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Genomic mutation profiling using liquid biopsy in Korean patients with prostate cancer: Circulating tumor DNA mutation predicts the development of castration resistance

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Purpose: To investigate germline and somatic mutation profiles in Korean patients with prostate cancer using liquid biopsy and solid tissue testing and to evaluate the prognostic value of circulating tumor DNA (ctDNA) in predicting castration resistance in patients with metastatic hormone-sensitive prostate cancer (mHSPC).

Materials and Methods: Plasma samples from 56 prostate cancer patients were subjected to next-generation sequencing (NGS) to identify germline mutations and ctDNA analysis using liquid biopsy to detect somatic mutations. Additionally, paired solid cancer tissues from 18 patients were subject to NGS to detect somatic mutations. The clinical parameters and ctDNA profiles of patients with mHSPC were analyzed to evaluate the prognostic value of ctDNA mutations with respect to predicting castration resistance using Cox proportional hazards regression analysis.

Results: Germline mutations occurred in 3.6% of the patients in this cohort, with mutations identified in *RAD50* (1.8%) and *BRCA1* (1.8%). Somatic mutations detected by liquid biopsy and solid tissue testing were common in *TP53* (12.5%), *PIK3CA* (3.6%), and *TMPRSS2-ERG* (3.6%). Of the 18 patients with paired tissue testing, two patients had at least one identical somatic mutation in both the liquid biopsy and solid tissue testing. In patients with mHSPC, the presence of ctDNA mutations could independently predict the castration resistance development (hazard ratio, 13.048; 95% confidential interval, 1.109–153.505; p=0.041).

Conclusions: Korean patients with prostate cancer showed a relatively low germline mutation rate compared to other ethnicities. The ctDNA mutations detected by liquid biopsy can predict the development of castration resistance in patients with mHSPC.

Keywords: Biomarkers; Circulating tumor DNA; Prostatic neoplasms; Prostatic neoplasms, castration-resistant

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INTRODUCTION

Prostate cancer (PCa), which is the most common non-skin malignancy in the United States of America (USA), accounts for 20% of all cancers among males. Rapid advances in genomic analysis over the last decade have enabled the identification of genetic factors that contribute to the development and progression of PCa [1-3]. With this rapid evolution of knowledge, germline and somatic testing in patients with PCa have been incorporated into the National Comprehensive Cancer Network guidelines in certain clinical scenarios due to treatment and family implications. However, most genetic studies have mostly been derived from Western populations, there have been relatively few studies on the genetic mutation profiles of Korean patients with PCa [4].

Blood from cancer patients may contain circulating tumor cells and circulating tumor DNA (ctDNA) shed from the primary tumor or different metastatic sites that circulate through the bloodstream [5]. This ctDNA can be detected using various genomic technologies, including next-generation sequencing (NGS) and polymerase chain reaction (PCR). This ctDNA analysis is considered a real-time "liquid biopsy" for patients with cancer [6]. In contrast to tissue biopsy, liquid biopsy is minimally invasive meaning that it is readily available at different time points. In PCa, liquid biopsy has predominantly been applied to the study of metastatic castration-resistant PCa (mCRPC) as this is a reliable method for estimating tumor burden and can be used as a prognostic indicator for treatment [7-11].

In this context, we investigated the germline and somatic mutation profiles in a relatively large number of Korean patients with PCa. Specifically, ctDNA was analyzed by liquid biopsy technique, and paired solid cancer tissues were also analyzed using NGS. In addition, we evaluated the prognostic value of ctDNA in the prediction of castration resistance in patients with metastatic hormone-sensitive PCa (mHSPC).

