Published in final edited form as:

Clin Genitourin Cancer. 2016 October ; 14(5): e515–e520. doi:10.1016/j.clgc.2016.03.019.

Circulating Tumor DNA as a Marker of Therapeutic Response in Patients With Renal Cell Carcinoma: A Pilot Study

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Keywords

Biomarker; Cell-free DNA; Kidney cancer; Next-generation sequencing; Treatment response

Introduction

Systemic therapies for metastatic renal cell carcinoma (RCC) have increased dramatically during the past several years.¹ Despite these therapeutic advances, no serum biomarkers are available to aid in treatment selection or to judge the response to therapy. Thus, patients undergoing active treatment of RCC have been followed up with cross-sectional imaging studies to judge the response. However, in the era of targeted and immune-based therapies, some have called into question the prognostic accuracy of this approach.² Additionally, early markers of tumor response would be welcome in a terrain of multiple therapies to optimize the sequencing of effective therapies and eliminate ineffective ones early in the treatment course. Circulating tumor DNA (ctDNA) is cell-free DNA derived from apoptotic or necrotic primary tumor cells, secretions from macrophages, or circulating tumor cells (CTCs).³ ctDNA is an inherently specific biomarker that is easily obtained from the peripheral blood, and it has been shown to be present in patients with multiple solid malignancies.⁴ In colorectal and breast cancer, it has been shown to correlate with tumor dynamics⁵ and disease-specific survival⁶ and to provide earlier evidence of response to treatment than conventional computed tomography imaging, CTCs, or conventional biomarkers.⁷ Previous studies have demonstrated the presence of cell-free hypermethylated DNA⁸ and non-tumor-derived DNA fragments⁹ in patients with RCC; however, no studies have correlated ctDNA with disease burden or therapy response. We sought to characterize

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Disclosure

L.A. Diaz is on the management team of and has a financial interest in Personal Genome Diagnostics, which performed the analysis for the present study. The remaining authors declare that they have no competing interests.

Supplemental Data

The Supplemental Table accompanying this article can be found in the online version at <http://dx.doi.org/10.1016/j.clgc.2016.03.019>.

the presence of ctDNA in patients with locally advanced or metastatic RCC and to correlate ctDNA with disease burden.

Sample preparation, next-generation sequencing, and digital polymerase chain reaction (PCR) were performed by Personal Genome Diagnostics (Baltimore, MD). In brief, DNA was extracted from formalin-fixed, paraffin-embedded tissue (DNA formalin-fixed, paraffin-embedded tissue kit; Qiagen, Venlo, The Netherlands), and targeted next-generation sequencing using the CancerSelect-R 203 gene panel (see Supplemental Table 1 available in the online version) was performed to identify the known mutations in 4 tumor specimens. The clinicopathologic characteristics of the samples are listed in Table 1.

Targeted sequencing identified unique mutations in RCC-related genes in all 4 patients (*VHL* in 1 patient, *BAP1* in 1 patient, *PBRM1* in 1 patient, and *NF* in 1 patient). In each patient, the specific identified mutation was then selected for ctDNA analysis. Plasma was collected prospectively in all patients before nephrectomy. The patients with detectable ctDNA underwent serial plasma collections. Circulating cell-free DNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (catalog no. 55114; Qiagen). The purified DNA concentration was determined using the Qubit dsDNA HS Assay Kit (catalog no. Q32854; Life Technologies). All extracted DNA was analyzed using droplet digital PrimePCR custom assays (BioRad, Hercules, CA), and the mutant fractional abundance was calculated as a percentage of the total DNA present.

PCR failed to detect the selected mutant alleles in 3 of the 4 cases; however, ctDNA was detected in 1 patient with metastatic disease with a substitution mutation resulting in a splice site donor of *VHL*. Notably, this patient had the largest primary tumor (13.5 cm) and the greatest pretreatment disease burden (30.4 cm summed length diameter). In this patient, the ctDNA decreased to undetectable levels after nephrectomy and the initiation of systemic therapy gemcitabine and sunitinib. However, the ctDNA level increased with disease progression. The dynamics of ctDNA correlated directly with the disease burden on computed tomography (Figure 1).

