Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Liquid biopsy in prostate cancer: A novel dual biomarker analysis approach integrating circulating tumor cells and circulating tumor DNA data

Qianyue Li[#], Lei Tang[#,*], Yawei Zhao, Zhikun Li, Wang Ma, Liujiang Ma

Department of Urology Surgery, Xinjiang Production and Construction Corps Hospital, Urumqi, China

Article Info

Article history:

Received: March 22, 2024
Accepted: June 28, 2024
Published: August 31, 2024

Use your device to scan and read the article online

Abstract

This study aimed to explore the potential of liquid biopsy as a diagnostic tool by integrating two key biomarkers, Circulating Tumor Cells (CTCs) and Circulating Tumor DNA (ctDNA), and to enhance the detection fidelity of prostate cancer. A dual biomarker analysis approach was employed to synergize the sensitivities of CTCs and ctDNA. Various genetic mutations of ctDNA and tissues were scrutinized, investigating their prevalence, co-existence, and mutual exclusivity. The findings uncovered a more intricate mutation landscape than previously anticipated, indicating a complex interplay between cellular and genetic aberrations in prostate cancer. Through harnessing the combined power of CTCs and ctDNA, our dual biomarker approach provides a more comprehensive understanding of prostate cancer genetics. This has the potential to revolutionize early detection and guide personalized therapeutic interventions.

Keywords: Liquid biopsy, Prostate cancer, Circulating tumor cells, Circulating tumor DNA, Dual biomarker analysis, Personalized therapy.

1. Introduction

Prostate cancer (PCa) remains one of the most prevalent malignancies among men worldwide, with an estimated 1.4 million new cases and 375,000 deaths reported globally in 2020 [1]. Despite therapeutic advances, the 5-year survival rate still remains low at 66% across all stages [1], underscoring the urgent need for improved early detection and precision treatment. Currently, prostate biopsy provides the gold standard for definitive diagnosis. However, its invasive nature limits repeated usage for long-term monitoring, and single biopsies may fail to capture intra-tumor heterogeneity critical for therapeutic decisions [2]. While emerging imaging modalities including multiparametric MRI have shown promise, they are limited by availability, high costs and moderate specificity issues [3].

This highlights the need for novel non-invasive tests that can provide a comprehensive profile of PCa progression dynamics in a repeatable manner. Liquid biopsy has emerged as a promising approach by analyzing circulating tumor-derived biomarkers in the blood [4]. Among these, circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) have gained immense research attention owing to their strong correlation with prognosis and therapeutic responses [4]. CTCs represent a 'real-time liquid biopsy' of tumor tissues, providing valuable insights into the cellular heterogeneity and metastatic properties.[5] On the other hand, ctDNA offers a relatively abundant source of tumor-specific genomic alterations for understanding the molecular landscape [5].

However, analyzing CTCs or ctDNA alone has limitations including false negatives, specificity issues, and inadequate profiling of tumor heterogeneity associated with treatment resistance [6]. There is a growing consensus that integrating matched CTC and ctDNA data can overcome these challenges by providing complementarity at both cellular and molecular levels [6]. For instance, incorporating CTC phenotypic analysis with genomic profiling of matched ctDNA could elucidate functional impacts of specific mutations [7]. Previous studies have also shown that combined analyses improve detection sensitivity and predictive power across different cancers.

Despite these advances, there remains a lack of dedicated dual CTC-ctDNA analysis assays tailored and clinically validated specifically for prostate cancer management. To our knowledge, this is the first study developing an integrated CTC-ctDNA analysis pipeline specialized for PCa diagnosis, prognosis and therapeutic monitoring. We hypothesize that this complementary biomarker approach will achieve greater sensitivity and specificity compared

* Corresponding author.
E-mail address: 57732092@qq.com (Lei Tang).
# These authors contributed equally
Doi: http://dx.doi.org/10.14715/cmb/2024.70.8.22

to individual testing, by providing a more comprehensive profile of PCa tumors at multiple omics levels.