MATERIALS AND METHODS

1. Study design and patient cohort

This study was approved by the Institutional Review Board of the Samsung Medical Center (approval number: 2017-03-097). All participants provided their informed consent for genetic analysis. This cohort was comprised of patients with PCa who underwent genomic analysis of germline mutation by NGS and somatic mutation by liquid biopsy and/or paired solid cancer tissue testing at the Samsung Medical Center between November 2017 and February 2020. Clinicopathological information and biological specimens (plasma samples and PCa tissues) collected at the time of prostate biopsy for initial cancer diagnosis or during systemic treatment were stored for all prospective participants throughout the duration of this study. The plasma samples from 56 of these patients were subjected to NGS to detect the germline hereditary genes, and liquid biopsy (Oncomine pan-cancer cell-free assay) to analyze somatic mutations in ctDNA. The paired solid cancer tissues from 18 patients were also subjected to solid tissue testing (Oncomine Comprehensive Assay) to detect somatic mutations. All the 18 patients had whole gland PCa in pre-biopsy magnetic resonance imaging, and the cancer tissues were obtained through transrectal ultrasonography-guided biopsies. Clinical and histological staging was performed based on the American Joint Committee on Cancer TNM staging system for PCa (eighth ed., 2017). This staging system defines tumors exhibiting regional lymph node metastasis (any T, N1, and M0) as stage IVA, and those exhibiting distant metastasis (any T, any N, and M1) as stage IVB.

To evaluate the prognostic value of ctDNA mutations in predicting the development of castration resistance in patients with mHSPC, patients who were initially diagnosed with stage IVB PCa and underwent androgen deprivation therapy (ADT) or chemo-hormonal therapy as first-line therapy were analyzed. Castration resistance was defined as PCa exhibiting clinical, radiographical, or biochemical progression despite castration level serum testosterone concentrations (<50 ng/dL) [12].

2. NGS assay for detecting germline mutations

DNA was extracted from the peripheral blood of patients. NGS libraries were constructed using this DNA and subjected to targeted sequencing with a customized panel (Celemics, Seoul, Korea) comprising 53 genes (Supplementary Table 1). DNA sequencing was performed on an Illumina MiSeq sequencer. The mean sequencing coverage depth was more than 100× for all case series. Paired-end reads (100 bp) were aligned to the hg19 reference human genome using the Burrows–Wheeler Aligner. The genes were annotated using ANNOVAR (http://annovar.openbioinformatics.org/en/latest). The pathogenicity of the germline variants was determined in accordance with established American College of Medical Genetics and Genomics and Association for Molecular Pathology consensus criteria and International Agency for Research on Cancer guidelines. At least two independent expert reviewers compared all variants against those reported in published literature and public databases, including ClinVar and variant-specific databases, and population frequency databases, including 1000 Genomes and the Exome Aggrega-

tion Consortium. All of the high- and moderate-penetrance variants which could be classified as pathogenic or likely to be pathogenic mutations are reported here.

3. ctDNA analysis using the Oncomine pan-cancer cell-free assay

DNA was extracted from plasma samples using the chemagic cfDNA (cell-free DNA) 2k Kit (PerkinElmer Inc., Waltham, MA, USA). This cfDNA was then used for library preparation. Each library was analyzed using the Oncomine pan-cancer cell-free assay (Thermo Fisher Scientific, Waltham, MA, USA). Library templating and sequencing was performed on the Ion Chef and the Ion GeneStudio S5 Plus, respectively. The Oncomine pan-cancer cell-free assay covers a wide range of gene alterations that impact therapeutic outcomes, with the panel in this study covering 56 genes (Supplementary Table 2).

4. Solid tissue testing using the Oncomine comprehensive assay

DNA and total RNA were extracted from cancer tissues using the Recover All™ Multi-Sample RNA/DNA Workflow (Invitrogen, Waltham, MA, USA), with all extractions performed in accordance with the manufacturer's instructions. The library was prepared using an Oncomine comprehensive panel (Thermo Fisher Scientific) comprising 143 genes and covering hotspot mutations in 73 genes, all the coding exons of 26 tumor suppressor genes, and copy number gains in 49 genes (Supplementary Table 3). DNA-specific amplicons were generated using 2,530 amplicons from two primer pools and clonal amplification of the libraries was performed using emulsion PCR and the Ion Chef System (Thermo Fisher Scientific). The prepared libraries were then sequenced on an Ion S5 XL Sequencer using an Ion 540 Chip. Eight samples were multiplexed per run on each Ion 540 Chip. Data were analyzed using Torrent Suite v (Thermo Fisher Scientific) and Ion Reporter v5.4 (Thermo Fisher Scientific). Oncomine Comprehensive v1-540-w2.3.1-DNA and Fusions-Single Samples were used to detect low-frequency somatic variants (single nucleotide variations, indels, and copy number alterations) and fusions. All of the detected variants were annotated using Oncomine Variant Annotator 5.4, which annotates the variants as follows: hotspots, when the variant is a predefined mutation in one of the 73 hotspot genes or 26 tumor suppressor genes; deleterious, when a variant creates a novel truncated mutation in one of the 26 tumor suppressor genes; amplification, when the minimum ploidy gain (at 5% confidence interval) is ≥2 in one of the 49 copy number gain genes.

