Circulating tumor cells in prostate cancer: Precision diagnosis and therapy (Review)

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Abstract. The primary cause of tumor-associated mortality in prostate cancer (PCa) remains distant metastasis. The dissemination of tumor cells from the primary tumor to distant sites through the bloodstream cannot be detected early by standard imaging methods. Circulating tumor cells (CTCs) represent an effective prognostic and predictive biomarker, which are able to monitor efficacy of adjuvant therapies, detect early development of metastases, and finally, assess therapeutic responses of advanced disease earlier than traditional diagnostic methods. In addition, since repeated tissue biopsies are invasive, costly and not always feasible, the assessment of tumor characteristics on CTCs, by a peripheral blood sample as a liquid biopsy, represents an attractive opportunity. The implementation of molecular and genomic characterization of CTCs may contribute to improve the treatment selection and thus, to move toward more precise diagnosis and therapy in PCa. The present study summarizes the current advances in CTC enrichment and detection strategies and reviews how CTCs may contribute to significant insights in the metastatic process, as well as how they may be utilized in clinical application in PCa. Although it is proposed that CTCs may offer insights into the prognosis and management of PCa, there are a number of challenges in the study of circulating tumor cells, and their clinical utility remains under investigation.

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Abbreviations: AA, abiraterone acetate; AR, androgen receptor; AR-V7, androgen receptor splice variant 7; CRPC, castration-resistant prostate cancer; CTC, circulating tumor cell; EMT, epithelial-mesenchymal transition; EpCAM, epithelial cell adhesion molecule; mCRPC, metastatic castration-resistant prostate cancer; OS, overall survival; PCa, prostate cancer; PCA3, prostate cancer antigen 3; PCTC, prostate cancer circulating tumor cell; PSA, prostate-specific antigen; PSMA, prostate specific membrane antigen; TMPRSS2-ERG, transmembrane protease serine 2 and ETS-related fusion gene

Key words: prostate cancer, circulating tumor cell, liquid biopsy, epithelial-mesenchymal transition, metastasis, prognostic, predictive

Contents

1. Introduction

Prostate cancer (PCa) is the most common urological malignancy and the second leading cause of male cancer-associated mortality in numerous developed countries (1). The rate of diagnosis differs between countries due to the difference in coverage of prostate-specific antigen (PSA) screening (2), but in populations with and without PSA screening, PCa is the cause of 1-2% of all mortality for men (3). Its greater prevalence in the West implicates lifestyle and environmental risk factors (4). Significant progress has been made in the treatment and understanding of the underlying biology (5-7). This includes the approval of several novel effective drugs that prolong life in men with advanced PCa, and the recognition that the terms hormone refractory and androgen-independent were misnomers. The majority of cancers remain hormone-driven despite castration resistance (8-13). Although the improvements in PCa detection and multiple treatments have led to a significant decrease in PCa-associated mortalities in the last three decades, the majority of men initially diagnosed with early-stage cancer eventually develop metastatic disease (14). Thus, PCa currently remains challenging to treat, and the aims of therapy are the improvement of overall survival (OS) time and quality of life.

Multiple clinical and pathological features, such as bone scan assay and quantitative imaging parameters (15), and laboratory biochemical indicators, such as PSA (16), currently guide treatment decision making in PCa. However, the lack of reliable prognostic and predictive factors and imperfect tools

to precisely diagnose and evaluate treatment success in the primary and metastatic setting continues to result in serious overtreatment and invalid treatment of patients (17). Novel strategies to improve diagnosis, earlier indications of treatment response and treatment failure, and improved definition of patient cohorts that will respond to a specific treatment in primary and metastatic PCa are therefore urgently required.

