OCT4-positive circulating tumor cells may predict a poor prognosis in patients with metastatic castration-resistant prostate cancer treated with abiraterone plus prednisone therapy

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Received March 31, 2023; Accepted June 30, 2023

DOI: 10.3892/ol.2023.14039

Abstract. Octamer-binding transcription factor 4 (OCT4) and circulating tumor cells (CTCs) are key factors associated with tumor metastasis and drug resistance in cancer. The present prospective study aimed to investigate the prevalence of OCT4-positive ($OCT4^+$) CTCs and the potential association with the clinical features and survival of patients with metastatic castration-resistant prostate cancer (mCRPC) treated with abiraterone + prednisone. In total, 70 patients with mCRPC treated with abiraterone + prednisone were enrolled in the present study and peripheral blood samples were collected prior to treatment initiation to determine CTC count via a Canpatrol system. RNA *in situ* hybridization was performed for $OCT4^+$ CTC quantification. Lactate dehydrogenase (LDH) was detected by automatic biochemical analyzer (AU54000, OLYMPUS). Results demonstrated that 34 (48.6%), 21 (30.0%) and 15 (21.4%) patients harbored $OCT4^+$ ($CTC^+/OCT4^+$) or OCT4-negative CTCs ($CTC^+/OCT4^-$) or were CTC-negative (CTC^-), respectively. Notably, $CTC^+/OCT4^+$ occurrence was associated with visceral metastasis and high levels of LDH. In addition, radiographic progression-free survival [rPFS; median, 15.0, 95% confidence interval (CI), 9.6-20.4 vs. not reached vs. median, 29.5, 95% CI, 18.6-40.4 months; P=0.001] and overall survival (OS) were significantly decreased (median, 27.3, 95% CI, 20.1-34.5 vs. not reached vs. not reached; P=0.016) in $CTC^+/OCT4^+$ compared with $CTC^+/OCT4^-$ and CTC^- patients. Subsequently, the adjustment was performed by multivariate Cox regression models, which revealed that $CTC^+/OCT4^+$ (vs. $CTC^+/OCT4^-$ or CTC^-) was independently associated with decreased rPFS [hazard ratio (HR), 3.833; P<0.001] and OS (HR, 3.938; P=0.008). In conclusion, $OCT4^+$ CTCs were highly prevalent in patients with mCRPC and associated with visceral metastasis and increased levels of LDH. Thus, the presence of $OCT4^+$ CTCs may serve as an independent prognostic factor for patients with mCRPC treated with abiraterone + prednisone.

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Key words: metastatic castration-resistant prostate cancer, octamer-binding transcription factor 4, circulating tumor cells, abiraterone, prognostic value

Introduction

Prostate cancer (PC) is the second most common malignancy after lung cancer and one of the leading causes of death in males worldwide. Notably, there are ~1,200,000 new cases and ~350,000 PC-associated deaths annually (1). In addition, the incidence of PC has risen by 2.75% in China over the past three decades (2,3). Although patients with metastatic PC are often treated with androgen deprivation therapy (ADT) and achieve initial treatment response, 10-20% of patients develop metastatic castration-resistant PC (mCRPC) (4-6).

Abiraterone is the first-line anti-androgen therapy in patients with mCRPC (7,8). However, the prognosis of abiraterone-treated patients with mCRPC remains suboptimal and mCRPC management is complex due to heterogeneity among patients (9). Thus, the identification of novel potential biomarkers is required for predicting survival in abiraterone-treated patients with mCRPC.

Circulating tumor cells (CTCs) originate from primary or metastatic tumor sites and enter the bloodstream, playing a key role in the formation of metastases (10,11). Alterations of specific biomarkers, such as breast cancer susceptibility gene 2 (BRCA2) and ezrin, in CTCs provide novel perspectives for tumor recurrence, metastasis, therapeutic efficacy and prognosis in patients with mCRPC (12,13). In addition, stem cell markers are abnormally expressed in CTCs (14,15). Notably, due to their direct origin from the tumor, CTCs may share similar characteristics with the tumor. Therefore, determination of biomarkers in CTCs may exhibit potential in predicting prognosis of patients with mCRPC in clinical practice (16).

Octamer-binding transcription factor 4 (OCT4), located on chromosome 6p21 in the human genome, is a stem cell marker that serves a crucial role in the carcinogenesis of several types of cancer, including pancreatic cancer, ovarian cancer and breast cancer (17-20). Previous studies demonstrated the prognostic value of OCT4-positive ($OCT4^+$) CTCs in patients with cancer (16,21). Notably, results of a previous study demonstrated that $OCT4^+$ CTCs are associated with advanced stage and distant metastasis in patients with non-small-cell lung

cancer (21). Another study indicated that prevalence of OCT4+ CTCs is increased in patients with pathologically confirmed muscle invasive bladder cancer compared with patients with non-muscle invasive bladder cancer (22). Moreover, previous studies demonstrated that OCT4 facilitates therapeutic resistance to ADT in PC; thus, OCT4 may exhibit potential as a biomarker for predicting survival in abiraterone-treated patients with mCRPC (23-25). To the best of our knowledge, however, research surrounding the clinical role of OCT4+ CTCs in patients with mCRPC treated with abiraterone + prednisone is limited.