5. Statistical analysis

Patient and disease characteristics were summarized as number (%) of patients or median (interquartile range) of values. The Kaplan–Meier curves describing the association between ctDNA mutations and castration resistance-free survival was then generated. We analyzed the prognostic value of ctDNA mutations in the prediction of castration resistance in patients with mHSPC using univariate and multivariate Cox proportional hazards regression analyses. Potential clinical predictors, including age, initial prostate-specific antigen (PSA) values, Gleason grade group (GGG) at the time of biopsy, presence of ctDNA mutations, disease volume (low-volume versus high-volume) based on the CHAARTED study [13], and treatment type (ADT alone versus chemo-hormonal therapy) were included in these analyses. High-volume disease was defined as the presence of visceral metastases and/or ≥4 bone lesions with ≥1 lesion outside the vertebral column and/or pelvis [13]. The cutoff points for continuous variables were determined using the median value for each variable. All statistical analyses were performed using Rex (Version 3.3.0; RexSoft Inc., Seoul, Korea) and a user interface for R (R Core Team, Vienna, Austria; https://www.r-project.org/). All p-values were determined using two-tailed tests. Differences were considered statistically significant when the p-value was less than 0.05.

RESULTS

1. Patient characteristics

Table 1 shows the baseline clinicopathological characteristics of the 56 patients subjected to the genomic analyses. Specimens from 52 of these patients were collected at the time of initial prostate biopsy, while the specimens from four patients were collected during systemic treatment. At the time of diagnosis, 11 (21.2%) patients exhibited regional lymph node metastasis (stage IVA) and 24 (46.2%) patients exhibited distant metastasis (stage IVB). Among the four patients whose specimens were collected during systemic treatment, there was one non-metastatic castration-resistant PCa (M0CRPC) patient and three mCRPC patients.

2. Prevalence of germline and somatic mutations

Of the 56 patients profiled, two (3.6%) patients each had one germline mutation (Tables 2, 3) in *RAD50* (splicing mutation: NM_005732.3:c.3036+1G>A) and *BRCA1* (nonsense mutation: p.Ser1007Ter). The analysis of ctDNA using liquid biopsy revealed 19 somatic mutations in 14 (25.0%) patients (Tables 2, 3). The highest prevalence of ctDNA somatic mutations was observed in patients with clinical stage IVB

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Table 1. Patient characteristics at the time of specimen sampling (n=56)

Characteristic	Value
Initial prostate biopsy (n=52)	
Age (y)	71 (65–77)
PSA level (ng/dL)	65 (23–235)
Prostate volume (cm^3)	39 (34–55)
PSAD (ng/dL/cm^3)	1.49 (0.47–5.49)
Family history of cancer	11 (21.2)
Prostate cancer	1 (1.9)
Gastric cancer	3 (5.8)
Colorectal cancer	1 (1.9)
Breast cancer	1 (1.9)
Endometrial cancer	1 (1.9)
Other cancers	4 (7.7)
Biopsy Gleason grade group	
1	3 (5.8)
2	1 (1.9)
3	3 (5.8)
4	19 (36.5)
5	26 (50.0)
Clinical stage	
Stage II	3 (5.8)
Stage III	14 (26.9)
Stage IVA (N1M0)	11 (21.2)
Stage IVB (M1)	24 (46.2)
During systemic treatment (n=4)	
Age (y)	77 (74–78)
PSA level (ng/dL)	6.2 (4.7–7.0)
Family history of cancer	0 (0.0)
Initial treatment	
Prostatectomy	1 (25.0)
Radiation therapy	1 (25.0)
Androgen-deprivation therapy	2 (50.0)
Status at specimen sampling	
M0CRPC	1 (25.0)
mCPRC	3 (75.0)

Values are presented as median (interquartile range) or number (%).
PSA, prostate-specific antigen; PSAD, PSA density; M0CRPC, non-metastatic castration-resistant prostate cancer; mCRPC, metastatic castration-resistant prostate cancer.