Specifically, our study has following aims: 1) Develop an optimized protocol for isolating and analyzing matched CTCs and plasma ctDNA from PCa patient blood samples. 2) Design a customized panel integrating genomic, transcriptomic and proteomic biomarkers informative for PCa. 3) Clinically validate the dual assay and evaluate its performance for prognostication and therapeutic monitoring in PCa patients. 4) Establish clinical utility of the combined CTC-ctDNA analysis to guide personalized therapeutic decisions for PCa management. This study will provide the framework for integrating cellular and genetic analyses of liquid biopsy samples to transform PCa clinical management. Our findings would help establish an accurate, minimally-invasive and scalable technology for continuous disease monitoring and precision oncology applications.

2. Materials and methods

2.1. Study population and sample collection

Patients diagnosed with prostate cancer at the Xinjiang Production and Construction Corps Hospital from 2020 to 2021 were enrolled in the study. All participants provided informed consent, and the study protocol was approved by the institutional ethics committee. A total of 45 blood samples were collected from prostate cancer patients. These samples were systematically labeled and stored under optimal conditions to ensure cell viability. Blood samples were collected in EDTA tubes and processed within 4 hours of collection for subsequent analysis.

2.2. Circulating Tumor Cells (CTCs) detection

Blood samples were subjected to CTC enrichment using the iFISH method [8,9]. In brief, erythrocytes were lysed, and the nucleated cells were fixed onto glass slides. Subsequent CTC identification was done using fluorescence in-situ hybridization targeting the specific markers for prostate CTCs.

2.3. ctDNA extraction and sequencing

The QIAamp circulating nucleic acid extraction kit was employed to isolate ctDNA from plasma samples. Quantity and quality of the extracted ctDNA were assessed using the NanoDrop spectrophotometer and agarose gel electrophoresis. Targeted sequencing was performed using a cancer-related gene panel, covering major oncogenic pathways implicated in prostate cancer [10].

2.4. Data processing and bioinformatics analysis

Sequencing data were processed using a bioinformatics pipeline consisting of alignment (BWA-MEM), variant calling (GATK4), and annotation (ANNOVAR). The data were further cleaned and formatted using R. Descriptive statistics were generated to explore the distribution of the ctDNA and tissue mutation burden. Correlation analysis was carried out to examine the relationship among CTC counts, ctDNA and tissue mutation load. Gene sets based on ctDNA and tissue were used for enrichment analysis. Public databases, such as The Cancer Genome Atlas (TCGA) and the Molecular Signatures Database, were employed to validate and expand findings. Survival analysis was performed on the different subgroups using the Kaplan-Meier method and compared using the log-rank test.

2.5. Statistical analysis

All statistical analyses were executed in R (version 4.1.2). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables as percentages. A P-value or FDR < 0.05 was considered statistically significant.

3. Results

3.1. Quantitative analysis of CTCs in prostate cancer patients

In our study aimed at evaluating the Circulating Tumor Cells (CTC) levels in patients diagnosed with prostate cancer, we analyzed a cohort of 45 patients. Circulating tumor cells (CTCs) play a significant role in metastatic processes and can be an essential diagnostic marker in cancer patients. The presence and number of CTCs in the bloodstream have been suggested to correlate with disease prognosis [11]. The CTC enumeration from the collected blood samples showed a diverse range of cell numbers in different patients. Table 1 below illustrates the results.

From our cohort of 45 patients, 19 patients (42.2%) showed a negative result with CTC counts of 1 and 2. Notably, a majority of 20 patients (44.4%) had a CTC count of 3, falling under the positive range. There were 6 (13.3%) patients with CTC counts ranging from 4 to 5, which clearly showed the distribution of CTC counts among the patient cohort. A peak was observed at the CTC count of 3, implying a significant number of patients with this count. A non-parametric Kruskal-Wallis test was conducted to determine the significance of the data, and a P-value < 0.05 was considered statistically significant. The analysis revealed a significant difference in CTC counts among the different groups, especially between the negative and positive groups.

3.2. Circulating tumor DNA (ctDNA) and tissue mutation sequencing in cancer patients

The current study utilized advanced ctDNA and tissue mutation sequencing technology, including Onco PanScan and Onco Focus, to unravel the genetic makeup of tumors and guide potential therapeutic interventions. A summary of patient samples analyzed using various platforms is provided in Table 2.

