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Evaluation of the use of plasma and urine as liquid biopsy for prognostic stratification in clear cell renal cell carcinoma (ccRCC)

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most common and aggressive subtype of renal tumors. It is usually asymptomatic, most often identified through incidental findings on imaging tests.

When diagnosed at an early stage, its prognosis is excellent, but about 25% of patients have advanced disease at diagnosis. The 5-year survival rate is 60%, dropping to 10% in metastatic cases.

Treatment consists of surgery and, for metastatic patients, systemic treatments and radiotherapy are used. Thus, tools for premature identification of high-risk patients is important for early therapeutic approach with the objective of greater treatment success.

Therefore, the aim of this study was to investigate liquid biopsy's clinical utility and potential for prognostic stratification by evaluating the presence of tumor DNA (tDNA) in serial plasma and urine samples from patients with ccRCC using Next Generation Sequencing (NGS).

Ethics committee approval: 2397/17

Methods

Untreated ccRCC patients recruited from 2017 to 2019 at A.C.Camargo Cancer Cencer's Urology Department.

Biological samples collection:

Tumor fragment and blood: at surgery.

Plasma and urine: **Baseline:** at surgery. **M1:** week 6 to 8. **M2:** month 6. **M3:** month 18. **M4:** month 30. (Monitoring)

Identification of somatic mutations in DNA from tumor tissue using NGS:

Custom panel: 28 genes often mutated in renal cell carcinomas *(NextSeq, Illumina)*.

If negative for somatic mutations in genes of the custom panel

Comprehensive Cancer Panel (CCP): 409 genes often mutated in cancer *(ION GeneStudio S5 Plus, Thermo Fisher)*.

Tracking of the tumor somatic mutations in DNA from plasma and urine using NGS:

Deep amplicon sequencing, at least 20.000x coverage depth *(ION GeneStudio S5 Plus, Thermo Fisher)*.

Mutations in plasma and urine were classified as:

- ***High confidence:*** VAF≥0.5% and ≥2x NTC
- ***Low confidence:*** VAF≥0.1% and <0.5% and ≥2x NTC

**VAF: variant allele frequency.*

Fisher's exact test was used to determine if there was a significant association between the presence of tDNA in plasma and urine samples and clinical data / patient outcome.

Results

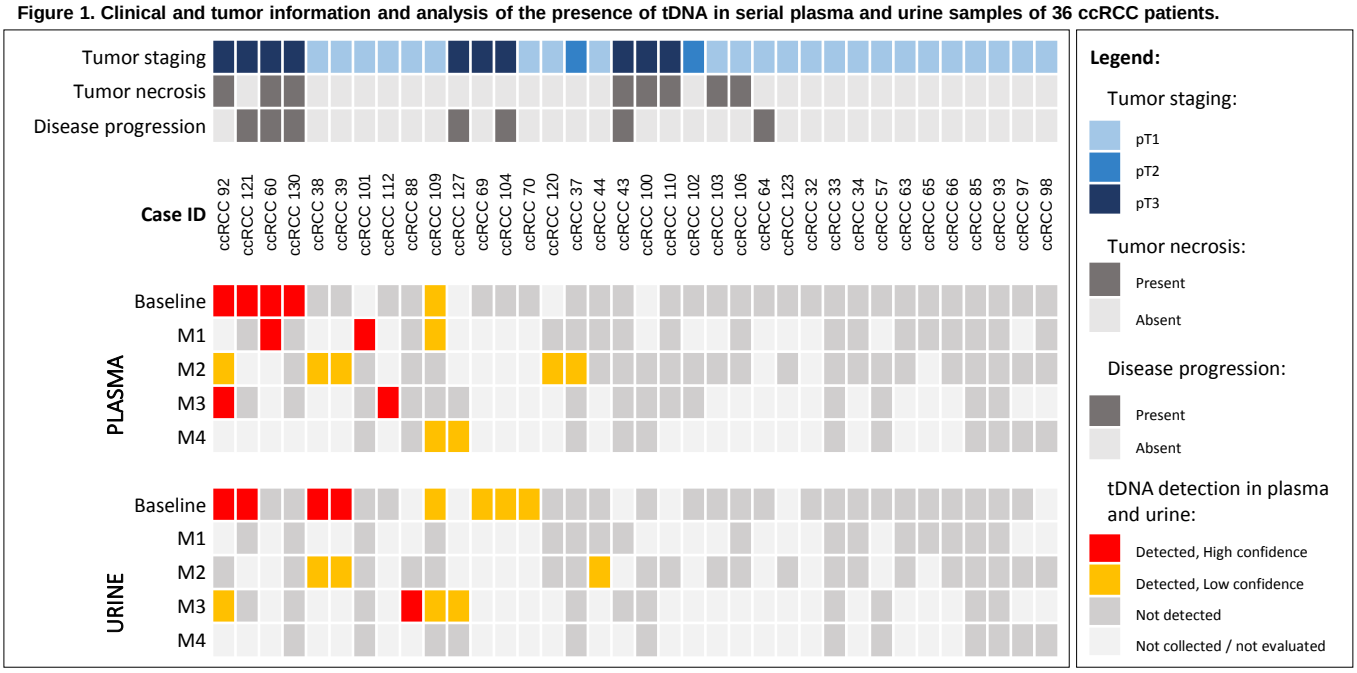
A total of 42 ccRCC cases were recruited for this study. Somatic mutations were identified in 36 ccRCC cases: 35 by the customized panel (the most frequent being the *VHL* gene, mutated in 67% of cases) and 1 by the CCP, in the *ROS1* gene. Most of these patients (72%) had localized disease (pT1 or pT2). During follow up, 7 patients presented metastasis, 2 progressed to death and 1 had another subtype of renal tumor.

In plasma, tDNA was positive in 16% of the baseline samples (5/32) and in 32% of the patients who had monitoring samples available (10/31), being 15% of the M1 samples (3/20), 19% of the M2 samples (5/26), 12% of the M3 samples (2/17) and 15% of the M4 samples (2/13). In urine, tDNA was detected in 23% of the baseline samples (7/30) and in 25% of the patients with monitoring samples (7/28), being none of the M1 samples (0/16), 13% of the M2 samples (3/23), 31% of the M3 samples (4/13) and none of the M4 samples (0/12). In tDNA positive samples, the mean variant allele frequency (VAF) in plasma was 1,79% (0.10%-10,66%) and 1.86% in urine (0.12%-11.56%).

tDNA positive baseline plasma samples with high confidence was significantly correlated with disease progression (p=0.0149, considering metastasis and/or ccRCC related death), tumor necrosis (p=0.0253) and tumor staging ≥pT3 (p=0.0019). There was no correlation between tDNA in baseline plasma samples and ISUP grading or surgical margin involvement, neither between urine baseline samples or plasma/urine monitoring samples and the clinical and tumor characteristics previously mentioned.

tDNA was positive simultaneously in plasma and urine in two cases in baseline samples (one metastatic), two cases in M2 samples (one with another subtype of renal cell carcinoma in the contralateral kidney) and 1 metastatic case in sample M3. Both cases that progressed to death (ccRCC 60 and ccRCC 104) presented tDNA in either plasma or urine of all samples collected: one presented tDNA in baseline and M1 plasmas, and the other in baseline urine.

Figure 1. Clinical and tumor information and analysis of the presence of tDNA in serial plasma and urine samples of 36 ccRCC patients.



Conclusions

These data show that it is possible to detect tDNA in plasma and urine in a fraction of ccRCC patients and that the presence of tDNA in plasma before surgery is associated with disease progression, presence of tumor necrosis and tumor staging ≥T3.

Contact

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