Table 2. Prevalence of germline and somatic mutations

Characteristic	Prevalence (%)	n
Germline mutation	3.6	2/56
Somatic mutation		
ctDNA analysis		
Total	25.0	14/56
Stage II	0.0	0/3
Stage III	28.6	4/14
Stage IVA	9.1	1/11
Stage IVB	33.3	8/24
M0CRPC	0.0	0/1
mCPRC	33.3	1/3
Solid tissue testing		
Total	27.8	5/18
Stage III	60.0	3/5
Stage IVA	0.0	0/2
Stage IVB	20.0	2/10
mCRPC	0.0	0/1

ctDNA, circulating tumor DNA; M0CRPC, non-metastatic castration-resistant prostate cancer; mCRPC, metastatic castration-resistant prostate cancer.

cancer and those with mCRPC (33.3% for both). The analysis of the 18 paired PCa tissue biopsy specimens revealed eight somatic mutations in five (27.8%) patients (Tables 2, 3). Of these 18 patients with concurrent tissue testing, one identical mutation was observed in both the liquid and tissue biopsy specimens from two patients. In one patient (patient No. PC18-1126) with stage IVB cancer, tissue biopsy specimen analysis revealed somatic mutations in *PTEN*, *TP53* (pLeu114Ter), and *TMPRSS2-ERG* (*E4*), while the liquid biopsy specimen analysis identified a somatic mutation in *PTEN*. In another patient (patient No. PC19-0816) with stage III cancer, the tissue biopsy specimen analysis revealed mutations in *TP53* and *BRCA1*, while the liquid biopsy analysis identified a somatic mutation in *TP53*.

Fig. 1 shows the somatic mutations detected in this study. Somatic mutations were most prevalent in *TP53* (12.5%), followed by *PIK3CA* (3.6%) and *TMPRSS2-ERG* (3.6%). Six types of gene amplification events were detected in four patients. Of these, *MYC* amplification, *FGFR3* amplification, and *MET* alteration—all of which are the subject of ongoing clinical trials [14]—were observed in 4.24%, 0.27%, and 0.9% of these cases, respectively.

3. Identifying the prognostic factors for predicting the development of castration resistance

Among the 24 patients with mHSPC, one patient was not treated and lost to follow-up. Therefore, 23 patients were evaluated to identify the prognostic factors responsible for the development of castration resistance. The clinicopathological data and the ctDNA mutation profiles of these patients are listed in Table 4. Kaplan–Meier curves using log-rank tests (Fig. 2) revealed that the presence of ctDNA mutations significantly reduced the castration resistance-free survival in mHSPC patients (p=0.010). The results of the univariate and multivariate analyses—evaluating the contribution of relevant variables to castration resistance development—are shown in Table 5. In the multivariate analysis using various clinical parameters, the presence of

Table 3. Clinical and genomic information of patients with germline/somatic mutations

Patient No.	Age (y)	Family history	PSA (ng/dL)	Biopsy GGG	Stage/CRPC	Germline mutation	ctDNA	Tissue
Germline mutation								
PC19-0709	67		36	5	3B	*RAD50*		
PC19-0816	77	Esophageal cancer	24	4	3A	*BRCA1*		
Somatic mutation								
PC18-0918	70	Breast cancer	155	5	IVB		U/D	*TMPRSS2-ERG*
PC18-0927	77		6	4	mCRPC		*CCND2*	N/T
PC18-1113	71		48	4	IVA		*PIK3CA, TP53*	N/T
PC18-1126	80		37	5	IVB		*PTEN*	*PTEN,TP53, TMPRSS2-ERG*
PC19-0211	77		7	5	III		*TP53, CCND3*	U/D
PC19-0402	63		467	4	IVB		*PIK3CA*	U/D
PC19-0709	67		36	5	III		U/D	*TET2*
PC19-0729	66	Multiple myeloma	540	3	IVB		*TP53*	U/D
PC19-0816	77	Esophageal cancer	24	4	III		*TP53*	*TP53, BRCA1*
PC19-0822	82	Liver cancer	74	5	IVB		*HRAS, TP53*	N/T
PC19-0826	81		2,137	4	IVB		*MYC, CCND1*	U/D
PC19-1008	84		364	5	IVB		*FGFR3, MET*	N/T
PC19-1016	67		2,232	5	IVB		*IDH1*	N/T
PC19-1204	74		325	5	IVB		*SMAD4*	U/D
PC19-1209	79		120	5	III		*SF3B1*	*MED12*
PC20-0204	65		33	4	III		*TP53*	N/T

PSA, prostate-specific antigen; GGG, Gleason grade group; CRPC, castration-resistant prostate cancer; ctDNA, circulating tumor DNA; U/D, mutation undetectable; N/T, no test.
Only patients with detected genetic mutation are presented in the table.

