Circulating Tumor DNA (ctDNA) in Kidney Cancer: A Narrative Review

Wesley Yip, A. Ari Hakimi✉

Memorial Sloan Kettering Cancer Center, New York, United States

Abstract

Circulating tumor DNA (ctDNA) has been investigated as a potential noninvasive biomarker for disease prognostication, monitoring, and treatment selection in various tumor types, including renal cell carcinoma (RCC). In this narrative review, we explore the current methods of ctDNA analysis and the use of ctDNA in both localized and metastatic RCC, focusing on plasma and urine samples. Additionally, we discuss several ongoing as well as upcoming clinical trials that incorporate ctDNA analyses into their study designs and outcomes. Despite the exciting potential of ctDNA in RCC, current assays still face significant limitations in sensitivity and detection limits.

Introduction

Kidney cancer ranks as the sixth most common cancer in men and the ninth most common in women[1]. Although most cases of renal cell carcinoma (RCC) present with localized disease, 25% to 50% of these patients eventually develop metastases[2]. Approximately 30% have metastatic disease at initial presentation[3]. Survival outcomes differ significantly between localized and metastatic disease, with overall survival ranging from 6 months to 5 years in the latter group[4]. Given these discrepant outcomes, accurate risk-stratification and disease prognostication are crucial for appropriate management. The Memorial Sloan Kettering Cancer Center (MSKCC)[5] and International Metastatic RCC Database Consortium (IMDC)[6] models are commonly used prognostic tools in the metastatic setting and were developed from retrospective studies examining clinical and laboratory factors in patients treated with systemic therapy. However, with an increasing number of investigations on the molecular landscape of RCC[7], these factors are not included in classic prognostic models. Moreover, while a multitude of therapeutic options are now available for advanced disease, there is minimal guidance for treatment selection.

Circulating tumor DNA (ctDNA) has emerged as a noninvasive method that can potentially influence both the diagnosis and treatment of RCC, offering an alternative or complement to tissue biopsies[8]. Liquid biopsies can be performed serially over the course of treatment and may better represent the tumor profile as a whole, compared to a single biopsy section, which can be limited by spatial heterogeneity within tumors[9,10]. A proposal exists to potentially incorporate ctDNA detection into standard TNM staging[11]. In this review, we discuss the current methods of ctDNA evaluation, the use of ctDNA in localized and metastatic settings, and future directions in urine-based studies and clinical trial correlatives.

ctDNA Overview

Human blood contains various materials, including cell-free nucleic acids (DNA and RNA), proteins, cells, and exosomes, which can be measured as a "liquid biopsy." These materials can originate from both benign and malignant sources, but the rapid turnover of malignant cells and the shedding of viable tumor cells from the tumor itself may increase the detection of these circulating tumor cells[12]. The half-life of ctDNA in the circulation is approximately

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Competing Interests

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Abbreviations

cfDNA cell-free DNA
NAC neoadjuvant chemotherapy
NMIBC non-muscle invasive bladder cancer

2 hours, compared with 16 minutes for nonmalignant cell-free DNA[13,14]. In an initial study evaluating cell-free DNA detection in RCC, 35 patients with RCC (all stages) were compared with 54 healthy controls. The study used quantitative real-time polymerase chain reaction (PCR) with two primer sets targeting a sequence of the actin-beta gene (ACTB). One primer set amplified both short and long DNA fragments (ACTB-106), while the other set amplified only long DNA fragments (ACTB-384). ACTB-106 levels represented DNA of apoptotic origin, whereas ACTB-384 levels indicated DNA from nonapoptotic cells. The study found that median ACTB-106 levels were two times higher and ACTB-384 levels were three times higher in RCC patients compared to healthy controls[15].

Several assays are available for assessing ctDNA, which typically target a small number of variants or aim for broad coverage[16]. Targeted assays can be useful for detecting specific variants associated with drug response and often use PCR, while broad coverage assays can detect large numbers of variants across multiple genes and typically use next-generation sequencing (NGS). It is crucial to draw blood samples into cell stabilization or EDTA tubes and process them within 6 hours of collection to avoid lysis of white blood cells and subsequent ctDNA dilution by normal leukocyte DNA[17]. NGS methods do not require prior knowledge of molecular mutations but are most expensive, time-consuming, and prone to false positives from sequencing artifacts. However, they are also more sensitive than PCR and can be more efficient by running multiple tests[18]. In addition, NGS may be better suited for assessing tumor mutational burden (TMB) compared to candidate gene analysis[19].