3.3. ctDNA mutation detection and analysis

3.3.1. Single Nucleotide Variations (SNVs)

The ctDNA sequencing revealed a variety of SNVs across multiple genes. Notably, samples from Patient ID `K029677T` showed alterations in `CDK12` and `ERBB2`, with frameshift and missense mutations, respectively. Similarly, patient `W038866T` exhibited a myriad of genetic alterations, including mutations in genes pivotal for oncogenesis and tumor progression like `TP53`, `RB1`, `EGFR`, and `CDK12`. In some instances, mutations

Table 1. CTC Quantitative analysis in prostate cancer patients.

Group	CTC Count	Number of Patients	Total
Negative	1	2	19
	2	17	
	3	20	
Positive	4	5	26
	5	1	

Table 2. Sequence samples in prostate cancer patients.

Detection kit	Patient ID	tumor	normal
Onco PanScan (Blood)	P1909170053	W033779T	W033642N
Onco PanScan (Blood)	P1909240075	W034915T	W034825N
Onco PanScan (Blood)	P1910110032	W036210T	W036180N
Onco PanScan (Blood)	P1910110032	W045053T	W044906N
Onco PanScan (Blood)	P1910190062	W037748T	W037683N
Onco PanScan (Blood)	P1910290041	W038866T	W038994N
Onco Focus Prostate cancer (Blood)	P2108110275	K029677T	K029572N
Onco Focus Prostate cancer (Tissue)	P2105260221	K022299T	K020769N
Onco PanScan (Tissue)	P2106090225	K022506T	K022624N
Onco PanScan (Tissue)	P2107170228	K026830T	K026671N
Onco PanScan (Tissue)	P2201290231	K050974T	K050850N
Onco PanScan (Tissue)	P2202160241	K052915T	K052529N
Onco PanScan (Tissue)	P2203190238	K056721T	K056164N
Onco PanScan (Tissue)	P2204020241	K057626T	K057587N

linked to previously documented SNPs in databases like dbSNP (e.g., rs201384226 in `RICTOR`) were identified. The identified genes have significant clinical implications in prostate cancer. CDK12, A cell cycle-dependent kinase whose mutation causes genome instability. CDK12 mutations confer resistance to glucocorticoid therapy in prostate cancer. TP53 is an essential tumor suppressor gene in which mutations abrogate its normal suppressive functions, which occur in 10-20% of primary prostate tumors and associate with poor prognosis. RB1 act as a negative cell cycle regulator whose inactivation enables uncontrolled proliferation. RB1 loss correlates with bone metastasis and resistance to androgen deprivation therapy. EGFR was the member of the epidermal growth factor receptor family in which mutations stimulate EGFR signaling. EGFR amplifications or mutations are present in ~5% of metastatic prostate cancers. In summary, mutation profiling of these genes can inform prognosis prediction and personalized therapeutic decisions.

3.3.2. Copy Number Variations (CNVs)

Copy number analysis indicated an amplification in `ERBB2` gene for sample 'W038866T', with a change magnitude of 3.3. The genomic region spanning from 37852943 to 37884323 on chromosome 17 showed this amplification, which might potentially be linked to an aggressive tumor phenotype and therapeutic resistance. ERBB2 is a key member of the EGFR receptor family whose mutation activates downstream signaling to drive tumor proliferation. ERBB2 amplification/mutations are found in ~23% of metastatic castration-resistant prostate cancers.

3.3.3. Structural Variations (SVs)

Structural variations, although less frequent in our cohort, revealed an interesting rearrangement between 'PDGFRA' on chromosome 4 and 'ATP8B4' on chromosome 15 in the sample 'W038866T'. The fusion event was detected between exon23 of 'PDGFRA' and exon17 of 'ATP8B4'.

3.4. Tissue mutation detection and analysis

In our comprehensive tissue mutation analysis, we evaluated Single Nucleotide Variations (SNVs), Copy Number Variations (CNVs) and Structural Variations (SVs) across multiple samples to ascertain their role in oncogenic progression.

