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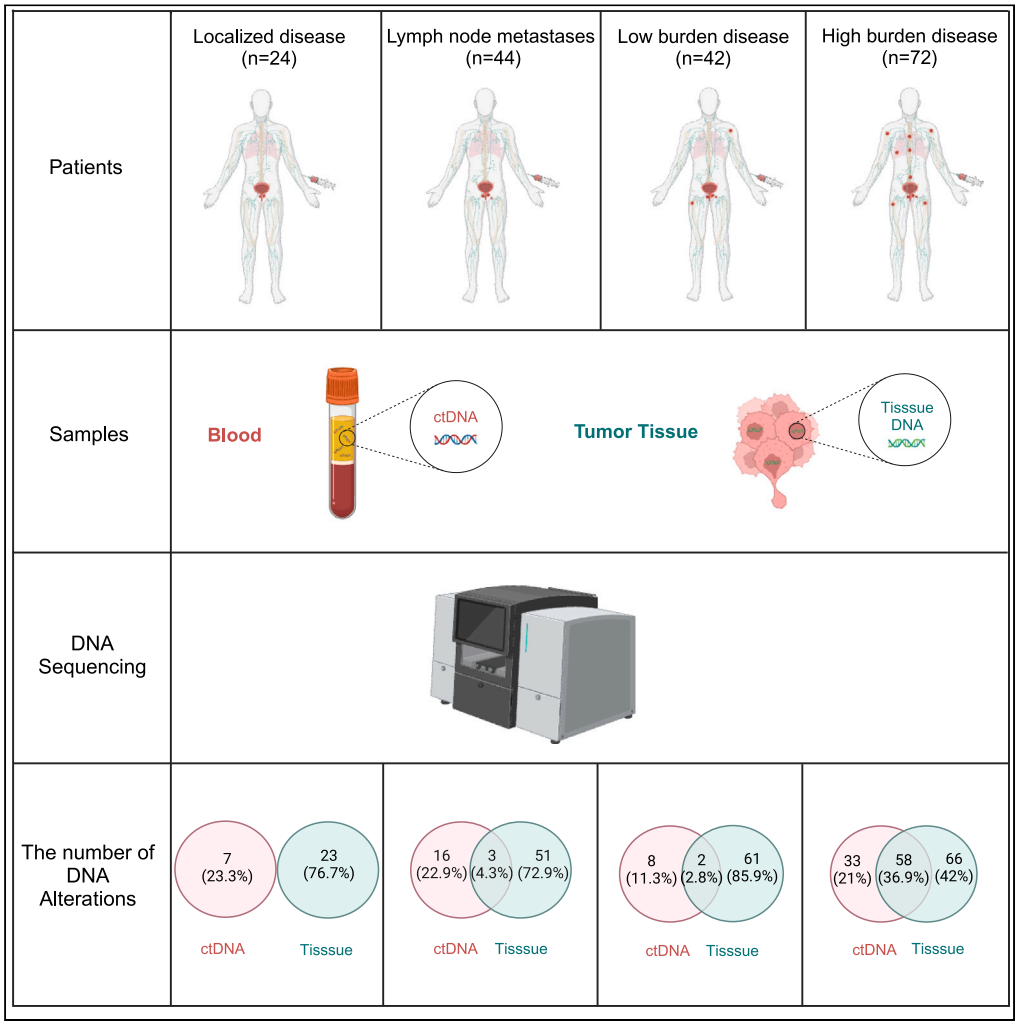

Bin Yang, Tingting Zhao, Baijun Dong, ..., Bo Peng, Wei Xue, Xudong Yao

yangbnju@gmail.com (B.Y.)
xuewei@renji.com (W.X.)
yaoxudong67@sina.com (X.Y.)

Highlights

The proportion of ctDNA in HSPC rises with disease progression

Genomic alterations in ctDNA and tissue complement in high-volume HSPC

Genomic alterations involving BRCA1/2, CDK12, TP53, PTEN or RB1 link to quicker castration resistance

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Circulating tumor DNA and tissue complementarily detect genomic alterations in metastatic hormone-sensitive prostate cancer

Bin Yang,[1,2,8,9,*] Tingting Zhao,[3,4,8] Baijun Dong,[5,8] Wei Chen,[6,8] Guanjie Yang,[1,2] Jun Xie,[7] Changcheng Guo,[1,2] Ruiliang Wang,[1,2] Hong Wang,[1,2] Longfei Huang,[4] Bo Peng,[1,2] Wei Xue,[5,*] and Xudong Yao[1,2,*]

SUMMARY

The clinical utility of circulating tumor DNA (ctDNA) in hormone-sensitive prostate cancer (HSPC) remains inadequately elucidated. This study presents the largest real-world cohort to conduct a concordance analysis between ctDNA and tissue-based genomic profiling in HSPC patients. The findings reveal diminished ctDNA abundance in cases with low tumor burden and demonstrate an increased concordance rate between ctDNA and tissue along with the progression of disease burden. Notably, a substantial number of exclusive genomic alterations (GAs) were identified either in ctDNA or tissue in high-volume metastatic disease. Integrating tissue and ctDNA analysis identified specific gene alterations (*BRCA1*, *BRCA2*, *CDK12*, *TP53*, *PTEN*, or *RB1*) associated with a shorter time to the progression to castration-resistant prostate cancer (CRPC), with an escalated CRPC risk correlated with cumulative GAs. This multicenter, real-world investigation underscores the complementary role of ctDNA and tissue in detecting clinically pertinent GAs, highlighting their potential integration into clinical practice for advanced prostate cancer management.