Clinical Applications—Localized

As CpG island hypermethylation has been found widely in cancer cells, Hauser et al. investigated hypermethylation in cell-free DNA in 8 genes (APC, GSTP1, ARF, p16, RAR-B, RASSF1, TIMP3, and PTGS2) known to be involved in RCC development and progression. Thirty-five patients with organ-confined RCC were compared to 54 healthy controls, and at least one gene was found to be methylated within serum cell-free DNA in 30 of 35 patients. Most genes were more frequently methylated in RCC patients than in the controls, with the exception of p16 and TIMP3. Combining the analysis of multiple genes increased diagnostic sensitivity[20]. Thus, CpG island hypermethylation could potentially have a role in the initial diagnosis of RCC.

In another study examining cell-free DNA methylation profiling, Nuzzo et al. used a cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) assay with high sensitivity in both localized and metastatic disease across a range of tumor types[21]. The authors analyzed 148 samples, of which 99 were RCC (88 clear cell, 11 papillary), 21 were bladder cancer, and 28 were from healthy controls. Of the RCC samples, 69 were derived from plasma, with 23 of these from patients with stage I-II disease. The investigators identified differentially methylated regions between patient groups and constructed a classifier based on the top 300 regions to assign a methylation score. They found that 97% of RCC samples had a higher median methylation score compared to control samples. While localized cases exhibited the lowest methylation scores, there was no statistically significant association between stage and methylation score ($P = 0.09$)[22].

In a study using a targeted deep sequencing platform, Kim et al. profiled genetic alterations from plasma and tumor tissue samples from 20 patients with RCC (10 localized, 10 metastatic) who underwent nephrectomy. Plasma samples exhibited variants in 40% of localized samples and 50% of metastatic samples, while tissue samples showed variants in 80% of localized samples and 70% of metastatic samples. The mutation patterns did not differ significantly between localized and metastatic samples. However, the investigators found that 53% of patients with mutations in tumor tissue had corresponding mutations in plasma samples across the entire cohort. Of the patients with metastatic disease, this concordance percentage was 71%, with the most common mutations occurring in *VHL, PBRM1,* and *KDM5C*. Patients with RCC demonstrated a higher median value of cell-free DNA compared to healthy controls ($P < 0.03$), but ctDNA levels could not differentiate between localized and metastatic disease[23].

Wan et al. evaluated 92 patients with clear cell RCC and measured plasma cell-free DNA levels before and after surgery using quantitative PCR. They found that patients with metastatic disease had significantly higher pretreatment levels than those with localized disease. Moreover, of patients with localized disease, those who experienced recurrence had higher pretreatment levels compared to those without recurrence[24].

Clinical Applications—Advanced

Pal et al. conducted a study involving 220 patients with advanced RCC who underwent ctDNA assessment using the Guardant360 platform, which encompasses 73 cancer-related genes. The authors found that 79% of patients had genomic alterations, with a median of one (interquartile range [IQR], 0-3) alteration per patient. The most frequent alterations included TP53, VHL, EGFR, NF1, and ARID1A. Patients receiving targeted therapy following first-line treatment had a higher mutation frequency compared to those who had first-line therapy alone, particularly for TP53, VHL, EGFR, NF1, and PIK3CA[25]. Notably, the frequency of several of these alterations was much higher than in prior tissue-based studies, such as The Cancer Genome Atlas (TCGA), although this may be due to a lower proportion of advanced disease in the TCGA cohort. However, mutations in several significant genes[26], such as PBRM1, BAP1, and KDM5C, were not included in the Guardant360 panel.

There may also be an association between ctDNA and RCC tumor burden. In a study of 34 patients with metastatic RCC, detectable ctDNA was found to be associated with a higher sum of longest diameter of measurable lesions, a surrogate for tumor burden[27]. A similar finding was reported in the study by Kim et al., where a statistically significant correlation ($P = 0.048$) was found between ctDNA and tumor burden based on primary tumor diameter on CT scans at the time of diagnosis[23].