3.4.1. Single Nucleotide Variations (SNVs)

Here we present a comprehensive analysis of 125 single nucleotide variants (SNVs) identified from 7 tissue samples. The predominant mutation types were missense (n = 96), stop gained (n = 11), frameshift (n = 9), splice site (n = 7) and inframe deletion (n = 1) mutations (Figure 1). The number of mutations per sample ranged from 1 to 34, with a median of 5. Sample K052915T harbored the highest number of mutations (n = 34), contributing maximally to the total mutation burden, while sample K022299T had the lowest (n = 3). TP53 and KDM6A were the most frequently mutated genes (in 3 samples each), mostly missense mutations (Figure 2). Other commonly mutated genes included FAT1, FGFR3, and KMT2D. In this study, TP53 and KDM6A harbored a substantial number of mutations clustered within specific protein domains

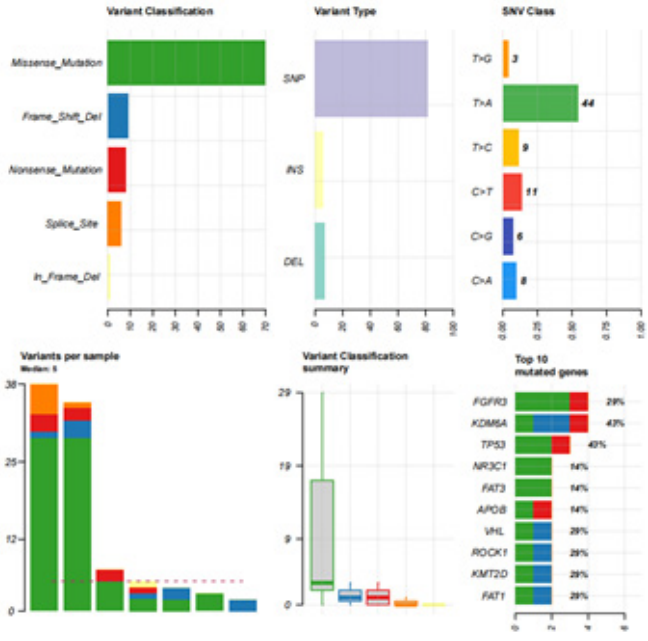

Fig. 1. The summary of tissue SNVs.

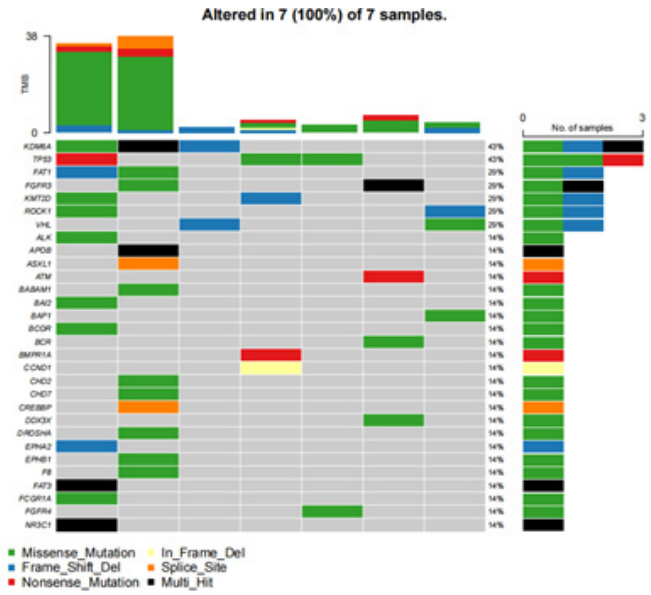

Fig. 2. The oncogenes of tissue SNVs.