Circulating tumor cells (CTCs) are cancer cells that are present in the blood of patients with solid cancers and are shed from existing tumor lesions into the bloodstream (18). Numerous laboratory studies and clinical trials in the past two decades have shown that CTCs may be used as a biomarker to predict disease progression and survival in patients with metastatic, advanced (19-22) or even early-stage PCa (23). High CTC numbers are associated with aggressive disease, increased metastasis and decreased time to relapse (22,24,25). In addition, CTCs isolated from patients with metastatic PCa (mPCa) may initiate metastasis in a xenograft model (26). A growing number of studies have shown that CTCs are a source of metastatic cells (27-29); CTCs have become an integral part of tumor staging criteria, which are currently focused on several types of tumors, including breast cancer (30), PCa (29), lung cancer (31) and colorectal cancer (32). Since blood collection is simple, convenient and minimally invasive, CTCs may be used as a real-time liquid biopsy for disease progression and survival (19). CTCs also have potential to guide therapeutic management (33), indicate tumor sensitivity to therapy, monitor therapy effectiveness or necessity, even in the absence of detectable metastases (33), offer insights into mechanisms of drug resistance (34) and promote new anti-drug research and development to a certain degree (35). CTCs may also be utilized as a surrogate endpoint marker in clinical trials (36), and may also become a promising tumor target. Despite the potential, the use of CTCs faces numerous challenges.

This review describes the current state of research into CTCs enrichment and detection strategies and clinical application, the evidence to demonstrate their diagnostic validity and therapeutic value, and their potential impact for future clinical trial design and therapeutic decision-making processes in PCa.

2. Enrichment and detection strategies for CTC

PCa circulating tumor cells (PCTCs) are present in the bloodstream at a low concentration, ranging between 1-10 cells per 10 ml in the majority of patients with cancer, which poses a serious challenge for any analytical system (18). The problem exists of looking for the proverbial 'needle in the haystack'. Thus, the detection and characterization of PCTCs requires highly sensitive and specific techniques, which consist of a combination of enrichment (isolation) and detection (identification) strategies, and these two steps are essential components of the identification process (23). It was not until recently that such sensitive molecular techniques were developed.

CTC enrichment approaches. CTCs can be isolated from blood cells based on cell size (37), density and positive immunoselection, such as epithelial cell adhesion molecule (EpCAM) (38) and prostate-specific membrane antigen (PSMA) (39) antibody-based enrichment of CTCs, or negative immunoselection, such as depletion of leukocytes by CD45 (40,41) antibody. Current CTC enrichment approaches are summarized in Table I.

Several membrane filter devices are available for CTC enrichment based on the differential cellular size, including isolation by size of epithelial tumor cells, micro electro-mechanical system-optics based microfilter, ScreenCell®, CellSieve™ and CellOptics® (42). Recently, Qin *et al* (43) used the resettable cell trap mechanism to separate cells based on their size and deformability using an adjustable aperture that can be periodically cleared to prevent clogging. This system was able to capture ≥5 CTCs in 18/22 (82%) patients with a mean count of 257 in 7.5 ml of whole blood, while the CellSearch system found ≥5 CTCs in 9/22 (41%) metastatic castration-resistant PCa (mCRPC) with a mean count of 25 in the 7.5 ml whole blood samples. Filtration by size consents to enrich CTCs from a wide range of tumors, but occasionally results in loss of smaller CTCs or clotting of filter pores by leukocytes (40). In addition, emerging CTC capture strategies typically distinguish these cells based on cultured cancer cells. However, CTCs were found to have significantly smaller size, larger nuclear-cytoplasmic ratio and more elongated shape (44), which may inevitably degrade the performance of the cell size-dependent capture system. Another morphology-based enrichment strategy is based on density gradient centrifugation using Ficoll-hypaque solution (45). Ficoll density gradient-dependent techniques are easy to operate, even if real losses of tumor cells have been observed (46).

