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Genomic Copy Number Alterations in Renal Cell Carcinoma with Sarcomatoid Features

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Abstract

Introduction and Objective—Sarcomatoid differentiation in renal cell carcinoma (sRCC) is associated with a very 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 (CNAs) in sRCC when compared to classical RCC subtypes.

Methods—Genomic copy number analysis was performed using single nucleotide polymorphism (SNP)-based microarrays on tissue extracted from the tumors of 81 patients who underwent renal mass excision, including 17 with sRCC.

Results—sRCC tumors exhibited significantly higher numbers of CNAs when compared to clear cell (ccRCC), papillary (pRCC) and chromophobe RCC (chRCC) (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 gains of 1q and 8q occurred in a significantly higher proportion of sRCC tumors compared to the other 3 histologies. Patients with sRCC tumors demonstrated significantly worse overall survival when compared to those without sRCC on Kaplan-Meier analysis ($p = 0.0001$). Patients with 9 or more CNAs also demonstrated significantly worse overall survival compared to those with fewer than 9 CNAs ($p = 0.004$).

Conclusions—Sarcomatoid differentiation in RCC is 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 non-sRCC tumors. Identification of candidate driver genes or tumor suppressor loci within these chromosomal regions may help identify targets for future therapies.

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Keywords

sarcomatoid renal cell carcinoma; chromosome aberrations; single nucleotide polymorphism; microarray analysis

Introduction

Sarcomatoid differentiation occurs in approximately 5% of renal cell carcinomas (RCCs) and may arise from any histologic subtype. Its presence portends extremely poor prognosis even when compared to other high grade RCCs, with median overall survival ranging from 4-12 months.¹ These outcomes stem from the fact that the majority of patients with sarcomatoid RCC (sRCC) present with metastatic disease, and to date effective systemic therapy has not yet been identified.

Evidence suggests that sarcomatoid differentiation is the result of a divergent clone, demonstrating unique patterns of allelic loss in comparison to its root RCC histologic subtype.² A better understanding of the genetic differences between sRCC and non-sRCC tumors may point to those changes essential for development of this aggressive phenotype, and ultimately lead to the identification of new therapeutic targets. In this study, we aimed to identify unique copy number alterations (CNAs) in sRCC when compared to non-sRCC tumors utilizing single nucleotide polymorphism (SNP)-based microarrays.

Materials and Methods

Study population

A total of 89 patients undergoing renal mass excision for RCC between November 2010 and July 2014 at our institution underwent single-nucleotide polymorphism (SNP) array analysis on portions their tumors. A single pathologist (E.D.) reviewed and confirmed the histologic identity of the tissue utilized for SNP array analysis. Any patient with sarcomatoid changes seen in the tissue sampled for SNP array was placed into the sarcomatoid RCC (sRCC) group. All other patients were placed in their respective groups based on the predominant histology present within the tissue sampled for SNP array. A total of 8 patients with sarcomatoid changes seen outside of the area sampled for SNP array were excluded from the final analysis in order to minimize the potential for inclusion of sRCC in the clear cell (ccRCC), papillary (pRCC) and chromophobe RCC (chRCC) groups. The mean and median number of CNAs seen in patients excluded were 7 and 4.5 respectively (range 1-27). Following these exclusions, the final study cohort consisted of 81 patients: 17 with sRCC, 34 with ccRCC, 24 with pRCC and 6 with chRCC. All patients provided written consent for inclusion in a prospectively-collected, IRB-approved Kidney Cancer Database which was queried to obtain baseline patient characteristics and outcomes data.