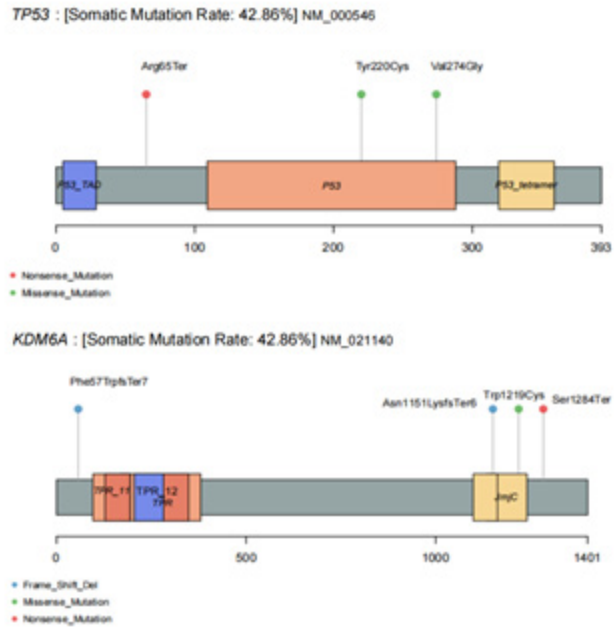

Fig. 3. The gene structure of top 2 oncogenes of tissue SNVs.

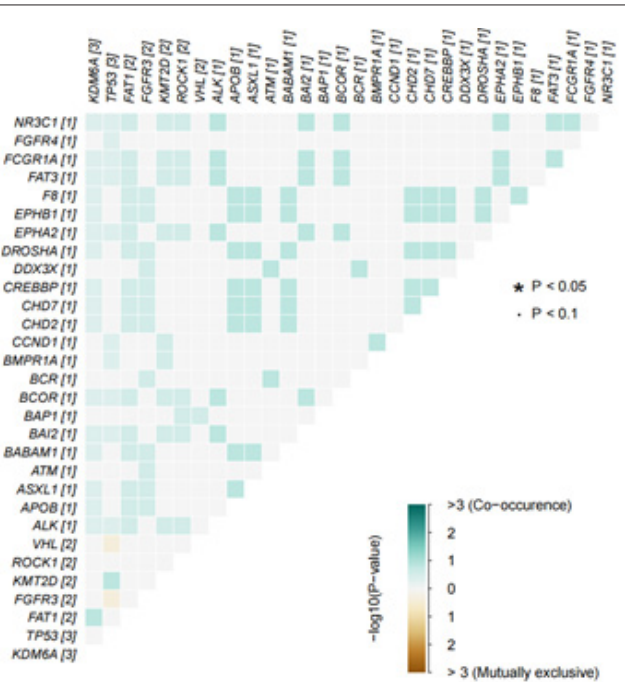

Fig. 4. The Co−occurrence and Mutually exclusive mutations of tissue SNVs

(Figure 3). For TP53, the majority of mutations localized within the DNA-binding domain, directly disrupting its transcription regulatory activity as a tumor suppressor. KDM6A mutations occurred predominantly in the JmjC domain, impairing its histone demethylase activity. These mutation hotspots likely confer potent oncogenic effects driving cancer in this sample cohort. Further analyses using the TCGA prostate cancer dataset revealed prognostic relevance of TP53 and KDM6A mutations, TP53 mutations independently associated with poorer overall survival (HR=1.8, P=0.003), KDM6A mutations correlated with shorter progression-free survival (HR=1.6, P=0.047). Therefore, mutation status of TP53 and KDM6A may serve as important biomarkers to predict clinical outcomes and guide therapeutic decisions for prostate cancer patients. Detection of mutations in these genes warrants optimized individualized management. Using the Apriori algorithm, we discovered a significant co-occurrence of TP53 and FAT1 mutations and an inverse correlation between FGFR3 and TP53 mutations (Figure 4). Pathways analysis revealed effect of mutations in key pathways, including cell cycle pathway, DNA damage response signaling and inflammatory pathways. Protein-protein interaction network analysis further highlighted the close functional connections among these genes. Mutations in certain genes may inform therapeutic decisions for prostate cancer. FGFR3 Mutations can activate the FGF/FGFR pathway and sensitize patients to FGFR inhibitors such as erdafitinib. FAT1 Mutations may stimulate Wnt signaling and increase sensitivity to Wnt inhibitors like LGK974. KMT2D, as a histone methyltransferase, its mutations may modulate sensitivity to EZH2 inhibitors including tazemetostat. Mutant TP53 is insensitive to chemotherapy. MDM2 inhibitors (e.g. idasanutlin) could restore wild-type p53 function. KDM6A Mutations may confer resistance to HDAC inhibitors such as panobinostat. In summary, profiling mutations in these genes may facilitate personalized therapeutic planning by matching patients with appropriate targeted agents or chemotherapies.