Immunoselection is the most commonly used approach for CTC enrichment, which relies on specific CTC markers that are detected by antibodies (39). Epithelial markers are expressed on epithelial tumors, but not on blood cells, and have therefore been used to isolate CTCs from blood cells (47). EpCAM and members of the family of cytokeratin (CK)s (CK8, CK18 and CK19) have been markers for positive selection in PCa (47). Thus far, the most successful approach belongs to the CellSearch® System (Veridex LLC, Raritan, NJ, USA), which employs ferromagnetic nanoparticles coupled to EpCAM antibodies for CTC capture, became the first validated CTC assay approved by the Food and Drug Administration (FDA) in 2008 (48,49). With this system, CTCs are isolated using ferrofluid covalently linked to an antibody against the surface of EpCAM (48). CTCs are differentiated from leukocytes by labeling the product with monoclonal antibodies against CKs (CTC markers) and CD45 (leukocyte marker) (38,48,49). The labeled sample is analyzed by an automated fluorescence detection system (CellTracks® Analyzer; Veridex LLC) (48,49). However, the limitation of the CellSearch System is that EpCAM-negative tumor cells may not be detected. Downregulation of EpCAM may occur during the epithelial-mesenchymal transition (EMT), a process linked to tumor cell invasion and dissemination, and to the stemness of cancer cells (50). Mesenchymal-based capture is a strategy that isolates cells based on osteoblast (OB)-cadherin cell surface expression (51). Using this method, OB-cadherin cellular events are detectable in men with mPCa and are less common in healthy volunteers. This method may complement existing epithelial-based methods and may be particularly useful in patients with bone metastases (51). For PCa, PSMA presents a compelling target for immunocapture, as PSMA levels increase in higher-grade cancers and metastatic disease

Table I. Advantages and disadvantages of CTC enrichment approach.

Technology and rationale	Advantages	Disadvantages	(Refs.)
Isolation by size	Easy and rapid; enrichment of CTCs from a wide range of tumors; feasible for EpCAM-negative CTCs	Loss of smaller CTCs or clotting of filter pores by leukocytes	(43)
Density gradient centrifugation	Operability; feasible for EpCAM-negative CTCs	Loss of some CTCs	(45,46)
Immunoselection	Visual confirmation of CTCs and quantitative	Costly and absence of standardized methods and reagents	(47-53)
Microfluidics device	Dependent or independent of tumor membrane epitopes; high recovery and efficiency; potential to recover CTCs for additional characterization	High technical requirement; absence of standardized methods; early in development	(58-60)

CTCs, circulating tumor cells; EpCAM, epithelial cell adhesion molecule.

and are specific to the prostate epithelium (39). In order to overcome this drawback, PSMA-based positive immunoselection was developed and may successfully capture the EMT CTCs (39). Santana *et al* (52) used monoclonal J591 antibodies and J415-antibodies that are highly specific for intact extracellular domains of PSMA on live cells in microfluidic devices for the capture of LNCaPs. The results showed that J591 outperforms J415 and a mix of the two for PCa capture, and that capture performance saturates following incubation with antibody concentrations of 10 μg/ml. In addition, negative selection for the antigens CD45 (expressed in leukocytes) and CD61 (expressed in megakaryocytes and platelets) may markedly reduce and avoid contamination by blood cells (53). The major advantage of immune-based separation is that CTCs can be directly visualized and quantified without requiring cell lysis. Its limitations include cost and variability, due to the absence of standardized methods and reagents (53).

Several other devices based on physical and/or biological properties of CTCs are available, including a NanoVelcro Chip assay (54), aptamer-conjugated graphene oxide membranes (55) and a microfluidics device that combines the multi-orifice flow fractionation and the dielectrophoresis/acoustophoresis cell separation techniques (56,57), among which the microfluidics device holds promise for CTC enrichment. Ozkumur *et al* (58) described an inertial focusing-enhanced microfluidic CTC capture platform, termed CTC-iChip, which is capable of sorting rare CTCs from whole blood at 10^7 cells/sec. Most importantly, the iChip is capable of isolating CTCs using strategies that are either dependent or independent of tumor membrane epitopes, and thus applicable to virtually all cancers. A technology that uses magnetic particles bearing tumor cell-specific EpCAM antibodies, self-assembled in a regular array in a microfluidic flow cell was reported recently (59), which could capture CTCs in 75% of patients with mPCa and 80% of patients with metastatic breast cancer, and showed similar or improved results compared with the CellSearch device in 10 out of 13 samples. In addition, a single inlet two-stage acoustophoresis chip could enrich PCa cells from white blood cells, DU145 spiked into blood were enriched from white blood cells at a sample flow rate of 100 μl min (-1), providing 86.5±6.7% recovery of the cancer cells with 1.1±0.2% contamination of white blood cells, and by increasing the acoustic intensity a recovery of 94.8±2.8% of cancer cells was achieved with 2.2±0.6% contamination of white blood cells (60). Notably, Sarioglu *et al* (61) developed a microchip technology (the Cluster-Chip) to capture CTC clusters independently of tumor-specific markers from unprocessed blood. CTC clusters are isolated through specialized bifurcating traps under low-shear stress conditions that preserve their integrity, and even two-cell clusters are captured efficiently (61).