Fig. 1. Somatic mutations detected by liquid biopsy and solid cancer tissue testing in 56 patients with prostate cancer. ctDNA, circulating tumor DNA. Only patients with detected genetic mutation are presented in the figure.

ctDNA mutations was the only independent predictive factor for the development of castration resistance (positive versus negative; hazard ratio, 13.048; 95% confidential interval, 1.109–153.505; p=0.041). ctDNA mutations in patients with castration resistance were observed in *MYC*, *CCND1*, *HRAS*, *TP53*, *FGFR3*, *MET*, and *IDH1*. Only ctDNA mutations in *PIK3CA*, *TP53*, and *SMAD4* were observed in patients who did not develop castration resistance during the study period.

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Table 4. Clinicopathological characteristics and ctDNA mutation status of patients with mHSPC (n=23)

Characteristic	Value
Age (y)	70 (60–81)
PSA level (ng/dL)	325 (60–747)
Biopsy Gleason grade group	
3	1 (4.3)
4	9 (39.1)
5	13 (56.5)
ctDNA mutation	
Negative	16 (69.6)
Positive	7 (30.4)
Disease volume	
Low-volume	8 (34.8)
High-volume	15 (65.2)
Treatment	
ADT alone	12 (52.2)
ADT+Docetaxel	11 (47.8)
Observation period (mo)	6 (4–14)
Progression to CRPC	8 (34.8)

Values are presented as median (interquartile range) or number (%).
ctDNA, circulating tumor DNA; mHSPC, metastatic hormone-sensitive prostate cancer; PSA, prostate-specific antigen; ADT, androgen deprivation therapy; CRPC, castration-resistant prostate cancer.

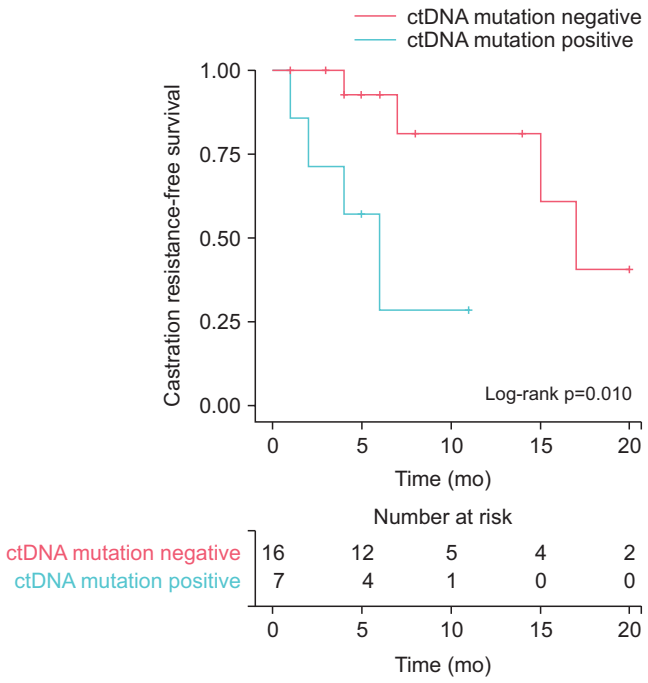

Fig. 2. Kaplan–Meier curve for castration resistance-free survival based on ctDNA analysis. ctDNA, circulating tumor DNA.

DISCUSSION

To the best of our knowledge, this is the first study to report germline and somatic mutation profiles using both liquid biopsy and solid tissue testing in a relatively large number of Korean patients with PCa. Additionally, this study investigated the clinical utility of liquid biopsy for predicting castration resistance in patients with mHSPC.