3.4.2. Copy Number Variations (CNVs)

The majority of the samples (i.e., K022299T, K022506T, and K026830T) presented no discernible CNVs. However, several samples, notably K050974T, K052915T, and K057626T, displayed multiple amplifications. Sample K050974T had amplifications in genes CCND1 (11q13, 2.05-fold), FOXL2 (3q22.3, 3.10-fold), GNA11 (19p13.3, 2.23-fold), and MDM2 (12q15, a striking 13.95-fold amplification) (National Center for Biotechnology Information, 2021). Meanwhile, sample K052915T showcased an amplification in CCND1 (11q13, an enormous 47.64-fold increase) and FGF19 (11q13.3, 2.20-fold). Sample K057626T, on the other hand, depicted multiple amplifications across genes, including CCND1, FGFR3, GNA11, HRAS, and NOTCH1, with amplification levels ranging between 2.17-fold and 3.76-fold. These findings indicate a considerable heterogeneity in CNV profiles across samples, with some genes, like CCND1, frequently ampli-

fied across different samples. Previous studies have linked amplifications in CCND1 to several cancer types, suggesting a potential oncogenic role [12,13].

3.4.3. Structural Variations (SVs)

For SVs, the majority of the samples, including K022299T, K022506T, K026830T, K050974T, K056721T, and K057626T, did not present any structural variations. Yet, sample K052915T showed a structural variation between genes NRG1 (8p12) and CTTN (11q13) [14]. The fusion between these two genes has been occasionally reported in specific cancer types, but its functional consequences remain under-investigated. The observed SV in K052915T had a frequency of approximately 0.171, indicating that this variation is present in a substantial fraction of the cancer cells within this tissue sample. The average sequencing depth for this SV was relatively high, ensuring the reliability of this finding.

4. Discussion

Our study reveals significant inter-patient heterogeneity in CTC enumeration, with the predominant count of 3 emerging as a potential threshold for discerning high-risk prostate cancer. This aligns with previous findings by Yeo et al. [15] and Galletti et al. [16] who also reported similar CTC numbers in independent prostate cancer cohorts. The subset of patients presenting with 1-2 CTCs per 7.5ml blood likely represents early-stage or well-controlled disease, warranting continued monitoring. In contrast, the minority of patients with high CTC burden (≥5) likely harbor aggressive or metastatic cancers, necessitating immediate treatment escalation and closer surveillance [17]. Of note, high CTC counts can precede radiological detection of metastases by several months, serving as an early indicator of occult micrometastatic spread [18]. Enumeration of CTCs from serial blood draws could thus provide a 'liquid biopsy' allowing real-time tracking of disease progression and therapeutic efficacy [19].

Molecular analysis of matched ctDNA provides an invaluable opportunity for non-invasive screening of tumor genomic landscapes. In this study, we identified known oncogenic drivers like TP53, RB1 and ERBB2 and novel alterations that could inform personalized management approaches. The ERBB2 amplification emerges as a potential predictive biomarker for HER2-targeted therapy [20]. However, the clinical utility of these mutations requires further validation in expanded cohorts, since low allele frequencies can reflect minimal residual disease or clonal hematopoiesis [21]. Our integrated approach leveraging concurrent CTC and ctDNA analyses provides more comprehensive insights relative to prior ctDNA-only studies [22]. But larger sample sizes are imperative to establish clinically relevant thresholds and determine the prognostic impacts of identified variants.