INTRODUCTION

Prostate cancer (PCa) is the second-most common cancer in males and is known for its heterogeneity.[1,2] Recent integrative genomic profiling studies have provided insights into pathogenesis and opportunities for precision medicine.[3–6] Specifically, studies have shown that advanced PCa with DNA repair defect could benefit from poly (adenosine diphosphate [ADP]- ribose) polymerase (PARP) inhibitors and platinum-based chemotherapies based on improved clinical response.[7–10] Additionally, an immune checkpoint inhibitor has been approved for advanced solid tumors with high microsatellite instability or deficient mismatch.[11] Genomic alterations (GAs) in *AR*, *TP53*, *RB1*, and *PTEN* have also been associated with poor clinical outcomes in PCa.[12–14]

Tissue biopsy is the gold standard for genomic testing but it is limited by temporal and spatial heterogeneity. As an emerging minimally invasive technique, plasma circulating tumor DNA (ctDNA) can capture molecular heterogeneity, identify GAs for targeted therapies, and monitor tumor recurrence and resistance in real time.[15] Detectable GAs from ctDNA have been identified as prognostic or predictive markers for patients with castration-resistant PCa (CRPC) in multiple studies.[3,16–18] However, the genomic characteristics and clinical application value of ctDNA in patients with hormone-sensitive PCa (HSPC) remain unclear. Therefore, this multicenter, real-world study aims to characterize the somatic molecular profiling, evaluate the concordance of GAs between ctDNA and matched tumor tissue, and investigate the clinical significance of GAs in patients with HSPC.

RESULTS

Patient characteristics

Overall, 182 patients with HSPC were included in this study, including 24 patients with localized PCa (defined as Group LP), 44 patients with lymph node metastases disease (defined as Group LND), 72 patients with high-volume metastatic HSPC (mHSPC) (defined as Group HVD),

[1]Department of Urology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China
[2]Urologic Cancer Institute, Tongji University School of Medicine, Shanghai, China
[3]Department of Urology, the Shanghai Tenth People's Hospital, School of Medicine, School of Life Sciences and Technology, Tongji University, Shanghai, China
[4]Research Institute, GloriousMed Clinical Laboratory (Shanghai) Co., Ltd., Shanghai, China
[5]Department of Urology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[6]Department of Urology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China
[7]Department of Urology, Shanghai Clinical College, Anhui Medical University, Shanghai, China
[8]These authors contributed equally
[9]Lead contact
*Correspondence: yangbnju@gmail.com (B.Y.), xuewei@renji.com (W.X.), yaoxudong67@sina.com (X.Y.)
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Table 1. Baseline characteristics of the study cohort

Parameter	Group LP(N = 24)	Group LND (N = 44)	Group LVD(N = 42)	Group HVD (N = 72)	Total(N = 182)
Median Age (IQR), y	65(56–69)	64(57–69)	67 (61–72)	68(63–72)	67(60–71)
Median PSA level (IQR), ng/mL	31 (11.1–57.9)	53(27–139.3)	100(30.8–188.4)	100(54.9–349)	82(30.9–186.1)
Gleason Score, n (%)					
≤7	11(45.8)	9(20.5)	4(9.5)	7(9.7)	31(17.0)
8	6(25.0)	18 (40.9)	9(21.4)	23(31.9)	56(30.8)
>8	7(29.2)	16(36.4)	28(66.7)	41(56.9)	92(50.5)
Unknown		1(2.3)	1(2.4)	1(1.4)	3(1.6)
Treatment before sample collection, n (%)					
Treatment-naïve	20(83.3)	27(61.4)	23(54.8)	59(81.9)	129(70.9)
ADT	2(8.3)	9(20.5)	10(23.8)	7(9.7)	28(15.3)
ADT +abiraterone	–	4(9.1)	4(9.5)	2(2.8)	10(5.5)
ADT+ chemotherapy	2(8.3)	4(9.1)	4(9.5)	4(5.6)	14(7.7)
ADT + abiraterone+chemotherapy	–	–	1(2.4)	–	1(0.5)

IQR, interquartile range; PSA, prostate-specific antigen; ADT, androgen deprivation therapy; ctDNA, circulating tumor DNA.

and 42 patients with low volume mHSPC (defined as Group LVD, including patients with bone metastases and without high-volume disease). The baseline characteristics of patients are shown in Table 1 and S1. The median age of all the patients was 67 years, and the median PSA level was 82 ng/mL. The Gleason score of 81.3% (148/182) of patients was ≥8. Before sample collection, 29.1% (53/182) of patients had received androgen deprivation therapy (ADT) monotherapy or combined ADT treatments. The median follow-up time was 487 days (range: 31–1704 days).

GAs in tumor tissue and ctDNA

Somatic GAs were detected in 70.9% (129/182) of patients in tumor tissue, with an average of 1.45 GAs per case (range, 0–7). Among the different types of GAs, single nucleotide variants (SNVs) accounted for 53.4% of the total GAs (n = 264), followed by insertions and deletions (InDels) (32.2%) and copy number variations (CNVs) (14.4%) (Table 2). The most commonly mutated genes observed in tumor tissue were *FOXA1*(34.6%), *TP53*(14.8%), *SPOP* (12.6%), *CDK12*(10.4%), and *BRCA2*(7.7%) (Figure 1A).

Moreover, ctDNA analysis revealed that 31.3% (57/182) of patients had at least one somatic alteration, with an average of 0.7 GAs per case (range, 0–7) (Table 2). The positive rate for ctDNA varied across different subgroups, with the highest rate observed in Group HVD (54.2%) (Table 2). SNVs and InDels were the most common types of GAs in ctDNA, accounting for 51.2% and 41.7% of the total GAs, respectively,

Table 2. List of somatic variant type in tumor tissue and ctDNA

	Group LP (N = 24)	Group LND (N = 44)	Group LVD (N = 42)	Group HVD (N = 72)	Total (N = 182)
Tissue					
Number of patients with GA≥1	16 (66.7%)	29 (65.9%)	29 (69.1%)	55 (76.4%)	129 (70.9%)
Total Number of GA (average/case, range)	23 (0.96, 0–4)	54 (1.23, 0–5)	63 (1.5, 0–5)	124 (1.72, 0–7)	264 (1.45, 0–7)
SNV	15	27	39	60	141
InDel	8	14	19	44	85
CNV	0	13	5	20	38
Blood					
Number of patients with GA≥1	2 (8.3%)	10 (22.7%)	6 (14.3%)	39 (54.2%)	57 (31.3%)
Total Number of GAs (average/case, range)	7(0.29, 0–6)	19 (0.43, 0–7)	10(0.23, 0–3)	91(1.26, 0–6)	127(0.7, 0–7)
SNV	4	7	1	53	65
InDel	3	11	9	30	53
CNV	0	1	0	8	9

ctDNA, circulating tumor DNA; GA, alteration; SNV, single nucleotide variant; InDel, insertion and deletion; CNV, copy number variation.