The present prospective study aimed to explore the prevalence of OCT4+ CTCs and the potential association of OCT4+ CTCs with clinical features and prognosis of patients with mCRPC treated with abiraterone + prednisone therapy.

Patients and methods

Subjects. From May 2018 to December 2021, 70 patients with mCRPC (aged from 55-89 years old) treated with abiraterone + prednisone were enrolled from Shanghai Songjiang District Sijing Hospital, Shanghai, China. The inclusion criteria were as follows: i) Diagnosed with PC via histological examination; ii) confirmation of CRPC. The CRPC diagnosis was according to the previous study (26); iii) confirmation of mPC via imaging technology; iv) aged >18 years and v) treated with abiraterone + prednisone. The following patient exclusion criteria were used: i) Presence of other primary malignant tumors; ii) absence of adequate organ and bone marrow function and iii) Eastern Cooperative Oncology Group performance status (ECOG PS) score >1 (27). The present study was approved by the Ethics Committee of Shanghai Songjiang District Sijing Hospital (approval no. 20180314sjyy01). All patients provided written informed consent.

Collection and detection of clinical features and samples. Clinical characteristics, such as age, therapeutic history, Gleason (28), International Society of Urological Pathology (ISUP) (29) and ECOG PS score, metastasis status and levels of prostate-specific antigen (PSA), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were obtained from all patients; the level of PSA was detected by electrochemiluminescence immunoassay analyzer (cat. no. E-170; Roche Diagnostics), and the levels of ALP and LDH were detected by automatic biochemical analyzer (cat. no. AU54000; Olympus Corporation). In addition, 10 ml peripheral blood samples were obtained from patients with mCRPC prior to treatment initiation. CTC counts in the peripheral blood samples were detected via a Canpatrol system, as previously described (30). CTC count ≥1 in 5 ml peripheral blood was defined as CTC-positive (CTC^+); CTC count <1 was defined as CTC-negative (CTC^-) (31,32). RNA *in situ* hybridization was used for determining OCT4 expression in CTC^+ samples (21). The capture probe sequences for OCT4 gene were the same as a previous study (21). Briefly, after washing three times with PBS, the probes for epithelial cell adhesion molecule (EpCAM; green color) and OCT4 (red color) were added and allowed to hybridize for 3 h at 40˚C. After washing 3 times with 0.1X SSC buffer (MilliporeSigma), CTCs were incubated with 0.5 fmol preamplification probes in the preamplification buffer (30% horse serum; 1.5% sodium dodecyl sulfate; 3-mM Tris-HCl; pH 8.0) for 30 min. at 40˚C. After washing with 0.1X SSC buffer, CTCs were incubated with 1 fmol amplification probes (sequences shown in Table SI). After washing, nuclei were stained with 4',6'-diamidino-2-phenylindole (DAPI; MilliporeSigma) for 5 min. The cells were observed and images captured under a fluorescence microscope at x400 magnification and counted by the clinicians. $CTC^+/OCT4^+$ was defined as ≥1 CTC expressing OCT4 and $CTC^+/OCT4^-$ was defined as no OCT4 expression observed in CTCs.

Treatment, follow-up and evaluation. Patients with mCRPC were treated with 28-day cycles of abiraterone + prednisone (abiraterone, 1,000 mg/day; prednisone, 10 mg/day). Treatment was discontinued following clinical disease progression, severe toxicity or death. Patients underwent follow-up once every 2 months in the first 6 months, then once every 3 months. The median and mean follow-up durations were 17.9 and 19.6 months, respectively, ranging from 2.1 to 42.5 months. The last follow-up date was August 2022. Based on follow-ups, radiographic progression was evaluated via modified Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 for soft tissue sites (33) or Prostate Cancer Clinical Trials Working Group 2 criteria for bone sites (34). The criteria of radiographic progression were as follows: i) appearance of ≥2 new lesions; ii) first observation of progression by bone scan and iii) progression of soft tissue lesions by computed tomography or magnetic resonance imaging (33,35). Radiographic progression-free survival (rPFS) and overall survival (OS) rates were determined.

Statistical analysis. SPSS (version 26.0; IBM Corp.) was used for data analysis and GraphPad Prism (version 7.01; GraphPad Software, Inc.; Dotmatics) was used for figure construction. The mean ± standard deviation and median (interquartile range) were used to show normal distribution continuous variables and skewed distribution continuous variables, respectively. The number (percentage) was used to show counting variables. Wilcoxon rank sum, χ^2 or Fisher's exact test was used for comparison. Kaplan-Meier curves were constructed to determine rPFS and OS and log-rank or Tarone-Ware tests were used. The small vertical lines in the Kaplan-Meier curve represented censored data, defined as patients who had an event during follow-up and those who had no event by the end of follow-up. All clinical characteristics were included in the Cox models. Factors associated with rPFS and OS were determined using univariate and forward-multivariate Cox regression analysis. In addition, multivariate Cox regression models with backward elimination methods were performed for validation. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics. The mean age of patients was 71.0±8.8 years (Table I). In total, 2 (2.8%), 27 (38.6%) and 41 (58.6%) patients were evaluated as Gleason score ≤6, 7 and

Table I. Clinical characteristics of patients with mCRPC.