SNP array analysis

Pathologic review of H&E stained tissue directly adjacent to the area utilized for SNP array was performed in order to ensure the region of tumor utilized was as phenotypically homogenous as possible, to maximize tumor percentage in the tissue sampled, and minimize

the presence of necrosis, stroma and normal tissue. The designated tumor tissue was then macrodissected from frozen section. The median percentage of tumor present in the tissue sampled was 90% (range 40-100%). Samples from November 2010 to March 2012 were analyzed using Cytogenetics 2.7M arrays, and samples from April 2012 to July 2014 were analyzed using Cytoscan HD arrays (both arrays from Affymetrix, Santa Clara, CA). Given the samples were processed for clinical purposes, extensive validation was performed to ensure uniformity between the old and new platform at the time of the switch in order to conform to CLIA standards. Total genomic DNA was extracted and SNP array analysis was performed as previously described.³⁻⁵ The SNP arrays were then scanned with an Affymetrix GeneChip Scanner 3000 7G. Copy number analysis was performed using the Affymetrix Chromosome Analysis Suite software (Figure 1).

Statistical analysis

All CNAs occurring within any particular histologic subtype at a pre-defined threshold frequency of 25% or higher were deemed significant. Univariate analysis was performed using Fisher's exact tests, t-tests and ANOVA where appropriate. Survival analysis was performed between groups utilizing Kaplan-Meier survival curves, the Wilcoxon test and Cox regression analysis. A threshold two-tailed p-value of less than 0.05 was utilized to determine statistical significance. Due to the small number of chRCC patients included in the study, sRCC CNAs occurring in $\geq 25\%$ which were not present in any of the 6 chRCC tumors were also considered "significant", despite p-values which slightly exceeded the 0.05 threshold.

Results

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

CNAs occurring in greater than 25% of samples within each histopathologic group are listed in Table 1. Copy number losses that occurred in a significantly higher proportion of sRCC tumors when compared to all non-sRCC tumors combined, as well as to each non-sRCC histology group independently, are summarized in Table 2. These included losses of chromosome arms 9q, 15q, 18p, 18q, and 22q. Gains of chromosome arms 1q and 8q occurred in a significantly higher proportion of sRCC tumors when compared to the other RCC histologies both separately and combined (Table 2). An illustration of the segments of chromosomes 1, 8, 9, 15, 18 and 22 found altered in individual sRCC tumors is provided in Figure 2.

Of the 17 tumors with sRCC present, 9 arose from a histologic background of ccRCC, 2 from pRCC, and 6 from unclassified RCC, including 2 with both ccRCC and pRCC features. Patients with sRCC tumors were more likely to have non-organ confined disease (i.e. pT3),

positive lymph nodes, and distant metastases when compared to non-sRCC patients ($p<0.0001$, $p=0.0004$ and $p<0.0001$ respectively; Table 1).

Of the sRCC patients, 7 (41%) died of disease at a median of 6.5 months (range, 0-20.8 months). On final follow up for the remaining sRCC patients, 7 (41%) were alive with metastatic disease and 3 (18%) had no evidence of disease. On Kaplan-Meier (KM) analysis, patients with sRCC tumors demonstrated significantly worse overall survival compared to those without sRCC ($p=0.0001$; Fig. 3). Patients whose tumors were found to have higher than the mean number of CNAs for the entire cohort (i.e., 9) also experienced worse overall survival compared to those with fewer CNAs ($p=0.004$). On Cox regression analysis sarcomatoid differentiation was an independent predictor of an increased risk of death (HR 6.7, 95% C.I. 1.4-33.0, $p=0.02$). Of the identified distinctive sRCC CNAs, only loss of 15q was more highly prevalent in sRCC patients experiencing disease-specific mortality compared to those alive at final follow up (86% vs 30%, $p=0.02$). Loss of 9q, 18p/q, 22q and gain of 1q and 8q were equivalently prevalent between those dying and not dying of sRCC.

Discussion

Sarcomatoid features are found in approximately 5% of kidney cancers and may arise from any histologic subtype.¹ The presence of sarcomatoid differentiation is associated with a very poor prognosis. Metastatic disease is common at presentation, and response to systemic therapies—even targeted therapies which have demonstrated efficacy in non-sRCC—is limited.^{6, 7} This phenotypic difference suggests that sarcomatoid differentiation develops due to genetic alterations independent of those fundamental to original RCC tumor formation. Identification of these unique genetic drivers may aid in the development of more effective systemic therapies for this aggressive phenotype.

