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European Association of Urology

Kidney Cancer

Genomic Characterization of Renal Cell Carcinoma with Sarcomatoid Dedifferentiation Pinpoints Recurrent Genomic Alterations

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Article info

Article history:
Accepted January 26, 2016

Associate Editor:
James Catto

Keywords:
Sarcomatoid
Renal cell carcinoma
Genomic
Sequencing
Profiling
p53

Abstract

Background: The genomic features underpinning renal cell carcinoma with sarcomatoid dedifferentiation (sRCC) are not well understood, and at present, there are no specific or effective therapies for sRCC.
Objective: To identify genomic alterations in patients with sRCC.
Design, setting, and participants: We conducted genomic profiling on paired epithelial and sarcomatoid areas of three sRCC cases. Genomic profiling was performed on another 23 sRCC patients harboring diverse epithelial components (total of 26 cases). Genomic profiling was conducted using a hybrid capture DNA next-generation sequencing assay of 236 cancer-related genes plus 19 genes frequently rearranged in cancer. Results were compared with 56 similarly sequenced cases of clear cell RCC (ccRCC) devoid of a sarcomatoid component, and with clear cell, papillary, and chromophobe renal cell carcinoma datasets from The Cancer Genome Atlas. Four additional ccRCC cases underwent whole exome sequencing.
Outcome measurements and statistical analysis: Genomic alterations in patients with sRCC and ccRCC were described, and their frequencies were compared using the Fisher exact test.
Results and limitations: Two of three patients with sRCC who underwent genomic profiling of both their epithelial and sarcomatoid components demonstrated identical mutational profiles, and a third case demonstrated commonly disrupted genes. Of the 26 sRCCs, *TP53* (42.3%), *VHL* (34.6%), *CDKN2A* (26.9%), and *NF2* (19.2%) were the most frequently altered genes. *NF2* mutations were mutually exclusive with *TP53* but not with *VHL* mutations. Limitations include the small sample size.

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http://dx.doi.org/10.1016/j.eururo.2016.01.051

Please cite this article in press as: Malouf GG, et al. Genomic Characterization of Renal Cell Carcinoma with Sarcomatoid Dedifferentiation Pinpoints Recurrent Genomic Alterations. Eur Urol (2016), http://dx.doi.org/10.1016/j.eururo.2016.01.051

Conclusions: We found that sRCC contains different driver mutations than ccRCC. The epithelial and sarcomatoid components of sRCC largely contain the same genomic features. On the basis of harboring either *TP53* or *NF2* mutations, sRCC can be divided into two groups. These findings may have implications for understanding the oncogenesis of sarcomatoid renal tumors and for defining systemic treatment options.
Patient summary: Next-generation sequencing of tumors from patients with sarcomatoid kidney cancer reveals mutations that differ from those in nonsarcomatoid patients. These findings have implications in understanding the pathobiology of sarcomatoid kidney cancer and indicate the need for a different treatment approach in these patients.

1. Introduction

Renal cell carcinoma with sarcomatoid dedifferentiation (sRCC) is a relatively rare but aggressive form of RCC, occurring in 5% of all RCC cases [1]. More than 75% of patients with sRCC present with metastatic disease [2,3], with a median overall survival <1 yr, with no recent improvements [2,4]. Although sRCC was initially described in 1968 as a separate disease entity, it was subsequently reclassified as a form of RCC, where the sarcomatoid component can coexist with the main epithelial RCC histologies. For patients with sRCC, the International Society of Urological Pathology (ISUP) recommended in 2013 that the underlying epithelial component should be reported as the primary tumor type [5].

With the advent of next-generation sequencing, large-scale retrospective studies of clear cell renal cell carcinoma (ccRCC) have identified frequent inactivating alterations of histone-modifying genes such as *SETD2*, *JARID1C*, and *PBRM1*, as well as a high frequency of *VHL* alterations, in ccRCC [6,7]. *NF2* mutations were reported at the frequencies of 1.7% in the study by Dalgliesh et al [6] and 1% in The Cancer Genome Atlas (TCGA) study [8]. Interestingly, these particular cases were related to ccRCC lacking *VHL* alterations (5 of 357 cases) and without a hypoxia expression phenotype [6]. Other studies have also recently characterized the genomic landscape of non–clear cell (chromophobe) RCC and identified recurrent mutations of *TP53* (32%) and *PTEN* (9%) [9].