CTC detection approaches. Following enrichment, CTCs require subsequent identification at the single-cell level and to be separated from normal blood cells. Detection of CTCs may be performed through immunology-based techniques or nucleic acid-based techniques.

Immunology-based techniques. Immunology-based techniques are the most common of the strategies and are effective for detection and isolation of CTCs. Immunological methods utilize labeled antibodies directed against epithelial or tumor-associated antigens, along with automated digital microscopy or flow cytometry, to identify and quantify CTCs (42). Numerous antigens have been used for this method, including EpCAM and different subtypes of CKs (62). However, not all CTCs express these markers, possibly as a consequence of the EMT process, and likely lead to false-negative results (62). In addition, tumor-specific antigens are expressed at an increased level in cancer cells compared with normal cells. PSA and PSMA are PCa-associated markers for detecting CTCs, and have been applied in antibody-based detection and isolation of CTCs (63-65).

EpCAM-based immunological assays are the most common of the strategies for CTC detection. Among the recently developed innovative techniques, the CellSearch® system is the most advanced commercially available technology with combined automated enrichment and immunostaining (48).

Currently, it is the only technology that has been approved by the USA FDA for the detection of patients with mPCa (22). The CD45 marker is employed to rule out white blood cells and increase detection specificity (66). A CTC is often defined as a $CK^+/CD45^-/DAPI^+$ intact cell (66). The nuclear dye DAPI is used to exclude cell fragments and debris false-positive may occur using CK as a marker (66). Notably, this technique allows quantitative measurement of CTCs and it may provide a more precise discrimination between patients (62).

In addition to the CellSearch® system, a variety of novel detection technologies have been developed. Myung *et al* (67) engineered a novel multifunctional surface, on the basis of the biomimetic cell capture, through optimized incorporation of multiple antibodies directed to cancer cell-specific surface markers, including EpCAM, human epidermal growth factor receptor-2 and PSA, which may lead to clinically significant, differential detection of CTCs that are rare and highly heterogeneous.

Nucleic acid-based techniques. Nucleic acid-based techniques have become the most widely used alternative to immunology-based techniques, which are considered to be more sensitive than protein-based approaches (68). Particularly, polymerase chain reaction (PCR)-based assays evaluate the amount of DNA from CTCs. However, the drawback of this technique is the inability to distinguish the DNA free in the blood from apoptotic cells, creating false-positive results (68). Therefore, the majority of research groups prefer reverse transcription (RT)-PCR assays to detect specific mRNA, since only viable CTCs produce mRNA (69-71). Several specific mRNA markers are used to identify PCTCs in nucleic acid-based methods (Table II).

To date, the mRNA encoding PSA has been the most extensively studied in clinical trials (63,69,72). Mohamadi *et al* (73) developed a velocity valley chip to efficiently capture magnetic nanoparticle-bound CTCs. The approach was successfully validated using samples collected from patients with PCa: CTCs and PSA mRNA sequences were detected in all cancer patient samples and not in the healthy controls. In addition, quantitative RT-PCR analysis of PSA and PSMA mRNA to detect circulating tumor cells improves recurrence-free survival nomogram prediction following radical prostatectomy (72). Furthermore, androgen receptor (AR), kallikrein-related peptidase (KLK)2, KLK3, PCa antigen 3 (PCA3), transmembrane protease serine 2 and ETS-related fusion gene (TMPRSS2-ERG), AR splice variant 7 messenger RNA (AR-V7) and prostate stem cell antigen mRNA are also utilized to identify PCTCs (63,69,74-76). However, the major limitations to these techniques are associated with the mRNA markers utilized, since they may be also present at low concentrations in normal blood, bone marrow cells and in other non-tumor cells (77). Therefore, to seek more specific mRNA markers is the key issue in future studies.