Characteristic	Patients with mCRPC (N=70)
Mean age, years, ± SD	71.0±8.8
History of prostatectomy (%)	
No	36.0 (51.4)
Yes	34.0 (48.6)
History of radiotherapy (%)	
No	29.0 (41.4)
Yes	41.0 (58.6)
History of hormone therapy (%)	
No	0.0 (0.0)
Yes	70.0 (100.0)
History of other therapy (%)	
No	62.0 (88.6)
Yes	8.0 (11.4)
Gleason score at initial diagnosis (%)	
≤6	2.0 (2.8)
7	27.0 (38.6)
≥8	41.0 (58.6)
ISUP grade (%)	
1	2.0 (2.8)
2	13.0 (18.6)
3	14.0 (20.0)
4	16.0 (22.9)
5	25.0 (35.7)
ECOG PS score (%)	
0	46.0 (65.7)
1	24.0 (34.3)
Bone metastasis (%)	
No	7.0 (10.0)
Yes	63.0 (90.0)
Lymph node metastasis (%)	
No	31.0 (44.3)
Yes	39.0 (55.7)
Soft tissue metastasis (%)	
No	59.0 (84.3)
Yes	11.0 (15.7)
Visceral metastasis (%)	
No	60.0 (85.7)
Yes	10.0 (14.3)
Median PSA, ng/ml (IQR)	32.1 (16.9-90.5)
Median ALP, IU/l (IQR)	88.6 (64.9-147.6)
Median LDH, IU/l (IQR)	217.7 (170.0-402.0)

mCRPC, metastatic castration-resistant prostate cancer; ISUP, International Society of Urological Pathology; ECOG PS, eastern cooperative oncology group performance status; PSA, prostate-specific antigen; IQR, interquartile range; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

≥8 at initial diagnosis, respectively. A total of 2 (2.8%), 13 (18.6%), 14 (20.0%) 16 (22.9%), and 25 (35.7%) patients were assessed as ISUP grade 1, 2, 3, 4 and 5, respectively. A total of 46 (65.7%) patients were evaluated as ECOG PS score 0 and the remaining 24 (34.3%) patients were assessed as ECOG PS score 1. In addition, 63 (90.0%), 39 (55.7%), 11 (15.7%) and 10 (14.3%) patients experienced bone, lymph node, soft tissue and visceral metastasis, respectively. The median [interquartile range (IQR)] PSA, ALP, and LDH were 32.1 (16.9-90.5) ng/ml, 88.6 (64.9-147.6) IU/l, and 217.7 (170.0-402.0) IU/l, accordingly.

CTC count and OCT4$^+$ CTC quantification. CTC count of patients is displayed in Fig. 1A. The median (IQR) CTC count was 3.5 (1.0-8.0) and the mean CTC count was 8.0±15.3. Moreover, 55 (78.6%) and 15 (21.4%) patients were assessed as CTC$^+$ and CTC$^-$, respectively. Among the 55 CTC$^+$ patients, 34 (61.8%) patients were evaluated as CTC$^+$/OCT4$^+$ and the remaining 21 (38.2%) patients were identified as CTC$^+$/OCT4$^-$ (Fig. 1B). The *in situ* hybridization images were presented in Fig. S1A and B.

Association between CTC count and OCT4$^+$ CTCs with patient characteristics. Elevated CTC count was associated with lymph node (P=0.011) and visceral metastasis (P=0.003), high levels of PSA (P=0.041) and low levels of LDH (P=0.026; Table II). CTC count was not associated with age, history of prostatectomy, radiotherapy or other therapies, Gleason score at initial diagnosis, ISUP grade, ECOG PS score, bone and soft tissue metastasis or ALP (all P>0.05; Table II).

CTC$^+$/OCT4$^+$ was associated with visceral metastasis (P=0.009) and high levels of LDH (P=0.032; Table III). Moreover, there was no association between CTC$^+$/OCT4$^+$ and patient characteristics, such as age, history of prostatectomy, radiotherapy or other therapies, Gleason score at initial diagnosis, ISUP grade, ECOG PS score, bone, lymph node or soft tissue metastasis and PSA or ALP levels (all P>0.050; Table III).

Prognostic value of CTC count and OCT4$^+$ CTCs. A total of 43 (61.4%) patients had PSA progression (defined as the first rise in PSA of 2 ng/ml and 25% above the lowest point). Among them, 36 patients had PSA and radiographic progression and seven patients had PSA progression alone. CTC$^+$ patients exhibited reduced rPFS compared with CTC$^-$ patients (P=0.041). Notably, the median [95% confidence interval (CI)] rPFS of CTC$^+$ and CTC$^-$ patients was 15.2 (9.1-21.3) and 29.5 (18.6-40.4) months, respectively (Fig. 2A). OS was decreased in CTC$^+$ compared with CTC$^-$ patients but this result was not statistically significant (P=0.060). Specifically, the median (95% CI) OS was 31.6 (25.4-37.8) months in CTC$^+$ and not reached in CTC$^-$ patients (Fig. 2B).