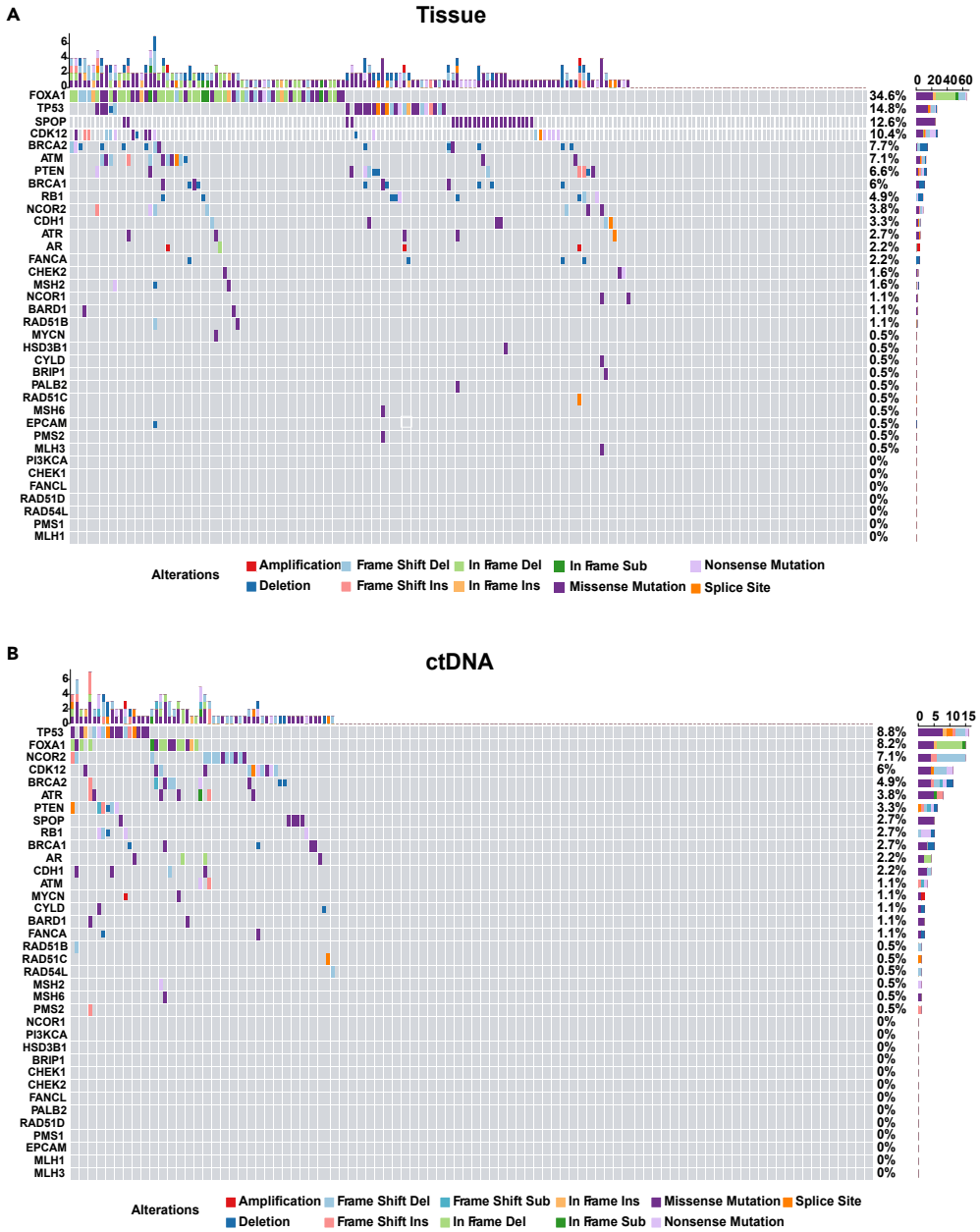

Figure 1. The landscape of somatic genomic alterations in tumor tissue and ctDNA among all patients with HSPC

(A) Somatic alterations in tumor tissue.

(B) Somatic alterations in ctDNA.

while CNVs accounted for only 7.1%. The top five genes with the highest mutation frequency in ctDNA were *TP53*(8.8%), *FOXA1*(8.2%), *NCOR2*(7.1%), *CDK12*(6%), and *BRCA2*(4.9%) (Figure 1B).

Comparison of GAs in tumor tissue and ctDNA

The comparison between somatic GAs detected in ctDNA and tumor tissue revealed that 23.9% (63/264) of GAs detected in tumor tissue were detected by ctDNA, while 49.6% (63/127) of GAs identified in ctDNA were also present in tumor tissue. Moreover, the proportion of GAs identified in both samples increased with disease progression. In Group LP, no shared GAs were found between ctDNA and tumor tissue (Figure 2A). A few GAs identified in tissue were also detected in ctDNA in either Group LND or Group LVD (Figure 2A). However, in Group HVD, 46.8% (58/124) of tissue-derived GAs were detected in ctDNA, and 63.7% (58/91) of ctDNA-derived GAs were also found in tumor tissue (Figure 2A).