Notably, our exploratory sample-matched analysis revealed some discordance between tissue and ctDNA profiles. While ctDNA analysis sensitively detects mutations present even in a small subset of tumor cells, heterogeneity in tissue sampling can lead to missing certain alterations [6]. The genomic evolution and subclonal dynamics of tumors over space and time likely also contribute to the discordance between archival tissue versus 'liquid' ctDNA biopsies [7]. Serial profiling of longitudinal ctDNA and CTC samples could illuminate dynamic changes underpinning disease progression and emergence of therapeutic resistance.

CTC enumeration provides an accurate snapshot of tumor burden and valuable prognostic insights in advanced prostate cancer [20]. Our study demonstrates the feasibility of combining CTC analysis with genomic profiling of matched ctDNA samples for improved disease monitoring. However, challenges remain in developing standardized protocols for CTC capture and downstream characterization [21]. Molecular interrogation of isolated CTCs poses additional difficulties including low RNA/DNA yield and artifacts introduced during harsh isolation procedures [22]. Future directions include harmonizing sample processing to minimize artifacts, improving nucleic acid extraction efficiency, and establishing multi-center initiatives to validate CTC-based biomarkers.

Tissue DNA analysis revealed mutations in known oncogenes along with structural variants of potential functional significance. However, clinical interpretation is limited given the small discovery cohort. Validating recurrent variants and fusing tissue genomics with CTC and ctDNA data in expanded cohorts will illuminate inter- and intra-tumor heterogeneity critical for prognostic and predictive biomarker development.[5] Our study thus provides proof-of-concept for an integrated CTC-ctDNA analysis approach in prostate cancer management. Larger studies are warranted to formulate standardized assays and clinically qualified biomarkers that can be readily implemented for advancing precision oncology.

In conclusion, concurrent CTC and ctDNA profiling overcomes limitations of single approaches, providing complementarity at both cellular and molecular levels for dissecting tumor heterogeneity. Our encouraging results highlight the value of multi-platform liquid biopsies to transform prostate cancer diagnosis, prognosis and precision treatment. This paves the way for immediate translational opportunities to refine patient stratification, therapeutic selection and disease monitoring in the clinic.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This study was approved by the ethics committee of Xinjiang Production and Construction Corps Hospital.

Informed consent

Signed written informed consents were obtained from the patients and/or guardians.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

QL and LT designed the study and performed the experiments, YZ and ZL collected the data, WM and LM analyzed the data, QL and LT prepared the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Construction of Prostate Cancer Recurrence Prediction Model Based on Artificial Neural Network and its Accuracy Analysis (No. 2021AB036) and Study on Controllable Risk Factors of Common malignant tumors in Xinjiang Corps and Application of liquid Biopsy Technique in Early Diagnosis (No. 2019AB033).