Compared with CTC$^+$/OCT4$^-$, rPFS was decreased in CTC$^+$/OCT4$^+$ patients (P=0.003). Median (95% CI) rPFS was 15.0 (9.6-20.4) months in CTC$^+$/OCT4$^+$ patients, and not reached in CTC$^+$/OCT4$^-$ patients (Fig. 3A). In addition, OS was decreased in CTC$^+$/OCT4$^+$ compared with CTC$^+$/OCT4$^-$ patients (P=0.049). Specifically, the median (95% CI) OS of CTC$^+$/OCT4$^+$ and CTC$^+$/OCT4$^-$ patients was 27.3 (20.1-34.5) months and not reached, respectively (Fig. 3B).

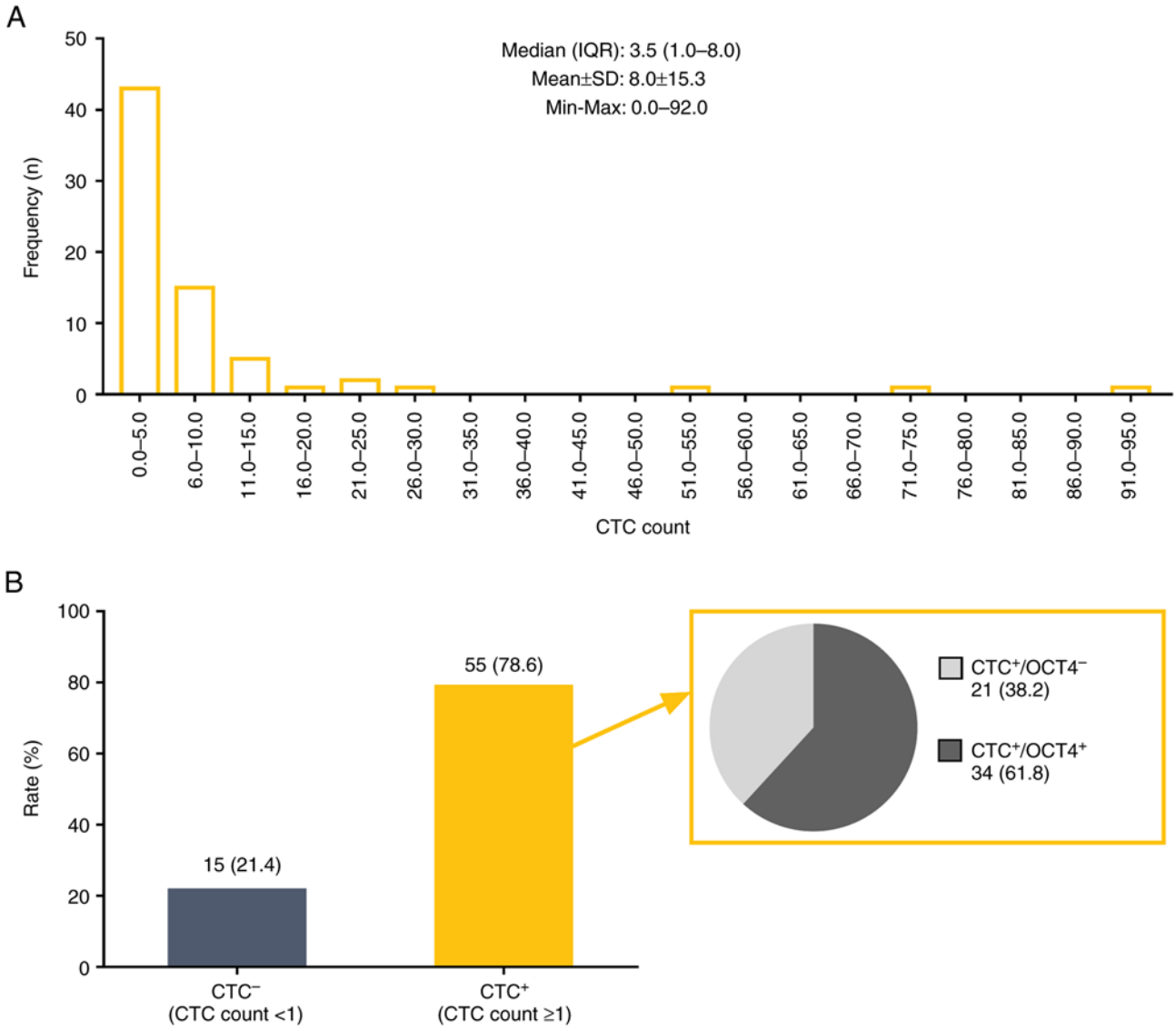

Figure 1. CTC count and OCT4+ CTCs in patients with metastatic castration-resistant prostate cancer treated with abiraterone + prednisone. (A) CTC count. (B) Proportion of CTC+, CTC-, CTC+/OCT4+ and CTC+/OCT4- patients. CTC, circulating tumor cell; OCT4, octamer-binding transcription factor 4.