Concordance with tissue-based genomic profiling was investigated by Kotecha et al. The study evaluated the cell-free DNA profiles of 110 patients with metastatic RCC compared to tumor tissue using established panel-based assays (cfDNA-IMPACT[28] and MSK-IMPACT[29], respectively). Tissue testing revealed 554 genomic alterations overall, of which 60% were from primary tumors and 40% from metastatic sites. The median number of genomic alterations per patient was 4, and all patients had at least one alteration. The most commonly identified alterations included VHL, PBRM1, SETD2, BAP1, and TP53. However, only 24 alterations were detected in 7 of 110 patients (6%) using standard thresholds. In a repeat analysis using expanded detection thresholds, the authors found 210 alterations in 74 of 110 patients (67%), predominantly in VHL, PBRM1, and TP53. The median time between tissue and blood analysis was 23 months (IQR 14 to 48)[30].

The time elapsed between assays may be important, as determined by Zengin et al. In their study of 839 patients with advanced RCC and ctDNA testing, the authors found that 112 patients also had tissue-based genomic alterations assessed by either a targeted NGS panel (FoundationOne or Tempus xT) or whole-exome sequencing. ctDNA assessment revealed at least one genomic alteration in 72% of patients, with TP53, VHL, and TERT being the most frequently mutated genes. Tissue samples showed that VHL, PBRM1, and SETD2 were the most commonly mutated genes. Of note, several commonly mutated genes in tissue samples were not assessed in the ctDNA panels (eg, PBRM1, SETD2, and KDM5C). In patients with gene mutations detected in both ctDNA and tissue, 34% of the alterations in tissue were also found in ctDNA. The percentage increased to 51% when samples were collected within 6 months of each other and further increased to 61% when collected within 3 months of each other[31].

ctDNA may find utility in disease monitoring and prognostication, as demonstrated in a study of 53 patients with clear cell RCC from the Osaka University Hospital. Thirty-nine of these patients had metastatic disease, of which 13 were untreated at the time of ctDNA analysis. The study investigators found that changes in mutant allele frequency of ctDNA closely mirrored the overall tumor burden over the course of treatment. Moreover, patients with short fragment sizes of cell-free DNA and/or positive ctDNA had a worse response to tyrosine kinase inhibitors compared to those with long fragment sizes and/or negative ctDNA. The size of cell-free DNA fragment size and ctDNA status showed a statistically significant correlation with cancer-specific survival but not progression-free survival[32].

The potential of ctDNA to predict tumor response to treatment is of particular interest, but limited data is available. A small study that followed 4 patients who received ipilimumab/nivolumab for metastatic RCC found that 3 patients had detectable ctDNA levels prior to treatment. Two of these patients had decreased ctDNA levels, along with a partial response to immune checkpoint inhibition. One of these patients with a partial response had a TP53 mutation, and the other had MTOR and ARID1A mutations. The remaining patient, who progressed during treatment, had increased levels of ctDNA over the course of therapy, with mutations in TP53, VHL, and PIK3CA[23]. The authors suggest that ctDNA may have efficacy as an early predictor of immune checkpoint blockade response, although the study's small size limits its conclusions.

Future Directions

Several groups are exploring the possibility of assessing ctDNA through urine, providing an even less invasive form of liquid biopsy. In a multicenter study involving 91 patients with renal tumors of all stages, urine samples were collected from 37 patients prior to surgery. The study found low overall ctDNA detection in plasma at 28% using an untargeted assay, which improved only to 55% with a targeted method. ctDNA was detected in urine in only 22% of patients. While plasma ctDNA detection correlated with lesion size and/or tumor thrombus, urine ctDNA detection did not. However, the study suggests that ctDNA results may overcome intratumoral heterogeneity in patients with detectable ctDNA in either plasma or urine[33].