While comprehensive genetic characterization has been performed in ccRCC as well as other histologic subtypes,⁸⁻¹¹ studies examining sRCC have been limited to date. Dal Cin et al. previously reported karyotypic alterations in four sRCC cases, of which two had complex karyotypes, and one demonstrated a deletion of 22q12 as its sole abnormality.¹² Brunelli et al. used fluorescence *in situ* hybridization (FISH) analysis to examine copy numbers of chromosomes 1, 2, 6, 10, and 17 in six sRCC tumors arising from chRCC; in contrast to chRCC, where these specific chromosomes are frequently lost, two-thirds of the sRCC tumors were found to have multiple gains of these five chromosomes.¹³

Jiang et al. used metaphase-based comparative genomic hybridization and reported that loss of 13q (75%) and 4q (50%) were the most common CNAs found among the 12 sRCC tumors examined.¹⁴ In our series, 13q loss was seen in 53% of sRCC tumors, however it was also found in 50% of chromophobe tumors as well as in one tumor each for ccRCC and pRCC. Davis et al. also reported a high rate of loss of all or part of chromosome 13 (86%) among 66 chRCC tumors examined.¹¹ Moore et al. found that 13q loss occurred in 25% of 400 ccRCC tumors examined, and was associated with worse grade and stage disease.¹⁵ Thus 13q loss may be more of a marker of initial histology, or more aggressive disease generally, and so it was not deemed unique to sRCC in this study. Similarly, 4q loss was seen in 41% of sRCC tumors in our study but was also found in 18% and 13% of ccRCC

and pRCC tumors, respectively. The difference in frequency of 4q loss between sRCC and the other two histologies was not statistically significant and thus in our study this CNA was not classified as unique to sRCC.

The final study examining CNAs in sRCC performed by Dagher et al. reported that loss of chromosome 9 and gain of chromosome 20 were independent predictors of the presence of a sarcomatoid component in ccRCC, based on karyotype analysis of 7 ccRCC specimens with sarcomatoid differentiation.¹⁶ The association between chromosome 9 loss and sRCC reported by Dagher et al. is mirrored in our study. Gain of chromosome 20 in our cohort was seen in 59% of sRCC tumors, but was also seen in 22% of non-sRCC tumors, including 50% of pRCC tumors; thus it could not be described as unique to sRCC tumors in our series.

To our knowledge, our study represents the largest to utilize SNP-based microarrays to examine CNAs in sRCC. We found that sRCC tumors have highly complex genomic profiles with numerous genomic imbalances which occurred at significantly higher frequencies than in ccRCC, pRCC and chRCC. Notably, among the non-sRCC tumors, four had metastatic disease at the time of presentation (3 ccRCC and 1 pRCC), with a mean number of CNAs of 7.0, suggesting that advanced disease alone does not account for the high number of CNAs seen in sRCC. Also, among eight non-sRCC patients with organ-confined disease at the time of surgery who later developed metastatic disease on follow-up, the mean number of CNAs was 7.8.

CNAs found to be more prevalent in sRCC tumors when compared to non-sRCC tumors included losses of 9q, 15q, 18p/q and 22q, and gains of 1q and 8q. Several of these regions have been found to be associated with worse disease and outcomes in ccRCC. Loss of chromosome arm 9q has been associated with higher grade and stage disease in ccRCC, as well as worse overall and recurrence-free survival.^{15, 17, 18} 18p/q loss has also been associated with higher grade and stage ccRCC.¹⁷ Gains of 1q and 8q have been associated with metastatic disease.^{19, 20}

Several RCC-related genes are located on the chromosomes that were found to be more frequently altered in sRCC tumors in this study. Loss of fructose-1,6-bisphosphatase (FBP1) on chromosome arm 9q has been shown to be necessary for hypoxia inducible factor (HIF)-mediated tumorigenesis in ccRCC, as FBP1 normally inhibits HIF function within the nucleus. Decreased FBP1 expression was shown by Li et al. to correlate with advanced tumor stage and poor prognosis in ccRCC.²¹ PML on chromosome arm 15q negatively regulates HIF1 α translation via repression of mammalian target of rapamycin (mTOR).²² Stabilization of PML by small C-terminal domain phosphatases (SCPs) has been shown to result in suppression of malignant features in ccRCC including tumor proliferation, migration and invasion, and to increase ccRCC response to mTOR inhibitors.²³

