Original Study

Clinical Relevance of Gene Copy Number Variation in Metastatic Clear Cell Renal Cell Carcinoma

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Abstract

The present study was performed on frozen tissue samples from 50 patients treated for metastatic clear cell renal cell carcinoma. Using the quantitative multiplex polymerase chain reaction of short fluorescent fragment method, several gene copy number variations present in tumor tissue were found to be associated with worse prognostic factors. Specifically, loss of 9p (CDKN2A), 9q (ALDOB), and 6q (PLG) was associated with poor prognosis factors. In addition, these copy number variations were associated with the prognostic factors.

Background: Gene copy number variations (CNVs) have been reported to be frequent in renal cell carcinoma (RCC), with potential prognostic value for some. However, their clinical utility, especially to guide treatment of metastatic disease remains to be established. Our objectives were to assess CNVs on a panel of selected genes and determine their clinical relevance in patients who underwent treatment of metastatic RCC. **Patients and Methods:** The genetic assessment was performed on frozen tissue samples of clear cell metastatic RCC using quantitative multiplex polymerase chain reaction of short fluorescent fragment method to detect CNVs on a panel of 14 genes of interest. The comparison of the electropherogram obtained from both tumor and normal renal adjacent tissue allowed for CNV identification. The clinical, biologic, and survival characteristics were assessed for their associations with the most frequent CNVs. **Results:** Fifty patients with clear cell metastatic RCC were included. The CNV rate was 21.4%. The loss of *CDKN2A* and *PLG* was associated with a higher tumor stage ($P < .05$). The loss of *PLG* and *ALDOB* was associated with a higher Fuhrman grade ($P < .05$). The loss of *ALDOB* was also associated with a worse Heng prognostic score (95% vs. 66%; $P = .029$) and lower 24-month survival rate (18% vs. 58%; $P = .012$). The loss of both *ALDOB* and *PLG* was frequent (32%) and was associated with a higher tumor stage and grade ($P < .05$). **Conclusion:** As expected, we showed that several CNVs were associated with clinical relevance, especially those located on *CDKN2A*, *PLG*, and *ALDOB*, in a homogeneous cohort of patients with clear cell metastatic RCC.

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Introduction

Kidney cancer is the 10th most common malignancy in Western countries with ~270,000 new cases diagnosed annually worldwide. Clear cell renal cell carcinoma (ccRCC) is the most frequent histologic subtype, representing 80% of cases.[1,2] Metastatic disease is observed in approximately one third of cases at diagnosis, and 30% of patients treated for a localized tumor will develop metastatic recurrence during follow-up.[3] During the past decade, the sequential use of targeted therapies (TTs) has become the treatment backbone for metastatic ccRCC, leading to significant improvement in overall survival. More recently, new immunotherapeutic agents have also shown promising outcomes.[4] Although several prognostic

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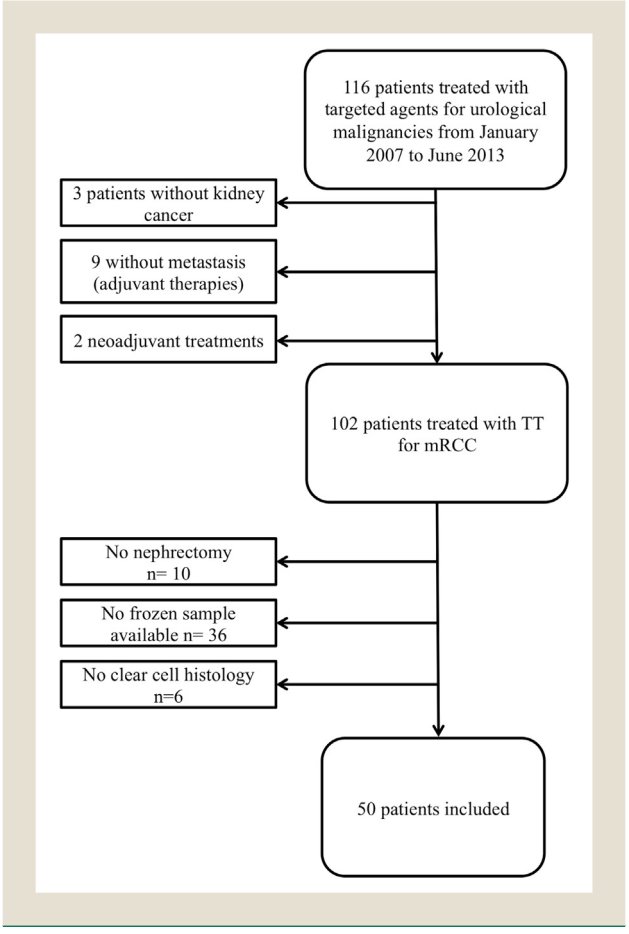
Figure 1 Flow Chart of the Study Population