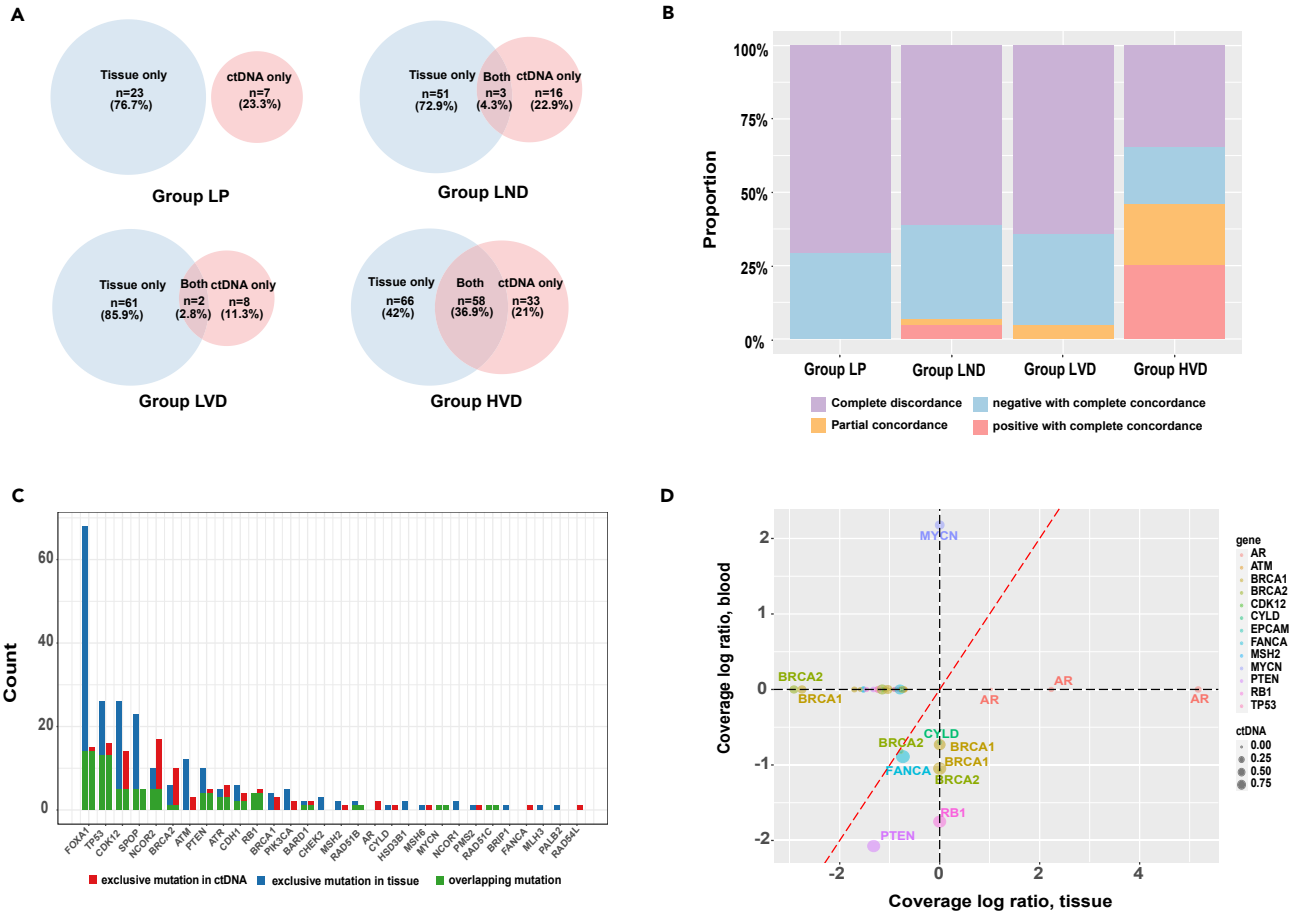

Figure 2. Comparison of somatic genomic alterations detected in tumor tissue and ctDNA

(A) The frequency of shared and exclusive alterations of tumor tissue and ctDNA in subgroups.

(B) Concordance on the individual patient level in subgroups.

(C) Concordance of genomic mutations of genes.

(D) Concordance of copy number variants.

Regarding the concordance at the individual patient level, four categories (definitions as shown in methods) were defined: negative with complete concordance (NCC), positive with complete concordance (PCC), partial concordance (PC), and complete discordance (CD). In Group LP, 29.2% of patients showed NCC, while the remaining 70.8% exhibited CD (Figure 2B). The percentages of NCC, PCC, PC, and CD for Group LN were 31.8%, 4.5%, 2.3%, and 61.4%, respectively (Figure 2B). Among patients in LVD, 30.9% were NCC, 4.8% were PCC, and 64.3% were CD (Figure 2B). In Group HVD, 19.4% of patients showed NCC, 25% had PCC, 20.8% showed PC, and 34.7% exhibited CD (Figure 2B).

At the gene level, 60 somatic mutations from 14 analyzed genes were detected in both ctDNA and tissue (Figure 2C). Four mutations of *RB1*, one mutation of *MYCN*, and *RAD51C* presented in tissue were fully identified in ctDNA. The number of mutations in tissue for *FOXA1*, *ATM*, and *SPOP* was significantly higher than that found in ctDNA ($p < 0.05$). Among 15 genes with no shared mutations in ctDNA and tissue, 6 genes (*CHEK2*, *HSD3B1*, *NCOR1*, BRIP1, *MLH3*, and *PALB2*) were only present in tissue sample, and 3 genes (*AR*, *FANCA*, and *RAD54L*) were only present in ctDNA. The remaining 7 genes (*ATM*, *BRCA1*, *CYLD*, *MSH2*, *MSH6*, *PMS2*, and *PIK3CA*) were detected in both samples but without shared mutations.

As for the CNVs, a total of 3 amplifications and 35 deletions were identified in tissue from 31 patients, while only 1 amplification and 8 deletions were detected in 8 ctDNA samples. The concordance for CNVs is low, with one deletion each for *BRCA2*, *PTEN*, and *FANCA* being shared in ctDNA and tissue (Figure 2D). Among 6 patients with a ctDNA fraction > 35%, 5 CNVs were identified in ctDNA and 4 were detected in tissue. Of those, 2 CNVs were found in both samples.