The prevalence of germline mutations in this cohort was 3.6%, with germline mutations detected in *RAD50* and *BRCA1*. The Korean patients with PCa in this study showed a lower germline mutation rate than that shown by patients of other ethnicities as reported in previous studies. A previous study on a Western population revealed that the prevalence of germline mutations among patients with metastatic PCa was approximately 11.8%, and that most germline mutations were detected in *BRCA2*, *ATM3*, *CHECK2*, and *BRCA1* [15]. Recently, a study on multi-racial and multi-ethnic populations examined the prevalence of germline mutations in 3,706 patients with PCa (2,594 Caucasian, 234 Jewish, 227 African American, 78 Hispanic, 73 Asian, and 401 males who did not belong to any of these racial groups) [16]. The incidence of the genetic alterations among the Caucasian (17.8%) and Jewish (22.2%) populations was higher than that in the other ethnic groups. In the Asian cohort of the study, the prevalence of germline mutations was 15.1% and mutations were most frequently detected in *BRCA2* (4.1%), *BRCA1* (2.7%), and *ATM* (2.7%).

We successfully performed ctDNA analysis at different disease stages using the Oncomine Pan-Cancer cell-free assay. The prevalence of ctDNA mutations (33.3%) in patients with metastatic diseases (stage IVB and mCRPC) was higher than that of patients with localized disease. The analysis of paired PCa tissue specimens revealed eight somatic mutations in five (27.8%) patients, and all these mutations were detected in patients with clinical stage III and IVB cancers. Meanwhile, the Oncomine pan-cancer cell-free assay for liquid biopsy and Oncomine comprehensive assay for solid tissue testing do not target the same genomic regions. Among 18 patients with paired analyses, two patients exhibited at least one concordant genomic mutation in both the liquid and tissue biopsy specimens. In one patient, as the *TP53* (pLeu114Ter) mutation was not covered in the Oncomine pan-cancer cell-free assay, the tissue biopsy analysis revealed mutations in *PTEN*, *TP53* (pLeu114Ter), and *TMPRSS2-ERG* (*E4*), while the liquid biopsy analysis identified a mutation in *PTEN*. In another patient, as *BRCA1* gene is not included in the Oncomine pan-cancer cell-free assay, the liquid biopsy analysis revealed a mutation in *TP53*, while the tissue biopsy analysis revealed mutations in *TP53* and *BRCA1*, as the Oncomine comprehensive assay identified somatic mutations in *BRCA1* (p.Ser1007Ter).

Table 5. Univariate and multivariate Cox proportional hazards regression analyses to determine the predictive factors for the development of castration resistance

Variable	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age ≥70 (y)	1.460 (0.353–6.040)	0.602	0.845 (0.121–5.898)	0.865
PSA ≥325 (ng/dL)	1.099 (0.265–4.559)	0.896	0.963 (0.116–8.024)	0.972
Biopsy Gleason grade group				
3–4	Reference		Reference	
5	6.005 (0.721–50.022)	0.097	8.327 (0.771–89.910)	0.081
ctDNA mutation				
Negative	Reference		Reference	
Positive	7.171 (1.260–40.816)	0.026	13.048 (1.109–153.505)	0.041
Disease volume				
Low-volume	Reference		Reference	
High-volume	1.716 (0.343–8.598)	0.511	0.620 (0.042–9.201)	0.728
Treatment				
ADT alone	Reference		Reference	
ADT+Docetaxel	0.537 (0.118–2.455)	0.423	0.848 (0.130–5.537)	0.864

HR, hazard ratio; CI, confidence interval; PSA, prostate-specific antigen; ctDNA, circulating tumor DNA; ADT, androgen deprivation therapy.

Liquid biopsy for analyzing ctDNA is a minimally invasive technique used to access real-time information with respect to disease burden, prognosis, and response to therapy. In the case of PCa, liquid biopsy has been mainly used to study patients with mCRPC. Several studies have demonstrated that circulating tumor cell counts and ctDNA mutations can predict the effect of treatment on survival in patients with mCRPC [7-10,17]. A recent study in Canada analyzed the ctDNA of 202 patients with mCRPC who underwent androgen receptor-directed therapies (abiraterone acetate or enzalutamide) and reported that mutations in *BRCA2* and *ATM* were associated with poor response to therapy. Wyatt et al. [11] reported that liquid biopsy specimen analysis of patients with mCRPC can reveal the genetic profile of metastatic cancer tissues. The authors collected plasma samples from 45 patients with mCRPC at the time of metastatic tissue biopsy and analyzed the somatic mutations identified by liquid biopsy and compared these to the mutations identified in the paired cancer tissue biopsy specimens. The copy number profiles were highly correlated between liquid and paired tissue biopsy specimens, with an 88.9% concordance rate in copy number calls for the clinically actionable genes [11]. On the other hand, our study showed a relatively low concordance rate of liquid and tissue analyses. A possible explanation of the low concordance rate could be earlier PCa stages of the patients included in our study compared to those of other previous studies. Approximately 33% of patients in this study were stage II or stage III diseases at the time of specimen sampling. There was a study that the liquid biopsy has limited applicability in patients with non-metastatic PCa. Klein et al. [18] performed cfDNA targeted sequencing (507 genes), cfDNA whole genome sequencing, and cfDNA whole genome bisulfite sequencing at different stages in multiple tumor types, including PCa. The overall sensitivity was estimated at 98% while specificity was less than 20% in the 65 patients with stage I–III PCa and 75% in the four patients with stage IV PCa [18].