Discussion

In the present initial report, we detected ctDNA in only 1 of 4 patients. Several reasons are possible for the low detection rate. First, RCC can demonstrate genetic heterogeneity, even within a single lesion¹⁰; thus, the tumor area sequenced could have different mutations than those of the clone that ultimately entered into the circulation. Similarly, the mutations against which we designed the PCR primers might have been branch mutations, rather than truncal mutations, representing only a subpopulation of tumor cells. Indeed, the only sample in which ctDNA was detected was when a truncal mutation, *VHL*, was targeted.¹⁰ Finally, the low detection rate might not have been an artifact but a reflection of the biology of the RCC. Gorin et al¹¹ recently demonstrated that only 27% of patients with metastatic RCC have detectable CTCs.

The major limitation of the present study was the small sample size. Future work will focus on expanding our cohort and optimizing ctDNA detection. From the present findings,

recruitment will focus on patients with a high tumor burden, and truncal mutations will be targeted in our future ctDNA analysis. Other limitations included sampling only 1 tumor area for sequencing and selecting only 1 mutation. However, if truncal mutations are selected, this limitation should not limit ctDNA detection. Finally, using a cancer gene panel rather than whole exome sequencing was a limitation. In this panel, although many of the most common RCC genes, such as *VHL*, *PBRM1*, *BAP1*, and others, were included, still others, such as *SETD2*, *KDM5C*, and *MTOR*, were not included.

Conclusion

In the present pilot study, ctDNA was detected in 1 of 4 patients (25%). In patients with detectable ctDNA at baseline, the ctDNA dynamics might correlate with the tumor burden. Future directions will work to optimize the detection of ctDNA in patients with metastatic RCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The present study was funded by the National Institutes of Health (Grant P30CA006973) and the Buerger Family Scholar fund.

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Clinical Practice Points

- Currently, no serum biomarkers are available for renal cell carcinoma (RCC).
- Circulating tumor DNA (ctDNA) has been shown to correlate with advanced disease and the response to therapy in several solid malignancies; however, little is known about the presence of ctDNA in patients with RCC.
- We characterized the presence of ctDNA in patients with locally advanced and metastatic RCC.
- Next-generation sequencing using a panel of 203 candidate genes identified tumor-specific mutations in the 4 queried tumor specimens. One of the identified mutations per patient was then selected and queried in the plasma.
- Serum polymerase chain reaction detected mutant ctDNA in 1 patient with metastatic disease, with a substitution mutation resulting in a splice site donor of *VHL*. In this patient, the ctDNA burden decreased after nephrectomy and the initiation of systemic therapy but increased with disease progression, indicating the potential utility of ctDNA as a marker of tumor response in select patients with RCC patients.
- Future directions will work to optimize the detection of ctDNA in patients with metastatic RCC.

Figure 1.
Correlation of Tumor Burden With Circulating Tumor DNA in 1 Patient With Metastatic Renal Cell Carcinoma

Table 1

Clinicopathologic Characteristics

Characteristic	Pt. No.			
	1	2	3	4
Gender	Male	Female	Male	Male
Age (years)	66	67	67	48
Stage	T3bN0M1	T3bN0M0	T1bN0M1	T3aN0M1
MSKCC risk group	Intermediate	NA	Intermediate	Intermediate
Primary tumor diameter	6.2	5.2	5.0	13.5
SLD	14.7	5.2	7.0	30.4
Gene	NF1	BAP1	PBRM1	VHL
Mutation in tumor	chr17_26587107-26587107_C_T	chr3_52417063-52417063_C_A	chr3_52626553-52626559_TTGAATA_	chr3_10158873-10158873_T_C
Consequence	Nonsense	Nonsynonymous coding	Frameshift	Splice site donor
Fraction mutant allele at baseline	ND	ND	ND	8.8%
Follow-up (mo)	6	53	25	10
Status at last follow-up visit	DOD	AWD	NED	DOD

Abbreviations: AWD = alive with disease; DOD = dead of disease; MSKCC = Memorial Sloan Kettering Cancer Center; NA = not available; ND = not detected; NED = no evidence of disease; Pt. No. = patient number; SLD = summed length of tumor diameter.