Discordant GAs in tumor tissue and ctDNA

The majority of somatic GAs were present exclusively in tissue or ctDNA (Figure 3; Table S2). Specifically, 201 out of 264 (76.1%) GAs were exclusively in tumor tissue, including 23(100%) in the Group LP, 51 (94.4%) in the Group LN, 61(96.87%) in Group LVD, and 66(53.2%) in Group

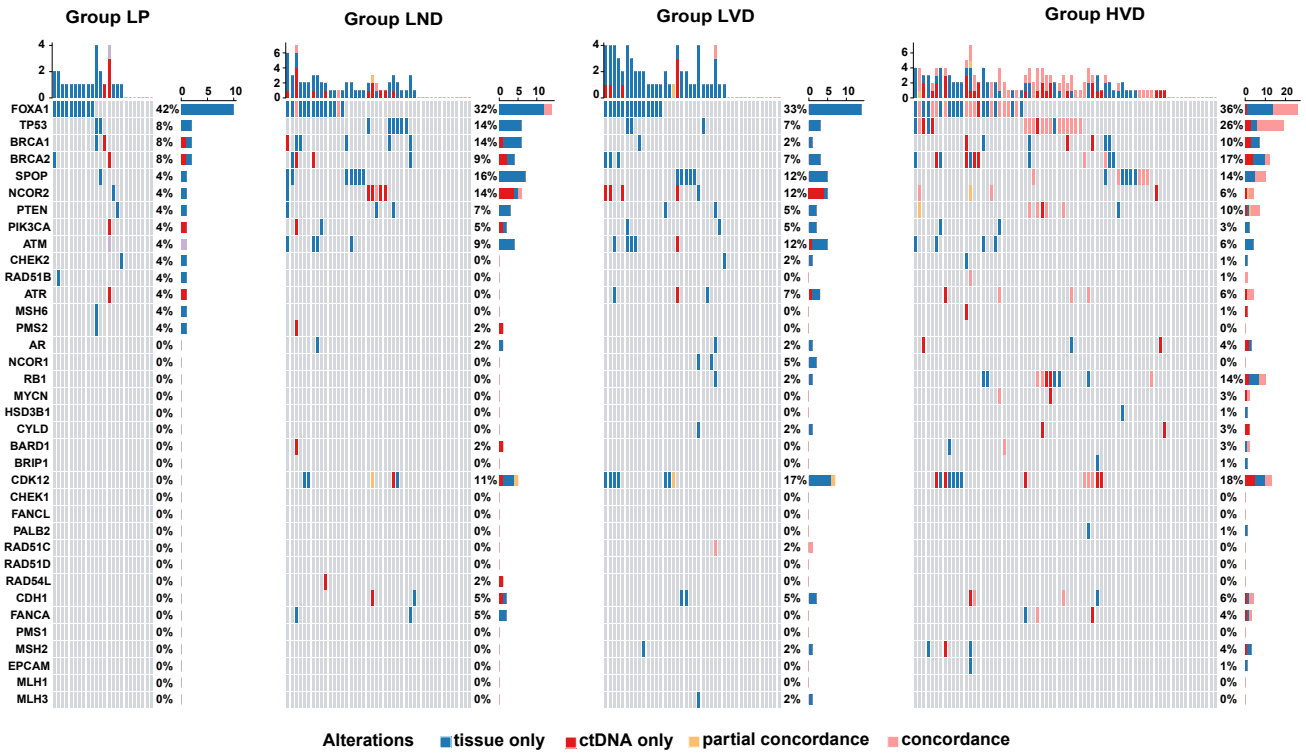

Figure 3. Oncoprint chart of comparison somatic alterations in tumor tissue and ctDNA

HVD. Meanwhile, 64 out of 127 (50.4%) GAs were exclusively detected in ctDNA, including 7 (100%) in Group LP, 16 (84.2%) in Group LN, 8(80%) in Group LVD, and 33(36.2%) in Group HVD.

Clinical outcomes of GAs in combined tumor tissue and ctDNA

The aforementioned analysis revealed the significant complementary role of ctDNA and tumor tissue in detecting somatic GAs in patients with high-volume mHSPC. To investigate the clinical relevance of these GAs, we integrated the results obtained from tumor tissue and ctDNA analysis. Among the Group HVD patients, 36 individuals were identified with pathogenic somatic alterations in genes *BRCA1*, *BRCA2*, *CDK12*, *TP53*, *PTEN*, or *RB1*, either in tissue or ctDNA. These patients exhibited a significantly shorter time to CRPC compared to those without such GAs (median time to CRPC, 12.2 months vs. not reach; HR: 2.97, 95%CI (1.35–6.52); $p < 0.005$; Figure 4A). The risk of developing CRPC was observed to escalate with the number of cumulative GAs in genes *BRCA1*, *BRCA2*, *CDK12*, *TP53*, *PTEN*, or *RB1*. Patients with multiple GAs had a significantly higher risk of developing CRPC compared to those with a single alteration or no alteration (single GA vs. negative, HR: 2.07, 95%CI (0.95–5.72); multiple GAs vs. negative, HR: 3.89, 95%CI (1.64–9.26); Figure 4B). After adjusting for clinicopathological factors, the results revealed an independent association between the presence of GAs in genes *BRCA1*, *BRCA2*, *CDK12*, *TP53*, *PTEN*, or *RB1* and time to CRPC (HR: 3.3, 95%CI (1.5–7.3); $p = 0.004$; Table 3).

DISCUSSION

This study presented the largest real-world cohort to investigate somatic genomic landscape, clinical application value of ctDNA across different disease stages of HSPC. The results confirmed low abundance of ctDNA in patients with localized and low metastatic burden HSPC. ctDNA analysis demonstrated the potential to capture GAs and exhibited higher concordance with tumor tissue in patients with high-volume mHSPC. The findings also highlighted that while there were shared GAs between ctDNA and tumor tissue, several clinically relevant GAs are exclusive to either in ctDNA or tumor tissue in high-volume mHSPC. Moreover, the integration of tumor tissue and ctDNA analysis in patients with high-volume metastatic disease revealed the clinically relevant implications of GAs in genes *BRCA1*, *BRCA2*, *CDK12*, *TP53*, *PTEN*, or *RB1*. The presence of these GAs was associated with a shorter time to CRPC, and the risk of CRPC development increases with the number of cumulative GAs.