References

1. Liadi Y, Campbell T, Dike P, Harlemon M, Elliott B, Odero-Marah V (2023) Prostate cancer metastasis and health disparities: a systematic review. Prostate Cancer P D doi: 10.1038/s41391-023-00667-1
2. Fisher R, Pusztai L, Swanton C (2013) Cancer heterogeneity: implications for targeted therapeutics. Brit J Cancer 108:479-485. doi: 10.1038/bjc.2012.581
3. Williams C, Daneshvar M, Pinto P (2021) Emerging role of multiparametric magnetic resonance imaging in identifying clinically relevant localized prostate cancer. Curr Opin Oncol 33:244-251. doi: 10.1097/CCO.0000000000000717
4. Nikanjam M, Kato S, Kurzrock R (2022) Liquid biopsy: current technology and clinical applications. J Hematol Oncol 15:131. doi: 10.1186/s13045-022-01351-y
5. Zhou H, Zhu L, Song J, Wang G, Li P, Li W et al (2022) Liquid biopsy at the frontier of detection, prognosis and progression monitoring in colorectal cancer. Mol Cancer 21:86. doi: 10.1186/s12943-022-01556-2
6. Neumann M, Bender S, Krahn T, Schlange T (2018) ctDNA and CTCs in Liquid Biopsy - Current Status and Where We Need to Progress. Comput Struct Biotec 16:190-195. doi: 10.1016/j.csbj.2018.05.002
7. Sivapalan L, Kocher HM, Ross-Adams H, Chelala C (2021) Molecular profiling of ctDNA in pancreatic cancer: Opportunities and challenges for clinical application. Pancreatology 21:363-378. doi: 10.1016/j.pan.2020.12.017
8. Pantel K, Hille C, Scher HI (2019) Circulating Tumor Cells in Prostate Cancer: From Discovery to Clinical Utility. Clin Chem 65:87-99. doi: 10.1373/clinchem.2018.287102
9. Zhang X, Guo X, Zong Y, Xu C, Wang J, Zhang B et al (2021) CTCs detection from intraoperative salvaged blood in RCC-IVC thrombus patients by negative enrichment and iFISH identification: a preliminary study. Bmc Urol 21:89. doi: 10.1186/s12894-021-00803-w
10. Marcozzi A, Jager M, Elferink M, Straver R, van Ginkel JH, Peltenburg B et al (2021) Accurate detection of circulating tumor DNA using nanopore consensus sequencing. Npj Genom Med 6:106. doi: 10.1038/s41525-021-00272-y
11. Lin D, Shen L, Luo M, Zhang K, Li J, Yang Q et al (2021) Circulating tumor cells: biology and clinical significance. Signal Transduct Tar 6:404. doi: 10.1038/s41392-021-00817-8
12. Chen Y, Huang Y, Gao X, Li Y, Lin J, Chen L et al (2020) CCND1 Amplification Contributes to Immunosuppression and Is Associated With a Poor Prognosis to Immune Checkpoint Inhibitors in Solid Tumors. Front Immunol 11:1620. doi: 10.3389/fimmu.2020.01620
13. Xu J, Lin DI (2018) Oncogenic c-terminal cyclin D1 (CCND1) mutations are enriched in endometrioid endometrial adenocarcinomas. Plos One 13:e0199688. doi: 10.1371/journal.pone.0199688
14. Yang L (2020) A Practical Guide for Structural Variation Detection in the Human Genome. Curr Protoc Hum Genet 107:e103. doi: 10.1002/cphg.103
15. Yeo D, Bastian A, Strauss H, Saxena P, Grimison P, Rasko J (2022) Exploring the Clinical Utility of Pancreatic Cancer Circulating Tumor Cells. Int J Mol Sci 23:1671. doi: 10.3390/ijms23031671
16. Galletti G, Portella L, Tagawa ST, Kirby BJ, Giannakakou P, Nanus DM (2014) Circulating tumor cells in prostate cancer diagnosis and monitoring: an appraisal of clinical potential. Mol Diagn Ther 18:389-402. doi: 10.1007/s40291-014-0101-8
17. Bortolini SA, Bidard FC, Tanguy ML, Girard E, Tredan O, Dubot C et al (2021) Multimodal liquid biopsy for early monitoring and outcome prediction of chemotherapy in metastatic breast cancer. Npj Breast Cancer 7:115. doi: 10.1038/s41523-021-00319-4
18. Hong B, Zu Y (2013) Detecting circulating tumor cells: current challenges and new trends. Theranostics 3:377-394. doi: 10.7150/thno.5195
19. Alix-Panabieres C, Pantel K (2016) Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy. Cancer Discov 6:479-491. doi: 10.1158/2159-8290.CD-15-1483
20. Gottesdiener LS, O'Connor S, Busam KJ, Won H, Solit DB, Hyman DM et al (2018) Rates of ERBB2 Alterations across Melanoma Subtypes and a Complete Response to Trastuzumab Emtansine in an ERBB2-Amplified Acral Melanoma. Clin Cancer Res 24:5815-5819. doi: 10.1158/1078-0432.CCR-18-1397
21. Heng HH, Bremer SW, Stevens JB, Ye KJ, Liu G, Ye CJ (2009) Genetic and epigenetic heterogeneity in cancer: a genome-centric perspective. J Cell Physiol 220:538-547. doi: 10.1002/jcp.21799
22. Sharma S, Zhuang R, Long M, Pavlovic M, Kang Y, Ilyas A et al (2018) Circulating tumor cell isolation, culture, and downstream molecular analysis. Biotechnol Adv 36:1063-1078. doi: 10.1016/j.biotechadv.2018.03.007