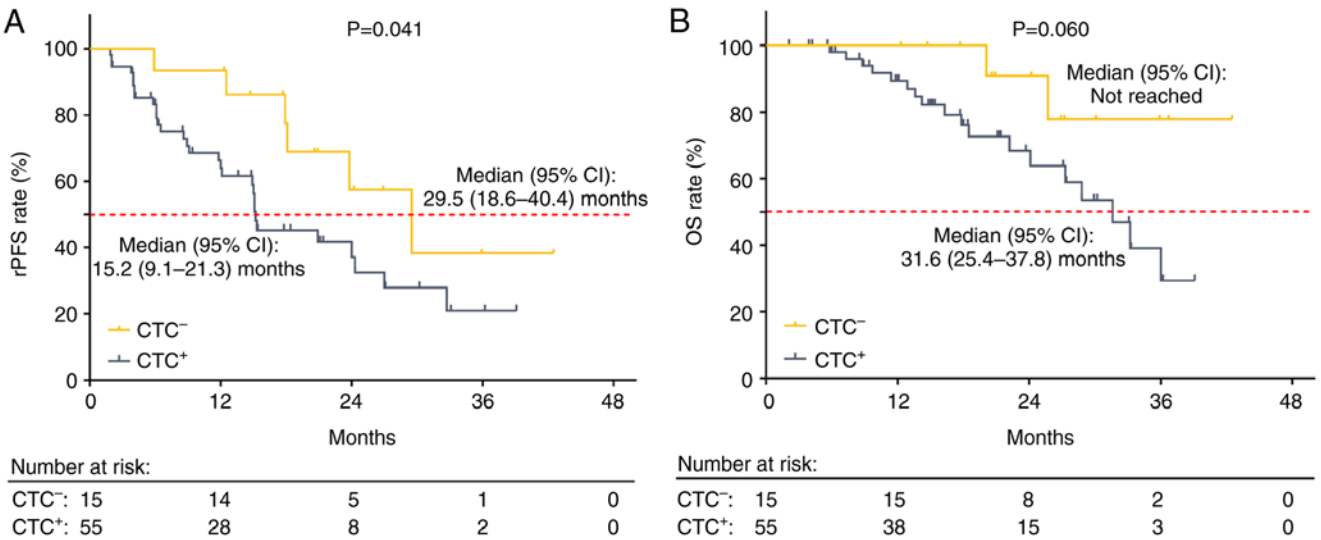

Figure 2. rPFS is decreased in CTC+ compared with CTC- patients. Kaplan-Meier analysis of (A) rPFS and (B) OS according to CTC status in patients with metastatic castration-resistant prostate cancer treated with abiraterone + prednisone. rPFS, radiographic progression-free survival; CTC, circulating tumor cell; OS, overall survival.

Table II. Comparison of CTC count in metastatic castration-resistant prostate cancer patients with different characteristics.

Characteristic	Median CTC count (IQR)	P-value
Age, years		0.619
<70	3.0 (1.0-7.0)	
≥70	4.0 (1.0-10.0)	
History of prostatectomy		0.303
No	3.5 (1.0-9.8)	
Yes	3.5 (0.0-7.3)	
History of radiotherapy		0.112
No	3.0 (0.0-6.5)	
Yes	4.0 (1.0-10.0)	
History of other therapy		0.662
No	4.0 (1.0-7.3)	
Yes	1.0 (0.3-13.8)	
Gleason score at initial diagnosis		0.272
≤7	2.0 (0.0-7.0)	
>7	4.0 (1.0-9.5)	
ISUP grade		0.278
1	3.5 (2.0-NA)	
2	2.0 (0.0-7.0)	
3	2.0 (0.0-8.5)	
4	3.5 (1.0-9.5)	
5	4.0 (1.5-9.5)	
ECOG PS score		0.276
0	3.0 (0.8-7.0)	
1	6.0 (1.0-11.5)	
Bone metastasis		0.324
No	1.0 (0.0-10.0)	
Yes	4.0 (1.0-8.0)	
Lymph node metastasis		0.011
No	2.0 (0.0-6.0)	
Yes	5.0 (1.0-13.0)	
Soft tissue metastasis		0.655
No	3.0 (1.0-7.0)	
Yes	4.0 (1.0-15.0)	
Visceral metastasis		0.003
No	2.5 (1.0-6.0)	
Yes	11.5 (6.0-28.5)	
PSA		0.041
Low	2.0 (0.0-7.0)	
High	4.0 (1.0-10.0)	
ALP		0.054
Low	2.0 (0.0-8.0)	
High	5.0 (2.0-9.0)	
LDH		0.026
Low	2.0 (0.0-6.0)	
High	6.0 (2.0-9.0)	

The low and high levels of PSA, ALP, and LDH were classified by median value. CTC, circulating tumor cell; ISUP, International Society of Urological Pathology; ECOG PS, Eastern Cooperative Oncology Group Performance Status; PSA, prostate-specific antigen; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; NA, not available.

Table III. Comparison of OCT4 expression in CTC in metastatic castration-resistant prostate cancer patients with different characteristics.