Additional tumor suppressor genes of interest located in the chromosome arms more frequently lost in sRCC tumors in this study include the SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1 gene (*SMARCB1*) and checkpoint kinase 2 gene (*CHEK2*) in chromosome arm 22q. *SMARCB1* inactivating mutations are found in rhabdoid tumors, which are aggressive pediatric soft tissue sarcomas

that often arise in the kidney.²⁴ The CHEK2 protein plays an important role in the DNA damage response in RCC as it phosphorylates the von-Hippel Lindau (vHL) protein, which ultimately results in transactivation of p53 and cell cycle growth arrest and apoptosis.²⁵

Proto-oncogenes of interest on chromosome arms 1q and 8q include MUC1 and MYC, respectively. Evidence suggests that sarcomatoid differentiation in RCC is representative of epithelial-mesenchymal transition (EMT) resulting in an increased capability for migration and metastasis.^{26, 27} MUC1 has been shown to be overexpressed in sRCC, resulting in EMT via upregulation of the transcriptional activity of SNAIL, a transcription factor known to activate EMT.²⁸ MYC activation has been demonstrated in ccRCC, where it contributes to tumor proliferation.²⁹ Investigation of all of these genes will help to further elucidate which, if any, contribute significantly to the development of sarcomatoid features, and provide information regarding potential therapeutic targets.

Several limitations of this study deserve mention. SNP-microarray analysis was performed based on the discretion of the surgeon or pathologist. Due to this, the potential for selection bias exists within the cohort examined. CNAs found in non-sRCC tumors in this study however matched up well with those known to occur most frequently in each histology (namely 3p loss and 5q gain in ccRCC, gain of chromosomes 3, 7, 12, 16, 17 and 20 in pRCC, and loss of chromosomes 1, 2, 6, 10, 13 and 17 in chRCC).^{8, 10, 11} This suggests that any selection bias present may be limited, and that the CNAs observed in the sRCC tumors in this study are likely to be representative of those that would be found in sRCC tumors in general.

Another limitation of this study was the small number of chRCC tumors included in the study. Again, the CNAs found most frequently in the few chRCC tumors mirrored those that were expected based on larger cohorts, and the CNAs seen more frequently in sRCC tumors did not overlap those seen in larger chRCC genetic studies.¹¹

Finally, in spite of being the largest study utilizing SNP-based microarrays to examine CNAs in sRCC tumors, the number of sRCC tumors included in the study in absolute terms was small. Combining data from multiple institutions would help to increase the pool of sRCC tumors, and allow for validation of the findings of the current study with more robust numbers.

Despite these limitations, the results of this study provide important insights into distinctive genomic changes in sRCC that may contribute to the development of this aggressive phenotype. Future studies aimed at identifying specific target genes within the chromosome arms altered in sRCC tumors may aid in the development of more effective systemic therapies for this highly aggressive form of RCC.

Conclusions

Sarcomatoid differentiation in RCC is associated with very high numbers of chromosomal imbalances. CNAs more prevalent in sRCC include losses of chromosome arms 9q, 15q, 18p/q, and 22q as well as gains of 1q and 8q. Identification of candidate driver oncogenes or

tumor suppressor loci in these regions may help to identify molecular targets for future therapies.

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Key of Definitions for Abbreviations

CNA	copy number alteration
SNP	single nucleotide polymorphism
sRCC	sarcomatoid renal cell carcinoma
ccRCC	clear cell renal cell carcinoma
chRCC	chromophobe renal cell carcinoma
pRCC	papillary renal cell carcinoma

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Figure 1.

Representative histopathology (left) and SNP array analysis (right) for (a) clear cell renal cell carcinoma and (b) renal cell carcinoma with sarcomatoid differentiation. Red arrows represent copy number losses and green arrows represent copy number gains. In panel (a), a loss of 3p and gain of 5q are seen. In (b), copy number losses of chromosomes 1p, 3p/q, 9, 14q, 17p, 18, 22 and gains of chromosome arms 1q, 3q, 8q, and 12p are seen.