In recent years, ctDNA is rapidly evolving and may provide clinical utility across the entire spectrum of cancer care, including cancer screening, prognosis, predicting response to targeted therapy, and monitoring treatment response and resistance.[15] Previous studies reported high ctDNA levels were observed in the majority of patients with metastatic (mCRPC).[17,19] Moreover, studies have demonstrated that high ctDNA levels are associated with increased tumor burden and worse clinical outcomes in PCa.[18] It has been shown that ctDNA levels

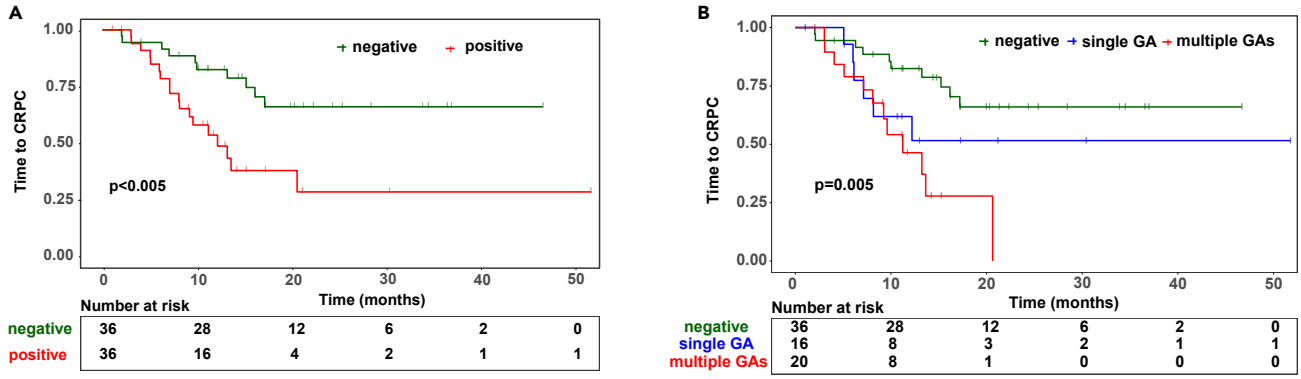

Figure 4. Kaplan-Meier analysis for time to CRPC in high-volume mHSPC depending on somatic *BRCA1/BRCA2/CDK12/TP53/PTEN/RB1* alteration status in either tumor tissue or ctDNA

(A) negative versus positive.

(B) negative versus single alteration and multiple alterations.

are low in localized PCa.[20,21] In the present real-world study, 31.3% of patients in ctDNA had detectable GAs. The number of GAs detected in ctDNA was lower than in tumor tissue. The detection rate of GAs in ctDNA was significantly lower in Group LP, LND, and LVD than in Group HVD.

Wyatt et al. reported a high concordance of 93.6% between GAs detected in tumor tissue and ctDNA in mCRPC.[19] In our prior investigation, we observed a moderate level of concordance in tumor and in ctDNA and depends on ctDNA proportion in aggressive-variant PCa.[22] In *de novo* mHSPC, a previous study identified 80% concordance between GAs detected in blood sample and tissue from the primary tumor.[23] The present study showed low abundance ctDNA in localized and low metastatic burden PCa. In patients with high-volume disease, 54.2% were ctDNA positive and 63.7% of GAs were shared with matched tissue. Estimating CNVs in samples with low ctDNA fraction remains a challenge with current technology.[18] Our data showed that fewer CNVs were detected in ctDNA compared with tissue, but the detectable rate for CNV increased when limited to ctDNA fraction > 35%. 2 out of 4 (50%) CNVs in tissue could be found in ctDNA. The relatively low concordant rate in our study may have been caused by several factors. Firstly, most of the patients had low burden disease, resulting in low ctDNA fraction in plasma. Secondly, other than biopsy specimens, radical surgical specimens were evaluated in a few patients. This may lead to high heterogeneity. Lastly, some patients with HSPC receive ADT monotherapy or combined treatments before plasma sample collection, which could reduce ctDNA abundance.

It should be noted that the detection of GAs in HSPC by either biopsy techniques alone is not entirely reliable. In fact, 76.1% of GAs were exclusively in tumor tissue, and 50.4% of GAs were uniquely identified in ctDNA. Interestingly, GAs in *FOXA1*, *SPOP*, and *ATM* were more frequently detected in tumor tissue than in ctDNA. Many significant findings in both ctDNA and tissue are worth mentioning. For example, patients with pathogenic GAs of *BRCA2*(n = 12), *BRCA1*(n = 7), *ATM*(n = 8), *CDK12*(n = 14), *CHEK2*(n = 1), which were exclusively identified in tissue, and those with *BRCA2*(n = 7), *BRCA1*(n = 2), *ATM*(n = 3), *CDK12*(n = 3), *RAD54L*(n = 1), which were exclusively detected in ctDNA, may benefit from PARP inhibitors and platinum-based chemotherapies.[7–10] Additionally, three patients had *AR* amplification in tissue, and one patient had *AR* mutation (p.W742L) in ctDNA, which were reported to have poor responses to next-generation hormonal therapy agents.[13]

Table 3. Univariate and multivariate Cox analysis for the time to CRPC

	Univariate analysis		Multivariate analysis	
Variables	HR (95%CI)	p	HR (95%CI)	p
Age (≥65 vs. <65 years)	1.1 (0.49–2.5)	0.79	0.87 (0.34–2.2)	0.76
PSA Level (≥100 vs. <100 ng/mL)	1.6 (0.75–3.6)	0.22	1.7 (0.71–4.2)	0.23
Gleason Score (>8 vs. ≤ 8)	1.5 (0.37–6.5)	0.56	1.5 (0.32–6.9)	0.6
Prior abiraterone or docetaxel treatment vs. No prior abiraterone or docetaxel treatment	0.6 (0.29–1.3)	0.19	0.7 (0.31–1.6)	0.38
BRCA1/BRCA2/CDK12/TP53/PTEN/RB1 alteration Positive vs. Negative	3 (1.4–6.5)	0.007	3.3 (1.5–7.3)	0.004

HR, hazard ratio; CI, confidence interval; PSA, prostate-specific antigen.