Characteristic	$CTC^+/OCT4^-$ (%)	$CTC^+/OCT4^+$ (%)	P-value
Age, years			0.348
<70	12.0 (57.1)	15.0 (44.1)	
≥70	9.0 (42.9)	19.0 (55.9)	
History of prostatectomy			0.304
No	10.0 (47.6)	21.0 (61.8)	
Yes	11.0 (52.4)	13.0 (38.2)	
History of radiotherapy			0.663
No	8.0 (38.1)	11.0 (32.4)	
Yes	13.0 (61.9)	23.0 (67.6)	
History of other therapy			0.664
No	18.0 (85.7)	31.0 (91.2)	
Yes	3.0 (14.3)	3.0 (8.8)	
Gleason score at initial diagnosis			0.431
≤7	9.0 (42.9)	11.0 (32.4)	
>7	12.0 (57.1)	23.0 (67.6)	
ISUP grade			0.630
1	0.0 (0.0)	2.0 (5.9)	
2	5.0 (23.8)	4.0 (11.8)	
3	4.0 (19.0)	5.0 (14.7)	
4	4.0 (19.0)	10.0 (29.4)	
5	8.0 (38.1)	13.0 (38.2)	
ECOG PS score			0.834
0	13.0 (61.9)	22.0 (64.7)	
1	8.0 (38.1)	12.0 (35.3)	
Bone metastasis			1.000
No	2.0 (9.5)	3.0 (8.8)	
Yes	19.0 (90.5)	31.0 (91.2)	
Lymph node metastasis			0.052
No	11.0 (52.4)	9.0 (26.5)	
Yes	10.0 (47.6)	25.0 (73.5)	
Soft tissue metastasis			1.000
No	18.0 (85.7)	28.0 (82.4)	
Yes	3.0 (14.3)	6.0 (17.6)	
Visceral metastasis			0.009
No	21.0 (100.0)	25.0 (73.5)	
Yes	0.0 (0.0)	9.0 (26.5)	
PSA			0.212
Low	11.0 (52.4)	12.0 (35.3)	
High	10.0 (47.6)	22.0 (64.7)	
ALP			0.304
Low	11.0 (52.4)	13.0 (38.2)	
High	10.0 (47.6)	21.0 (61.8)	
LDH			0.032
Low	13.0 (61.9)	11.0 (32.4)	
High	8.0 (38.1)	23.0 (67.6)	

The low and high levels of PSA, ALP, and LDH were classified by median value. CTC, circulating tumor cell; ISUP, International Society of Urological Pathology; ECOG PS, Eastern Cooperative Oncology Group Performance Status; PSA, prostate-specific antigen; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

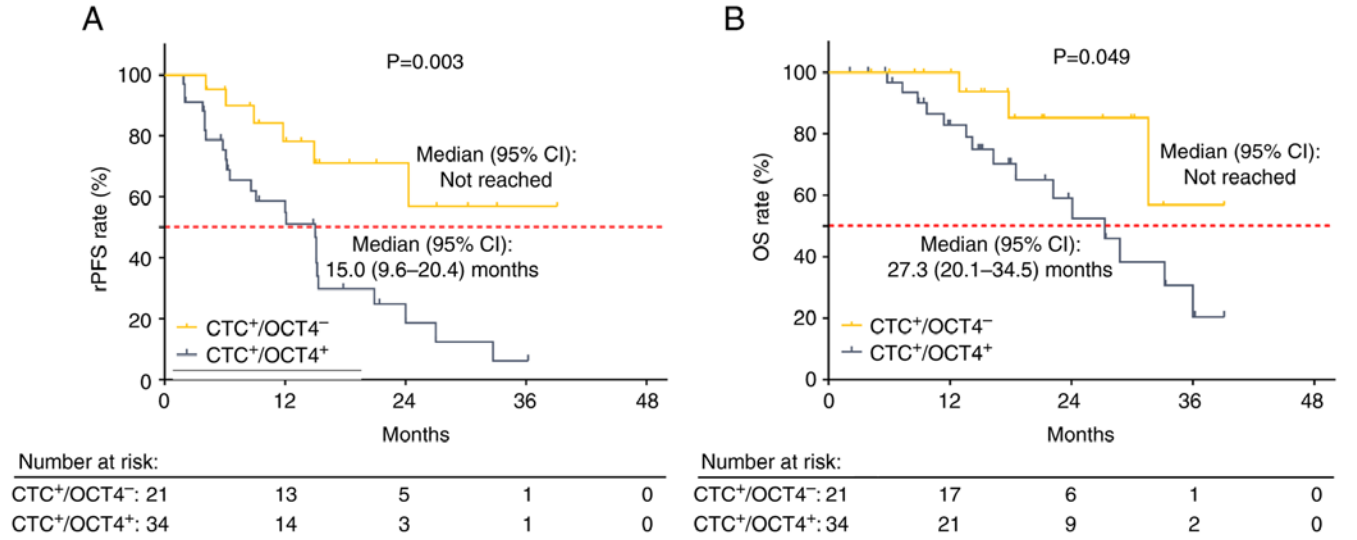

Figure 3. rPFS and OS are decreased in CTC+/OCT4+ compared with CTC+/OCT4- patients. Kaplan-Meier analysis of (A) rPFS and (B) OS according to CTC/OCT4 status in patients with metastatic castration-resistant prostate cancer treated with abiraterone + prednisone. rPFS, radiographic progression-free survival; OS, overall survival; CTC, circulating tumor cell; OCT4, octamer-binding transcription factor 4.