In the study by Nuzzo et al., cfMeDIP-seq was performed on 30 urine samples from patients with RCC, with the majority of patients being at stage I–II (67%). When comparing the urine cell-free DNA in these patients to healthy controls, the authors found a mean area under receiver operating characteristic curve of 0.86 (95% CI 0.83 to 0.89). Although these results are not as accurate as those from plasma, the authors believe that the assay performance can be enhanced by enriching for tumor-derived DNA and incorporating tumor methylation data into the analysis[22].

Clinical Trials

Completed

NCT02960906: The BIOmarker Driven Trial with Nivolumab and Ipilimumab or VEGFR tKi in Naïve Metastatic Kidney Cancer (BIONIKK) was a randomized phase 2 study involving 202 patients with treatment-naïve metastatic RCC who received nivolumab, ipilimumab/nivolumab, or TKI based on molecular subgroups. Among the exploratory outcome measures, the study aimed to determine whether the mutation and methylation status of ctDNA can be correlated with the clinical evolution and progression-free survival rates. The primary endpoint of investigator-assessed objective response rates per molecular/treatment group has been reported, while the results of the exploratory analyses, including ctDNA, are planned for future publication[34].

NCT03469713: In the phase 2 study of nivolumab and stereotactic body radiotherapy in patients with metastatic RCC (NIVES), 69 patients were enrolled and received nivolumab for at least 6 months with 30 Gy of radiation in 3 consecutive fractions (10 Gy per fraction) to a metastatic site. A planned exploratory analysis is evaluating *JAK1*, *JAK2*, and *B2M* mutations in ctDNA from plasma samples, as these may be associated with acquired resistance to checkpoint inhibition. Similar to the BIONIKK trial, the primary outcomes of the NIVES trial have been published[35], but the ctDNA data are eagerly awaited.

Recruiting

NCT05059444: Guardant Health is currently recruiting approximately 1000 patients with a wide range of cancer types including RCC to their Observation of ResiduAl Cancer with Liquid Biopsy Evaluation (ORACLE) study to determine whether their Guardant Reveal assay can be used for detecting recurrences of early-stage solid tumors. The study is recruiting patients with high-risk RCC, defined as grade 3–4 and stage II-IV who are being treated with curative intent. Patients with limited/resectable distant metastases are also eligible for inclusion.

NCT04295174: The KIDSTAGE observational study conducted by Odense University Hospital has completed patient recruitment. The study uses positron emission tomography/computed tomography (PET/CT) and ctDNA for monitoring tumor burden and disease progression in patients with RCC of any stage. The primary outcomes include investigating the utility of ctDNA for disease monitoring and whether dual time point fluorodeoxyglucose (FDG) PET/CT can be used for staging. The goal of 70 patients recruited has been met, and with a study timeframe of 3 years, the results are expected soon.

NCT03786796: The ORCHID phase 2 study of olaparib plans to enroll 20 patients with metastatic RCC harboring specific DNA repair gene mutations who have received prior treatment with either an immune checkpoint inhibitor or anti-VEGF therapy. The primary outcome measure is objective response or stable disease at 6 months, and ctDNA reversion mutations at clinical progression will also be evaluated.

NCT05329532: The phase 1/2 study of the Modi-1/Modi-1v vaccine (ModiFY) is recruiting patients with triple negative breast cancer, human papillomavirus–negative head and neck squamous cell carcinoma, high-grade serous ovarian carcinoma, or RCC and evaluating treatment as monotherapy and in combination with immune checkpoint inhibitor therapy. ctDNA will be measured throughout the study and up to 12 weeks after the final treatment dose.

NCT04609293: An observational study in China aims to evaluate the efficacy of combination therapy with camrelizumab, apatinib, and hypofractionated radiotherapy in patients with locally advanced, metastatic, or recurrent RCC. Secondary outcome measures include ctDNA analysis and whole exome sequencing of the primary tumor before and after treatment.

Conclusion

ctDNA holds promise as a noninvasive biomarker for disease prognostication, monitoring, and treatment selection. It may also provide valuable insights into changes in tumor profiles over time and overcome intratumoral heterogeneity. However, improvements in assay design are needed to increase sensitivity and detection limits. Prospective evaluation of ctDNA is being investigated through correlative studies in ongoing and upcoming clinical trials.

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