Classical tumor suppressor genes, including *TP53*, *PTEN*, and *RB1*, are associated with aggressive disease and poor clinical outcomes in PCa.[12,18] *CDK12* mutations are reported to be more frequent in Chinese PCa than in Western populations.[3] *CDK12*-mutated PCa showed a high risk of metastasis and short overall survival.[24] Additionally, *BRCA* mutations in PCa are linked to adverse prognostic features and poor clinical outcomes.[25] In our study, the combined analysis of tumor tissue and ctDNA revealed that patients with any pathogenic GAs in genes *BRCA1*, *BRCA2*, *CDK12*, *TP53*, *PTEN*, or *RB1*, experienced a shorter time to CRPC, and the risk of CRPC development increases with the number of cumulative GAs.

Tissue-based genomic profiling is widely acknowledged as the gold standard for guiding therapy decisions in advanced cancer. Nevertheless, it is constrained by limited tissue availability and its incapacity to capture the intricate intratumor spatial and temporal variations. In contrast, ctDNA holds a distinct advantage as it provides access to a "genomic pool" originating from various metastatic sites within the patient. Our study has demonstrated ctDNA- and tumor tissue-based genomic profiling are complementary in aggressive-variant PCa and HSPC.[22] However, it is imperative to acknowledge that ctDNA testing results may still be susceptible to various biological factors. A notable challenge associated with ctDNA testing is the potential for false negatives, particularly in cases of low tumor burden. Our study observed that only 16.4% of patients with a low tumor burden exhibited detectable alterations in ctDNA. Another significant concern in ctDNA testing revolves around clonal hematopoiesis of indeterminate potential, especially in genes associated with *ATM* and *TP53*, which display a high mutation frequency in PCa. Lastly, the reliability of ctDNA testing is compromised when the sample contains a low tumor fraction, hindering the precise assessment of variant allele fractions and the analysis of copy number alterations. Future technological development to address these challenges will be key to advancing the application of ctDNA testing in the routine clinical setting.

The study highlights the complementary role of ctDNA and tumor tissue analysis in detecting clinically relevant somatic GAs in high-volume mHSPC and underscore the potential of incorporating both approaches in clinical practice. These findings hold promise for improving the precision of cancer management and ultimately enhancing patient outcomes in the context of advanced PCa treatment.

Limitations of the study

The study is commendable in its efforts to shed light on the genomic landscape and clinical application value in ctDNA of HSPC. However, it is important to acknowledge and address several limitations that might have influenced the findings and interpretation of the results. Firstly, the retrospective, real-world nature of the study may introduce bias in data collection and imbalances in group sizes, with the sample size in certain groups being significantly smaller than others. Secondly, the distinction between patients who received treatment and those who were treatment-naïve was not made. This could have an impact on the analysis and interpretation of the data, as treatment status may affect the presence and characteristics of ctDNA. Furthermore, the heterogeneity between biopsy and radical surgical specimens was not taken into account when assessing concordance between ctDNA and tumor tissue samples. This heterogeneity could introduce variability in the results and compromise the accuracy of the concordance analysis. Lastly, the decision not to limit the assessment of concordance to patients with detectable ctDNA may have implications for the generalizability of the results. Despite these limitations, the study offers valuable real-world data on the genomic landscape in ctDNA of different disease stages of HSPC. The findings emphasize the complementary role of ctDNA and tumor tissue analysis in detecting clinically relevant GAs and underscore the potential of incorporating both approaches in clinical practice in advanced PCa.

MATERIALS AVAILABILITY

This study did not generate new unique reagents.

Data and code availability

- The raw sequencing data are not openly available due to patient privacy, ethical and legal issues. Data are available on request sharing by sending requests to the lead author Bin yang (yangbnju@gmail.com), which will need the approval of institutional ethical committees. The remaining data are available in the manuscript, supplemental information.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this work is available from the lead contact upon request.

Experimental model and study participant details

Study design and patients

Between December 2016 and April 2021, patients with localized, regional, and distant metastatic HSPC in Shanghai Tenth People's Hospital, Renji Hospital of Shanghai Jiaotong University School of Medicine, and the First Affiliated Hospital of Wenzhou Medical University who underwent genomic testing on blood samples and matched tumor tissue from biopsy or radical prostatectomy were identified. In total, 182 male patients, aged 35 to 67, were included. All individuals were Han Chinese descent and originated from China. Blood samples were collected within one week after diagnosis or one week before surgery. The median time between tumor tissue and plasma ctDNA collection was 3 days. Patients were categorized into four groups based on the extent of metastasis: Group LP (localized PCa), Group LND (lymph node metastases only), Group HVD (high-volume disease, according to CHAARTED trial criteria),[26] and Group LVD (low volume disease, including patients with bone metastases and without high-volume disease). The Institute Ethics Committee approved this study, and written informed consent was obtained from all participants before their inclusion.

Method details

DNA sequencing

DNA of tumor formalin-fixed paraflin-embedded samples was extracted using a QIAamp DNA FFPE Tissue Kit (Qiagen, Inc., Valencia, CA, USA). For blood samples, plasma was isolated by centrifugation at 1,600×g for 10 min and then further centrifuged at 16,000×g for 10 min. ctDNA was extracted from plasma using a QIAamp Circulating Nucleic Acid Kit (Qiagen, Inc., Valencia, CA, USA). Genomic DNA was extracted from white blood cells using a Blood Genomic DNA Mini Kit (Cwbiotech, Beijing, China). All the procedures were following the manufacturer's instructions. Extracted DNA samples were then used for library preparation and quantification guided by KAPA Hyper Prep protocols (Kapa Biosystems, Inc.). Two custom-designed DNA enrichment panels were used, with NimbleGen SeqCap EZ choice probe pool (Roche) used to capture the coding regions of 620 genes and xGen Lockdown Probe Pool (Integrated DNA Technologies, Coralville, IA) used to capture the coding regions of 50 or 66 genes (GloriousMed Clinical Laboratory Co., Ltd.). The libraries were then purified using AM-Pure XP (Beckman) and quantified using a Qubit dsDNA HS Assay Kit (ThermoFisher). The final library was sequenced on the IlluminaNextseq500 or the NovoSeq 6000 platform (Illumina, San Diego, CA).