Figure 2.

Copy number alterations found more frequently in sarcomatoid RCC cases in this study included losses of 9q, 15q, 18p/q and 22q, as well as gains of 1q and 8q. This figure maps out the altered segments of each of these chromosome arms on a case-by-case basis, with each line representing extent of loss (lines to left of individual chromosome ideograms) or gain (lines to the right) in an individual sarcomatoid RCC tumor.

Figure 3. Overall survival comparison using Kaplan-Meier analysis. (a) sarcomatoid RCC patients (red) vs. non-sarcomatoid RCC patients (blue), $p=0.0001$. (b) patients whose tumors showed 9 or more copy number alterations (red) vs fewer than 9 copy number alterations (blue), $p=0.004$.

Table 1

Summary of group characteristics separated by tumor histology.

	SARCOMATOID	CLEAR CELL	CHROMOPHOBE	PAPILLARY	P-VALUE
n	17	34	6	24	
Mean number of CNAs (range)	18.0 (8-48)	5.8 (2-14)	7.2 (1-28)	6.5 (1-16)	<0.0001
Most Common Losses	1p (59%), 3p (88%), 4p (35%), 4q (41%), 5q (29%), 6p (35%), 6q (65%), 8p (47%), 9q (88%) , 9q (88%) , 10q (47%), 13q (53%), 14q (65%), 15q (53%) , 18p (47%) , 18q (53%) , 21q (47%), 22q (59%) , Y (29%)	3p (100%), 6q (35%), 8p (26%)	1p (83%), 2 (50%), 6q (83%), 10 (67%), 13 (50%), 17 (67%), 21 (50%), X (50%)	Y (63%)	
Most Common Gains	1q (35%) , 2q (35%), 3q (35%), 5p (39%), 5q (35%), 7 (59%), 8p (29%), 8q (47%) , 12 (35%), 16p (35%), 17q (47%), 20q (59%)	5q (65%)	None	3q (38%), 7 (79%), 12q (29%), 16 (38%), 17 (75%), 20 (50%)	
Percent High Grade Disease	94%	47%	17%	38%	0.02
Non-organ Confined Disease (pT3)	88%	35%	33%	17%	<0.0001
Positive Lymph Nodes on Presentation	47%	3%	0%	8%	0.0004
Metastatic Disease on Presentation	82%	9%	0%	4%	<0.0001
Mean Follow-up (mo)	10.9	19.7	19.5	17.2	0.17

Bolded/underlined CNAs in the sarcomatoid RCC column highlight those CNAs which occur at a significantly higher frequency in sarcomatoid RCC tumors when compared to the other 3 histology types. Abbreviation: CNA = copy number alteration.

Table 2

Summary of significantly different copy number gains and losses seen in sarcomatoid RCC tumors when compared to all non-sarcomatoid RCC tumors as well as to each RCC histology separately.

CNA	sRCC	Non-sRCC	p-value	ccRCC	p-value	chRCC	p-value	pRCC	p-value
9q-	88%	11%	<0.0001	12%	<0.0001	17%	0.003	8%	<0.0001
15q-	53%	3%	<0.0001	0%	<0.0001	0%	0.048	8%	0.003
18p-	53%	11%	0.0005	9%	0.001	0%	0.048	17%	0.02
18q-	59%	9%	<0.0001	9%	0.0003	0%	0.02	13%	0.003
22q-	59%	8%	<0.0001	0%	<0.0001	0%	0.048	21%	0.02
+1q	41%	3%	0.0002	6%	0.004	0%	0.12	0%	0.0009
+8+	47%	2%	<0.0001	0%	<0.0001	0%	0.058	4%	0.002

Abbreviations: CNA = copy number alteration; RCC = renal cell carcinoma; sRCC = sarcomatoid RCC; ccRCC = clear cell RCC; chRCC = chromophobe RCC; pRCC = papillary RCC.