the histological identity of the tissue used for SNP array analysis. Any patient with sarcomatoid changes seen in the tissue sampled for SNP array was placed in the sRCC group. All other patients were placed in a group based on the predominant histology present in the tissue sampled for SNP array. Eight patients with sarcomatoid changes seen outside the area sampled for SNP array were excluded from final analysis to minimize the potential for including sRCC in the ccRCC, pRCC and chRCC groups. A mean of 7 CNAs (median 4.5, range 1 to 27) were seen in excluded patients. Following these exclusions the final study cohort consisted of 81 patients, including 17 with sRCC, 34 with ccRCC, 24 with pRCC and 6 with chRCC. All patients provided written consent for inclusion in a prospectively collected, institutional review board approved kidney cancer database, which was queried to obtain baseline patient characteristics and outcome data.

SNP Array Analysis

Pathological review of hematoxylin and eosin stained tissue directly adjacent to the area used for SNP array was performed to ensure that the region of tumor used was as phenotypically homogenous as possible, maximize the tumor percent in the tissue sample and minimize the presence of necrosis, stroma and normal tissue. The designated tumor tissue was then macrodissected from frozen section. The median percent of tumor present in the tissue sample was 90% (range 40% to 100%).

Samples from November 2010 to March 2012 were analyzed using Cytogenetics 2.7M arrays (Affymetrix®) and samples from April 2012 to July 2014 were analyzed using CytoScan® HD arrays. Because the samples were processed for clinical purposes, extensive validation was performed to ensure uniformity between the old and new platforms at the time of the switch to conform to CLIA (Clinical Laboratory Improvement Amendments) standards. Total genomic DNA was extracted and SNP array analysis was performed as previously described.[3–5] The SNP arrays were then scanned with a GeneChip® Scanner 3000 7G. Copy number analysis was performed using ChAS (Chromosome Analysis Suite, Affymetrix) (fig. 1).

Statistical Analysis

All CNAs occurring in any particular histological subtype at a predefined threshold frequency of 25% or higher were deemed significant. Univariate analysis was performed using the Fisher exact test, the t-test and ANOVA when appropriate. Survival analysis was performed between groups using Kaplan-Meier survival curves, the Wilcoxon test and Cox regression analysis. A threshold of 2-tailed $p < 0.05$ was used to determine statistical significance. Due to the small number of chRCC patients included in study sRCC CNAs occurring in 25% or greater, which were not present in any of the 6 chRCCs, were also considered significant despite p values that slightly exceeded the 0.05 threshold.

RESULTS

A mean of 8.7 CNAs (median 6, range 1 to 48) were seen across the entire 81-patient cohort. A significantly higher mean number of CNAs was seen in sRCC compared to ccRCC, pRCC and chRCC (18.0 vs 5.8, 6.5 and 7.2, respectively, $p < 0.0001$, table 1). This remained true even when limiting comparison of the mean number of CNAs to sRCC vs 26 high grade nonsRCCs and vs 5 Fuhrman grade 4 nonsRCCs only (mean 18.0 vs 6.1 and 7.6, $p < 0.0001$ and 0.03, respectively).

Table 1 lists CNAs that occurred in greater than 25% of samples in each histopathological group. Copy number losses were noted in a significantly higher proportion of sRCCs vs all nonsRCCs combined as well as vs each nonsRCC histology group independently (table 2). They included losses of chromosome arms 9q, 15q, 18p, 18q and 22q. Gains of chromosome arms 1q and 8q were observed in a significantly higher proportion of sRCCs than in the other RCC histologies separately and combined (table 2). Figure 2 shows the segments of chromosomes 1, 8, 9, 15, 18 and 22 that were found to be altered in individual sRCC tumors.

Nine of the 17 tumors with sRCC arose from a histological background of ccRCC, 2 arose from pRCC and 6 arose from unclassified RCC, including 2 with ccRCC as well as pRCC features. Patients with sRCC were more likely to have nonorgan confined disease (ie pT3 or greater), positive lymph nodes and distant metastases compared to patients with nonsRCC ($p < 0.0001$, 0.0004 and < 0.0001, respectively, table 1).

Seven patients (41%) with sRCC died of the disease at a median of 6.5 months (range 0 to 20.8). At final followup of the remaining patients with sRCC 7 (41%) were alive with metastatic disease and 3 (18%) had no evidence of disease. On Kaplan-Meier analysis patients with sRCC demonstrated