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Commentary

Liquid biopsy in renal cell carcinoma

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Liquid biopsy has emerged as a powerful, noninvasive tool for the detection and monitoring of cancer. It relies on identifying tumor markers, such as circulating tumor cells (CTCs), cell-free tumor DNA (ctDNA), cell-free RNA (cfRNA), exosomes, as well as tumor-derived metabolites and proteins in biofluids.[1] Prior studies in individuals with renal cell carcinoma (RCC) have reported negative correlations between ctDNA levels and clinical outcomes, including cancer-specific survival,[2] progression-free survival (PFS),[2,3] and overall survival (OS).[3] In this issue of *The Oncologist*, Correa et al build upon these foundations using a bespoke whole-exome sequencing (WES)-informed ctDNA assay (Signatera RUO, Natera, Inc.), demonstrating its prognostic value for RCC recurrence in patients who underwent nephrectomy.[4] The authors conducted deep WES on formalin-fixed, paraffin-embedded (FFPE) tumor samples and matched normal blood samples from 45 patients to identify somatic single nucleotide variants present in the tumor but not in germline DNA. By assaying cell-free DNA for these patient-specific, bespoke panel of mutations, the authors detected ctDNA in 18 of 36 (50%) patients tested preoperatively and in 12 of 45 (27%) after nephrectomy. Preoperative ctDNA detection was not correlated with disease grade but was associated with a larger primary tumor. The authors showed that the pre- and post-operative detection of ctDNA was significantly associated with a shorter recurrence-free survival (hazard ratio (HR) 2.7, $P = 0.046$ and HR 3.23, $P = 0.003$, respectively). These findings represent a step toward guiding the use of adjuvant therapies in patients who are at high risk of relapse or not cured by nephrectomy based on ctDNA levels. However, despite employing a bespoke approach optimized for sensitivity, the ability to detect ctDNA was limited. Only 27% (12 of 45) of patients had ctDNA detected at any time after surgery in a population that ended up with a 60% rate (27 of 45) of established recurrences. A warranted question thus emerges: could emerging liquid biopsy technologies improve sensitivity by looking beyond somatic mutations?

The state of liquid biopsy in kidney cancer

ctDNA

Clinical ctDNA assays, including the one employed by Correa et al, have largely focused on detecting somatic mutations, which in some cancers can provide prognostic and predictive information to guide therapy. However, the application of ctDNA assays in RCC has been challenging, mainly due to the low levels of ctDNA shed into the bloodstream in RCC. Previous efforts using tumor-agnostic assays had achieved detection rates of up to 40% only, even in cases of widely metastatic disease.[2,5] The detection of ctDNA also depends on parameters such as the sequencing depth, the size of the gene panel used, and the technical sensitivity of the assay, which may not enable the detection of variants present at a very low variant allele frequency.[6-8] In one of the most extensive evaluation of ctDNA in metastatic RCC (mRCC), Zengin et al identified genomic alterations in 71.8% of 920 samples.[9] However, this study did not rule out the presence of germline and clonal hematopoiesis variants, both of which are relatively common in mRCC and may contribute to false positive findings in cfDNA variant analysis.[10] ctDNA-level dynamics have also being explored and found to be associated with tumor load, progression, and response to therapy.[11,12] For instance, using a bespoke assay, Chehrazi-Raffle et al accurately differentiated partial and complete responses among patients with mRCC receiving immunotherapy.[12]

In the emerging field of "fragmentomics," the fragmentation patterns of cfDNA have also been investigated as diagnostic and prognostic markers in patients with RCC. Two studies by Yamamoto et al revealed that shorter cfDNA fragments were associated with a shorter PFS ($P = .004$ and $P = .006$, respectively).[2,13] Notably, in patients with mRCC, the cfDNA mutation status, fragment size, and proportion of cfDNA fragments were all associated with prognosis ($P = .010$, $P = .011$, and $P = .007$, respectively). However, these associations were not observed in another high-risk group.[2] Using a machine-learning approach for examining fragmentomics in a standard cfDNA targeted cancer gene panel, Taylor et al obtained a 10-fold cross-validation AUC of 0.93 for identifying RCC in a cohort of patients with metastatic cancers.[14]

Epigenetic approaches to liquid biopsy

Epigenetic approaches have recently gained traction, in part because they may be more sensitive than mutation-based approaches. The epigenome offers a large set of features that can distinguish ctDNA from cell-free DNA released by

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Table 1. Comparison of liquid biopsy approaches in renal cell carcinoma.

Techniques	Advantages	Limitations
Tumor-agnostic ctDNA assays	Mature technologies in clinical use Fairly cost-effective	Paucity of actionable mutations Limited sensitivity at low tumor fraction
Bespoke ctDNA assays	Higher sensitivity enabled by knowledge of patient-specific mutations.	Requires tumor tissue to identify patient-specific mutations Paucity of actionable mutations
Epigenetic approaches	May provide actionable information beyond mutations, including histologic subtypes and gene expression DNA methylation profiling may improve sensitivity	Maturing technology, additional benchmarking of sensitivity required Higher cost Does not identify actionable mutations
Circulating tumor cells	Direct sampling of tumor cells	Challenging detection especially in early stage High cost Requires specialized technology