Importantly, regarding the finding of our study, a recent report has demonstrated the clinical utility of the liquid biopsy in detecting genomic instability of cancer. Liu et al. [19] investigated the data from 853 patients in a publicly available data set and 259 patients from an independent cohort of non-small cell lung cancer patients and demonstrated that the presence of allele heterogeneity defined by liquid biopsy can predict unfavorable overall survival. The authors mentioned that it is difficult to fully obtain comprehensive information on tumor heterogeneity; however, ctDNA allele heterogeneity analysis by liquid biopsy provides an easily-accessible biomarker for predicting cancer prognosis. A key finding of our study is that ctDNA mutations can serve as a potential biomarker for predicting the development of castration resistance in patients with mHSPC. Multivariate Cox proportional hazard regression analysis revealed that the presence of ctDNA mutations detected by liquid biopsy was an independent predictor of castration resistance development (hazard ratio, 13.048). Our current liquid biopsy technique is not able to provide comprehensive genomic information about cancer, but this technique can provide ad-

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ditional information, which can be used to supplement solid tissue testing. Furthermore, with a minimally invasive test, this liquid biopsy enables the prediction of castration resistance development by analyzing the genomic instability in patients with mHSPC, which manifests as genetic mutations in ctDNA.

This study has several limitations. First, this study was based on retrospective analysis with all the data supplied by a single institution, which may cause selection bias and the possibility of residual confounding variables. Second, the number of patients who were subjected to paired solid tissue testing was relatively small. Although the number of patients with tissue testing was small, the data are valuable references for evaluating somatic mutation profiles in Korean patients with PCa. Finally, the target regions used in the liquid biopsy and tissue somatic testing vary. Hence, gene mutations detected using liquid biopsy panels do not represent all genetic alterations in patients with cancer. However, this study demonstrated that the presence of ctDNA mutations was the only independent predictive factor for the development of castration resistance in patients with mHSPC, and that ctDNA mutation significantly reduced castration resistance-free survival. Further multicenter studies on the prognostic value of these genetic instabilities and external validation using independent datasets are needed to validate these findings.

CONCLUSIONS

In this study, Korean patients with PCa showed a lower germline mutation rate than other ethnicities. Although it should be noted that the ctDNA analysis using liquid biopsy is not completely representative of the genomic changes within cancer, however, we were able to confirm that the presence of ctDNA mutations detected by liquid biopsy can predict a shorter time to the development of castration resistance in patients with mHSPC. The ctDNA analysis by liquid biopsy might be a useful biomarker for risk stratification in future clinical studies of patients with mHSPC.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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AUTHORS' CONTRIBUTIONS

Research conception and design: Eunhae Cho and Hwang Gyun Jeon. Data acquisition: Joung Eun Lim, Joongwon Choi, and Hwang Gyun Jeon. Statistical analysis: Eunhae Cho and Jiwoong Yu. Data analysis and interpretation: Junnam Lee, Eunhae Cho, and Jiwoong Yu. Drafting of the manuscript: Jiwoong Yu and Eunhae Cho. Critical revision of the manuscript: Eunhae Cho and Hwang Gyun Jeon. Obtaining funding: Hwang Gyun Jeon. Administrative, technical, or material support: Eunhae Cho, Joung Eun Lim, and Junnam Lee. Supervision: Eunhae Cho, Minyong Kang, Hyun Hwan Sung, Byong Chang Jeong, Seong Il Seo, Seong Soo Jeon, Hyun Moo Lee, and Hwang Gyun Jeon. Approval of the final manuscript: Hwang Gyun Jeon.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.4111/icu.20200406.

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