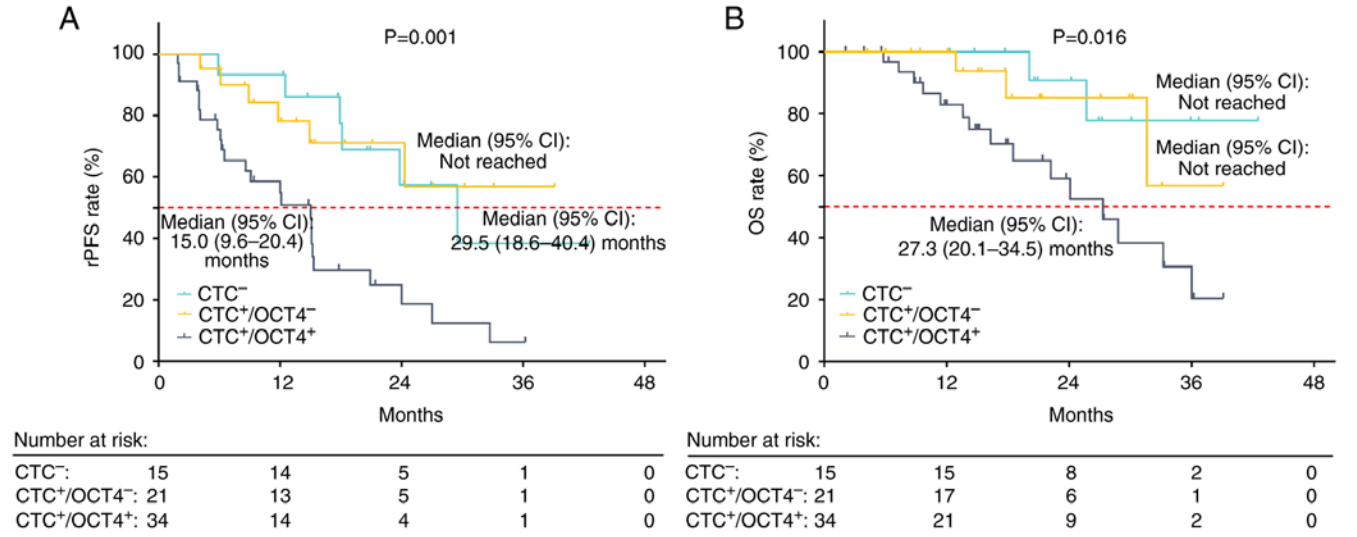

Figure 4. rPFS and OS are decreased in CTC+/OCT4+ patients. Kaplan-Meier analysis of (A) rPFS and (B) OS according to CTC+/OCT4+, CTC+/OCT4- and CTC- in patients with metastatic castration-resistant prostate cancer treated with abiraterone + prednisone. rPFS, radiographic progression-free survival; OS, overall survival; CTC, circulating tumor cell; OCT4, octamer-binding transcription factor 4.

rPFS (P=0.001; Fig. 4A) and OS (P=0.016; Fig. 4B) were decreased in CTC+/OCT4+ compared with CTC- and CTC+/OCT4- patients.

Independent risk factors for rPFS and OS. Forward-multivariate Cox regression models demonstrated that CTC+/OCT4+ (vs. CTC+/OCT4- or CTC-) was independently associated with decreased rPFS [hazard ratio (HR), 3.833; P<0.001] and OS (HR, 3.938; P=0.008). ECOG PS score (1 vs. 0) was also independently associated with reduced rPFS (HR, 2.163; P=0.033) and OS (HR, 2.750; P=0.032; Table IV).

Further multivariate models were established to validate the findings of the forward-multivariate Cox model. Multivariate model 1 included factors with P<0.05 in the univariate model; CTC+/OCT4+ vs. CTC+/OCT4- or CTC- (P=0.005) was an independent risk factor, while LDH (P=0.127) was not an independent risk factor for decreased rPFS (Table SII). Multivariate model 2 included factors with P<0.1 in the univariate model; CTC+/OCT4+ vs. CTC+/OCT4- or CTC- (P=0.006) and ECOG PS score 1 vs. 0 (P=0.048) were independently associated with decreased rPFS. However, LDH was not an independent risk factor for decreased rPFS (P=0.180; Table SII). Multivariate model 3 included all factors and used a backward elimination method; CTC+/OCT4+ vs. CTC+/OCT4- or CTC- (P<0.001) and ECOG PS score 1 vs. 0 (P=0.033) were independently associated with decreased rPFS (Table SII). Concerning OS, multivariate models 1, 2 and 3 all showed that CTC+/OCT4+ vs. CTC+/OCT4- or CTC- and ECOG PS score 1 vs. 0 were independently associated with shorter OS (all P<0.050; Table SIII).

Table IV. Multivariate Cox regression models of rPFS and OS in patients with metastatic castration-resistant prostate cancer.