Although several recurrent gene mutations have been described in ccRCC and chromophobe RCC, patients with sRCC were theoretically excluded from these landmark studies. Thus the genomic landscape of patients with sRCC remains largely unknown. A few groups have examined candidate genes in small groups of patients. A recent study used RNA sequencing in six patients with sRCC to elucidate differential expression profiles and found aurora kinase-driven mammalian target of rapamycin (mTOR) pathway activation as a potential therapeutic target [10]. Very few studies investigating the genomic makeup of sRCC have been conducted to date.

In the current study, we identified recurrent genomic alterations in sRCC using a genomic profiling assay designed to identify clinically relevant genomic alterations (CRGAs) that suggest a potential benefit from targeted therapy. We initially conducted genomic profiling in three patients using matched epithelial (ccRCC) and sarcomatoid components of sRCC, and then we expanded the study and analyzed the genomic profiles of 23 sRCC cases prospectively assayed in the course of clinical care. Finally, the results were compared with 56 nonsarcomatoid cases for internal validation and with TCGA data for external validation.

2. Patients and methods

2.1. Patients

Ten samples from seven patients treated at The University of Texas MD Anderson Cancer Center (MDACC) for sRCC were used. Formalin-fixed paraffin-embedded (FFPE) tissues from nephrectomies were used (three paired clear cell/sarcomatoid and four sarcomatoid, for a total of 10 samples, all of which were sequenced at Foundation Medicine, Inc. [FMI]). Deidentified genomic profiles of 19 sRCC samples and 56 ccRCC samples assayed at FMI were also included, as described later. Dedicated genitourinary pathologists reviewed all cases, confirmed the presence of sarcomatoid dedifferentiation, and identified the epithelial component in the tumor if present. All patients provided written consent for genetic analysis. Waiver of consent was obtained for patients who were not alive at the time of the study. The MDACC institutional review board approved this study.

2.2. Genomic profiling on DNA extracted from formalin-fixed paraffin-embedded samples

Targeted sequencing was performed on three paired sRCC cases (samples from kidney primary tumor) from MDACC using both the epithelial (clear cell) and sarcomatoid components. In addition, 23 nonpaired sRCC and 56 nonsarcomatoid ccRCC cases were sequenced at FMI at a high coverage of about 700× on average. Experienced pathologists with a subspecialized interest in urologic pathology reviewed all samples. FFPE tissues were collected into extraction tubes and processed. Technical details of the targeted sequencing were as follows. First, hybridization capture was performed for 3230 exons of 236 cancer-related genes and 37 introns from 19 genes commonly rearranged in cancer (Supplementary Table 1) using ≥50 ng of DNA from FFPE samples that were sequenced with high uniform coverage. Second, paired-end sequencing (49 × 49 cycles) was performed by using HiSeq instruments (Illumina, La Jolla, CA, USA) in a Clinical Laboratory Improvement Amendment (CLIA) laboratory (FMI). As described previously [11], sequence data from genomic DNA were mapped to the reference human genome (hg19) by using the Burrows-Wheeler aligner [12] and was processed using SAMtools (http://samtools.sourceforge.net) [13] and Picard (http://picard.sourceforge.net). All classes of genomic alterations including base substitutions, small insertions and deletions (indels), copy number amplifications and deletions, and gene fusions and rearrangements were determined by a custom data analysis pipeline as described previously [11]. Base substitutions and indels were detected by using custom tools optimized for mutation calling in tumor samples on the basis of statistical modeling of sequence quality scores and local sequence assembly. Variations were filtered by using the Single Nucleotide Polymorphism Database 135 and a

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