nonmalignant cells. For instance, Nuzzo et al demonstrated that the analysis of the cfDNA methylome from plasma and urine has the potential to identify patients with RCC, including patients with early-stage disease.[15] Moreover, Lasseter et al showed that cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq) was able to detect all 40 of their mRCC cases, with an 88% specificity in 34 control subjects.[10] More recently, a proof-of-concept work by Baca et al profiled histone modifications in plasma using cell-free chromatin immunoprecipitation sequencing (cfChIP-seq). Histone modifications are key features of the epigenome that contribute to gene regulation. cfChIP-seq enabled the investigators to infer activity of therapeutically targetable transcription factors from plasma, including HIF2α in clear cell RCC.[16] In addition, El Zarif et al used cfChIP-seq in a small proof-of-concept study to detect RCC with sarcomatoid differentiation, a clinically actionable, aggressive phenotype where immunotherapy is standard.[17] These studies suggest that epigenomic techniques are promising methods to detect RCC and may also provide phenotypic information such as histologic subtypes and activity of targetable genes and TFs, both of which are overlooked in ctDNA variant analysis. Validation of these observations in larger studies and prospective cohorts is warranted.

Other approaches

Liquid biopsy techniques are not limited to the analysis of ctDNA. Markers such as serum and urinary cfRNA, as well as CTCs, have been investigated as biomarkers of patient survival. Serum miR-122-5p and miR-206 levels (both $P < .005$) were found to correlate significantly with higher grade and stage, as well as shortened OS.[18,19] Urinary miR-328-3p levels ($P = .042$) similarly associated with shorter survival. Additionally, through a process known as cellular seeding, primary and metastatic tumors may excrete cells into peripheral blood. These CTCs have the potential to migrate to secondary sites and promote tumor metastasis and progression.[20,21] Basso et al provided prospective evidence indicating that patients with mRCC treated with tyrosine kinase inhibitors, who had 3 or more CTCs at baseline, experienced a significantly shorter median OS of 13 months compared to 52.8 months for patients with fewer than 3 CTCs. However, CTC counts did not correlate with treatment response.[22] One limitation of using CTCs as a biomarker is that their detection is often challenging when the burden of disease is low, which may limit their utility for early cancer detection and recurrence monitoring.[23] Furthermore, proteomic analyses have revealed associations between the levels of some circulating proteins and prognosis. Kidney Injury Molecule-1 (KIM-1), for instance, was found to be a biomarker of minimal residual disease and is prognostic of recurrence in patients with RCC.[24]

The path to clinical utility

Liquid biopsy techniques are starting to identify genomic, transcriptomic, and epigenomic features which may eventually help guide treatment of RCC. For instance, if these approaches become sufficiently sensitive to detect minimal residual disease, they could identify patients who benefit most from adjuvant immunotherapy,[25] following paradigms in urothelial and colorectal cancers.[26,27] However, current ctDNA bespoke approaches, including the one employed by Correa et al, may still not be sufficiently sensitive to distinguish patients who are cured with surgery from those with minimal residual disease where adjuvant immunotherapy may be helpful. Moreover, the variability in assays without benchmarking on a uniform set of samples makes it difficult to compare findings across studies. Finally, given the paucity of actionable mutations, the utility of liquid biopsy in RCC may be enhanced by employing epigenetic approaches that may be able to infer actionable, non-mutational features, such as histologic subtypes, sarcomatoid differentiation, and HIF2α activity. Table 1 summarizes the characteristics of different liquid biopsy techniques. Finally, future efforts should focus on the integration of liquid biopsy into clinical trials to test clinical validity and utility in the management of RCC.

Conflicts of interest

Sylvan Baca is a co-founder and shareholder of Precede Biosciences. Toni K. Choueiri reports institutional and/or personal, paid and/or unpaid support for research, advisory boards, consultancy, and/or honoraria past 5 years, ongoing or not, from: Alkermes, Arcus Bio, AstraZeneca, Aravive, Aveo, Bayer, Bristol Myers-Squibb, Calithera, Circle Pharma, Deciphera Pharmaceuticals, Eisai, EMD Serono, Exelixis, GlaxoSmithKline, Gilead, HiberCell, IQVA, Infinity, Ipsen, Jansen, Kanaph, Lilly, Merck, Nikang, Neomorph, Nuscan/PrecedeBio, Novartis, Oncohost, Pfizer, Roche, Sanofi/Aventis, Scholar Rock, Surface Oncology, Takeda, Tempest, Up-To-Date,

CME events (Peerview, OncLive, MJH, CCO and others), outside the submitted work. Institutional patents filed on molecular alterations and immunotherapy response/toxicity, and ctDNA. Equity: Tempest, Pionyr, Osel, Precede Bio, CureResponse, InnDura Therapeutics, Primium Committees: NCCN, GU Steering Committee, ASCO (BOD 6-2024-, ESMO, ACCRU, KidneyCan). Medical writing and editorial assistance support may have been funded by Communications companies in part. No speaker's bureau. Mentored several non-US citizens on research projects with potential funding (in part) from non-US sources/Foreign Components. The institution (Dana-Farber Cancer Institute) may have received additional independent funding of drug companies or/and royalties potentially involved in research around the subject matter. T. K. Choueiri is supported in part by the Dana-Farber/Harvard Cancer Center Kidney SPORE (2P50CA101942-16) and Program 5P30CA006516-56, the Kohlberg Chair at Harvard Medical School and the Trust Family, Michael Brigham, Pan Mass Challenge, Hinda and Arthur Marcus Fund and Loker Pinard Funds for Kidney Cancer Research at DFCI. The other authors indicated no financial relationships.

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