Bioinformatics analysis

Sequencing adapters were trimmed by Trimmomatic from the raw data.[27] The reads after adapter trimming were then aligned with the human reference genome (hg19) by BWA.[28] Duplicated reads were removed by Picard. Mapped reads were also realigned to the genome by Genome Analysis Tool Kit.[29] Somatic mutations and germline mutations were called by Mutect2 and GATK's Haplotype Caller with a paired workflow and GATK respectively. Variants were then annotated by ANNOVAR and self-development code.[30] An in-house script was used to verify the human identity concordance of paired samples. Somatic copy number alterations were also detected by GATK.

Somatic mutations from ctDNA samples were filtered with the following rules: 1) 10 allele reads support; 2) 1% allele frequency; 3) supporting reads should be below 4 in the WBC control; 4) mutation frequency should be 5 times higher than in the WBC control; 5) mutations should not occur more than 2 times in the PoN; 6) no significant strand bias (GATK parameter FS > 60 for SNP and FS > 200 for indel). Similar filtering rules were applied for somatic mutations from FFPE samples except for allele frequency which was required to be over 5% and mutation frequency which was required to be 8 times higher than in the white blood cell (WBC) control. Functional filtering removed variants located in non-coding regions and synonymous mutations were removed for downstream analysis. A log2 ratio of more than 0.6 was considered a copy gain event for the AR gene. A log2 ratio less than −0.7 was considered a copy loss. Germline variants called by GATK on WBC samples were first filtered with a threshold of minimum coverage of 50x and allele frequency of over 30%.

Variants not on coding regions and synonymous mutations annotated with ANNOVAR were filtered out. Further, variants with over 0.1% population minor allele frequency annotated by the ExAC database were considered less functional and ignored in the downstream analysis. Mutations were considered deleterious if they were nonsense/stop-gains, frameshift insertions and deletions, and ±1, 2 splice-site variants , or were reported as pathogenic or likely pathogenic in the ClinVar database.

ctDNA fractions (ctDNA%) were estimated based on the allele fractions of somatic mutations.[23] In diploid chromosomes, the mutant allele fraction (MAF) and ctDNA% are related asMAF = (ctDNA×1)/[(1 - ctDNA)×2 + ctDNA×1], and so ctDNA = 2/(1/MAF +1). In haploid chromosomes, ctDNA = MAF. The final ctDNA% estimate for a sample was based on the somatic mutation that yielded the highest ctDNA% estimate.

Concordance assessment

The concordance of somatic GAs between ctDNA and tumor tissue was categorized into four categories for each patient: (1) negative with complete concordance (NCC), where both ctDNA and tumor tissue did not detect any GAs; (2) positive with complete concordance on (PCC), where both ctDNA and tumor tissue had at least one alteration and the total number of GAs in ctDNA were completely matched those in tumor tissue; (3) partial concordance (PC), where ctDNA have one concordant alteration and at least one discordant alteration in the same gene as tumor tissue; and (4) complete discordance (CD), where there were no overlapping GAs between ctDNA and tumor tissue.

Quantification and statistical analysis

The statistical analysis was conducted in R programming language (v.3.6). All analyses focus on 36 clinically relevant genes in PCa (Table S3). Gene unique to one biopsy type was identified using two-sided Fisher exact tests. Time to CRPC was calculated from the time of initial treatment to the diagnosis of castration resistance, following the European Association of Urology (EAU) guidelines (2022 edition). We estimated survival curves using the Kaplan-Meier method and identified differences between subgroups using the log rank test. Cox regression analyses were used to assess the association of GAs with time to CRPC and calculate the respective hazard ratio (HR) and 95% confidence intervals (CI). A p value less than 0.05 was considered statistically significant.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.108931.

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AUTHOR CONTRIBUTIONS

X.Y., W.X., and B.Y. designed and supervised the study. B.Y., B.D, W.C, G.Y., J.X., C.G., R.W., H.W., and B.P. collected samples and acquired the clinical data. B.Y., T.Z., and L.H. analyzed and interpreted the data. B.Y. and T.Z. drafted the manuscript. B.Y., T.Z., B.D., and W.C. revised the manuscript. All authors approved the final version of the manuscript.

DECLARATION OF INTERESTS

L.H. was employed by GloriousMed Clinical Laboratory Co., Ltd.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Blood and Tumor tissue samples from biopsy or radical prostatectomy of 182 patients	This study	Table S1
Chemicals, peptides, and recombinant proteins		
QIAamp DNA FFPE Tissue Ki	Qiagen	56404
QIAamp Circulating Nucleic Acid Kit	Qiagen	55114
Blood Genomic DNA Mini Kit	Cwbiotech	CW2087M
nuclease-free water	invitrogen	AM9932
Tris-EDTA buffer solution	sigma	93283-500ML
20X PBS Buffer	Sangon	B548117-0500
KAPA Hyper Prep Kit	Roche	KK8504
KAPA HiFi Hot Start Ready Mix	Roche	KK2602
Universal P5/P7 Primer	IDT	105513669
Hieff NGS DNA Selection beads	Yeasen	12601ES56
xGen Lockdown Probe Pool	IDT	customized
NimbleGen SeqCap EZ choice kit	Roche	customized
Software and algorithms		
Trimmomatic	Bolger et al., 2014[27]	https://github.com/usadellab/Trimmomatic
Burrows-Wheeler Alignment tool	Li et al., 2009[28]	https://bio-bwa.sourceforge.net/
Genome Analysis Toolkit	McKenna et al., 2010[29]	https://github.com/broadinstitute/gatk/releases
ANNOVAR	Wang et al., 2010[30]	https://www.openbioinformatics.org/annovar/annovar_download_form.php
R v3.6	R project	https://www.r-project.org/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact: Bin Yang (yangbnju@gmail.com).