A, rPFS

Variable	P-value	HR	95% CI	
			Lower	Upper
$CTC^+/OCT4^+$ vs. $CTC^+/OCT4^-$ or CTC^-	<0.001	3.833	1.887	7.784
ECOG PS score, 1 vs. 0	0.033	2.163	1.063	4.402

B, OS

Variable	P-value	HR	95% CI	
			Lower	Upper
$CTC^+/OCT4^+$ vs. $CTC^+/OCT4^-$ or CTC^-	0.008	3.938	1.428	10.858
ECOG PS score, 1 vs. 0	0.032	2.750	1.090	6.935

rPFS, radiographic progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; CTC, circulating tumor cell; OCT4, octamer-binding transcription factor 4; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Discussion

According to a previous study, OCT4, as a cancer stem cell marker, is elevated in PC compared with normal prostate and benign prostatic hyperplasia tissue, indicating its cancer specificity in PC (23). Previous studies have quantified CTCs and demonstrated value of CTCs in predicting survival for patients with mCRPC (36-39). Results of the present study demonstrated that elevated CTC count was associated with lymph node and visceral metastasis and high levels of PSA and LDH in patients with mCRPC treated with abiraterone + prednisone. Further Kaplan-Meier curves demonstrated that CTC^+ was associated with decreased rPFS in patients with mCRPC treated with abiraterone + prednisone. This may be because CTCs in the blood may reflect the ability of cancer cells to detach from primary or metastatic sites to new sites, exacerbating the progression of PC (40). Lymph node and visceral metastasis and high levels of PSA and LDH may result in a worse survival (11). Therefore, CTC^+ was associated with shortened rPFS in patients with mCRPC treated with abiraterone + prednisone. Patients with lymph node metastasis or visceral metastasis are more likely to exhibit CTC^+ and $CTC^+/OCT4^+$ (21), but in fact, *in situ* hybridization images are quite similar in patients with CTC^+ and $CTC^+/OCT4^+$ no matter what the metastasis status.

Previous studies have demonstrated a potential association between OCT4 and disease features and prognosis of patients with PC (23,41). For example, increased OCT4 levels are associated with elevated TNM stage and distant metastasis in patients with PC (23). In addition, a previous study used $OCT4^+$ tumors from palliative transurethral resection prostate specimens and the results demonstrated that increased tumor OCT4 was associated with increased T stage and PSA recurrence in post-docetaxel-treated patients with mCRPC (41). Since blood samples are more convenient to obtain (compared with radiology) and CTCs as a marker in blood-based liquid biopsy have been extensively explored (10,42), it would be helpful for providing a monitoring option for cancer prognosis to identify the clinical role of $OCT4^+$ CTC in patients with mCRPC treated with abiraterone + prednisone, which has yet to be reported. Here, $CTC^+/OCT4^+$ was associated with visceral metastasis and high levels of LDH in patients with mCRPC treated with abiraterone + prednisone. In addition, $CTC^+/OCT4^+$ vs. $CTC^+/OCT4^-$ or CTC^- was independently associated with reduced rPFS and OS. OCT4 may play a role in promoting stemness, epithelial-mesenchymal transition, proliferation and metastasis of tumor cells via numerous signaling pathways (such as PI3K/AKT/mTOR pathway and notch signaling pathway), further promoting tumor progression (20,43,44). Moreover, OCT4 promotes malignancy and drug resistance of cancer cells, leading to disease progression (17). Thus, $CTC^+/OCT4^+$ vs. $CTC^+/OCT4^-$ or CTC^- was independently associated with reduced rPFS and OS in patients with mCRPC treated with abiraterone + prednisone. As PSA progression is less accurate than radiographic progression in patients with mCRPC (45), the present study only utilized rPFS to investigate the prognostic value of $OCT4^+$ CTCs. rPFS did not differ between CTC^- and $CTC^+/OCT4^-$ patients. The present results may have been biased, as rPFS rates were initially higher in CTC^- compared with $CTC^+/OCT4^-$ patients and one CTC^- patient died in the 30th month. The present study was limited by short follow-up duration and the censored data in the $CTC^+/OCT4^-$ group led to a high rPFS rate. Thus, rPFS did not differ between CTC^- and $CTC^+/OCT4^-$ patients. Further investigation with a larger sample size and longer follow-up period are required to validate the findings of the present study.

Notably, $OCT4^+$ CTC levels were determined prior to treatment initiation but $OCT4^+$ CTC levels following abiraterone treatment are yet to be elucidated. Further *in vivo* and *in vitro* investigations are required to determine the mechanisms underlying OCT4 in regulating drug resistance, as the present

study only investigated the prognostic value of OCT4+ CTC in patients with mCRPC treated with abiraterone + prednisone. The present study aimed to investigate the prognostic value of OCT4 expression in CTCs and did not enroll non-cancer patients (who have no CTCs) as controls; the lack of control group was a limitation. As a result, the OCT4 tumor-specificity in mCRPC needs validations in further study with a health control group.

In conclusion, OCT4+ CTCs were highly prevalent and associated with visceral metastasis and increased LDH levels. Thus, OCT4+ CTCs may exhibit potential in predicting prognosis of patients with mCRPC treated with abiraterone + prednisone.

Acknowledgements

The author would like to thank Dr Qiongying Ma and Dr Ziyi Sheng (Department of Nursing, Shanghai Songjiang District Sijing Hospital) for their assistance in data entry and peripheral blood collection.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YM was conceived the study, analyzed and interpreted data, constructed figures and wrote and reviewed the manuscript. YM confirms the authenticity of all the raw data. The author has read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committee of Shanghai Songjiang District Sijing Hospital, Shanghai, China (approval no. 20180314sjyy01). All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The author declares that they have no competing interests.

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