Abbreviations: mRCC = metastatic renal cell carcinoma; TT = targeted therapy.

models using clinical and pathologic features have been developed, the use of molecular markers that reflect tumor biology has not yet been validated in patients with metastatic ccRCC.[5-7]

Integrated molecular analysis of ccRCC has shown that chromosomal instability (CIN) is a major pathway of carcinogenesis.[8] Using whole genome and exome sequencing, gene array expression, copy number variation (CNV), and methylation analysis on 106 ccRCC cases, CIN and CNV have been found to be involved in several carcinogenesis pathways such as PI3K-AKT-mTOR, cell cycle, and SWI-SNF complex signaling. The most frequent CNVs were loss of 3p (94%), gain of 5q (65%), gain of 7q (41%), loss of 8p with or without loss of 8q (20%), and loss of 9p (25%), 14q (27%), and 18q (11%). Moreover, based on extensive analysis of 10 primary tumors and their corresponding metastases, CNVs located on 3p, 5p, 8p, and 14q were also reported as an early molecular event, which occurred in approximately two thirds of the tumors.[9]

Despite these findings, the clinical relevance of CNV has not yet been clearly established owing to conflicting results and study biases, including a mix of localized and metastatic disease.[10-16] In a study of 77 patients with localized RCC, microsatellite analysis of loss in the region of gene *CDKN2A* (9p) showed that the presence of this CNV was associated with poor outcomes.[17] In a recent study focusing on the effect of 4 ccRCC molecular subtypes in 53 patients with metastatic ccRCC (m-ccRCC), the overall rate of CIN was similar among all groups, with, however, a greater frequency of gain on 2p12, 2p23, and 8q21.13 in the fourth subtype, which was associated with resistance to sunitinib.[18] Taken together, all these results suggest that detection of several main CNVs could play a key role in m-ccRCC. However, to the best of our knowledge, data regarding the CNV characteristics in m-ccRCC patients treated with TTs are scarce.

The objective of the present study was to analyze the characteristics of a panel of 14 CNVs detected in primary tumor tissue from patients with m-ccRCC treated with TTs and to evaluate their clinical relevance.

Patients and Methods

Patients

All patients with m-ccRCC treated at our center with a first-line TT from January 2006 to June 2013 and with available frozen samples of both tumor and nonmalignant tissue were included in the present study (Figure 1). The treatment was sunitinib (50 mg/d for 4 or 6 weeks), sorafenib (800 mg/d), or temsirolimus (25 mg/wk through intravenous perfusion). The patients were systematically followed up on days 1, 14, and 28 of the first cycle and at least monthly during TT exposure.[19-22] All common clinical and biologic baseline data, prognostic scores (Heng and Memorial Sloan Kettering Cancer Center scores[7,23]), histologic characteristics of the resected primary tumor, and patient outcomes were collected retrospectively. The tissue samples were collected immediately after surgical excision of the primary tumor and stored frozen at −80°C. All patients included in the present study provided written informed consent for frozen storage and genetic research on their tissue samples.

Disease progression was defined according to the findings from computed tomography evaluation using Response Evaluation Criteria In Solid Tumors.[24] Overall survival (OS) was defined from treatment initiation to the date of death from any cause. Progression-free survival (PFS) was defined as the interval from treatment initiation to the date of disease progression or treatment discontinuation.

Selection and Analysis of CNV Panel

A panel of CNV was designed to include the most frequently reported CNVs involved in ccRCC carcinogenesis, namely loss of 3p, 9p, 14q, and gain of 5q.[10,11,14-16] We also integrated the detection of 10 other CNVs according to their potential clinical relevance and a reported frequency of ≥ 10%: loss of *KIF1B* (1p36.2), *PDCD1* (2q37.3), *VHL* (3p25.3), *PLG* (6q26), *CDKN2A* (9p21.3), *ALDOB* (9q21.3), and *SLC7A8* (14q11.2) and gain of *MCM2* (3q21.3), *LPCAT1* (5p15.33), *PDGFRB* (5q33.1), *PTTG1* (5q35.1), *EGFR* (7p11.2), *MYC* (8q24.21), and *CDK4* (12q13.2-q14.1; Supplemental Material; available in the online version).

CNVs were detected using the somatic quantitative multiplex polymerase chain reaction (PCR) of short fluorescent fragment (QMPSF) method, as previously described.[25,26] In brief, the QMPSF method is based on the simultaneous amplification of short target genomic sequences using dye-labeled primers. PCR was performed with DNA extracted from both frozen tumor and normal tissue samples. For each patient, electropherograms obtained