In addition to the aforementioned types of commonly used CTC detection techniques, certain novel technologies are also in rapid development, particularly sensor-based detection technologies, which show high sensitivity and specificity (78). Sioss *et al* (78) selected PCA3 RNA as a marker and developed a nanoresonator chip-based sensor for detection of prostate circulating tumor cells. Similarly, Ivanov *et al* (79) described a chip-based method using nanostructured microelectrodes and electrochemical readout, which confirmed the identity of isolated CTCs and successfully interrogated them for specific biomarkers. Recently, a light addressable potentiometric sensor was exploited in the label-free detection of CTCs in PCa, which may be a potential platform for CTC detection and may provide a powerful tool for downstream analysis (80).

Table II. Specific mRNA markers for detection of prostate cancer circulating tumor cells.

mRNA markers	(Refs.)
PSA	(72,73)
PSMA	(72)
PCA3	(75)
PSCA	(69)
AR	(74)
AR-V7	(76)
TMPRSS2-ERG	(63,75)
KLK2	(69)
KLK3	(69,75)

PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; PCA3, prostate cancer antigen 3; AR, androgen receptor; AR-V7, androgen receptor splice variant 7; TMPRSS2-ERG, transmembrane protease serine 2 and ETS-related fusion gene; KLK, kallikrein-related peptidase.

Overall, in the last two decades, several promising CTC enrichment and detection methods have been developed. These strategies should be validated in appropriately sized clinical trials in order to evaluate their quality, validity and clinical practicability.

3. Clinical application of CTCs in PCa

CTCs have an important role in the progression and metastasis of PCa, and represent tumor characteristics of individual patients well. Therefore, CTCs may be used as an ideal biomarker for prognosis, prediction and clinical management of patients with PCa.

Prognostic and predictive biomarker. Utilizing multiple blood tests, CTC enumeration and characterization may provide prognostic and predictive value on PCa, particularly in metastatic and advanced PCa (47,81-83). As early as 2005, Moreno *et al* (84) demonstrated that in patients with metastatic PCa, CTC enumeration with the cut-off of 5 CTCs was a predictive factor superior to other clinical variables. They also demonstrated that increased CTC numbers have been found in patients with bone metastasis compared with patients with visceral spread, which suggests a prognostic potential for CTC detecting in monitoring patients for bone disease in PCa (20-22). Furthermore, in patients with CRPC, post-treatment CTC counts were a stronger prognostic factor for survival than a 50% decline in PSA [receiver operating characteristic (ROC) AUC 0.87 vs. 0.62] (19,85). Therefore, for patients with CRPC with no metastases and slow-rising PSA, CTC detection

would be complementary to PSA testing, which may be useful in facilitating decisions about additional imaging requirements and for early diagnosis of bone metastases, allowing for earlier intervention with treatments. In addition, CTC counts appear to be an earlier and more sensitive predictor for survival and treatment response compared with current objective response criteria (OR) in patients with mCRPC (86). Recently, Marín-Aguilera *et al* (87) analyzed the molecular profiling of peripheral blood from 43 patients with mCRPC with known CTC content in order to identify genes that may be associated with PCa progression. The analysis revealed a two-gene model (selenium binding protein 1 and matrix metalloprotein 9) with a high significant prognostic ability [hazard ratio (HR), 6; 95% confidence interval (CI), 2.61-13.79; P<0.0001]. Notably, a phase III SWOG-led therapeutic trial demonstrated that increased CTC telomerase activity was associated with HR, 1.14 (P=0.001) for OS in patients with baseline CTC count ≥5 (47% of patients), subsequent to adjusting for other clinical covariates, including CTC counts and serum PSA at study entry (88).

However, CTCs were identified in 21% of patients with PCa prior to radical prostatectomy, which was similar to the rate of CTC detection in control (non-cancer) patients (89). Consistently, Loh *et al* (90) collected samples of peripheral blood (7.5 ml) drawn from 36 men with newly diagnosed high-risk non-metastatic PCa, prior to any initiation of therapy, and analyzed for CTCs using the CellSearch® method. They demonstrated that patients with high-risk, non-metastatic PCa present infrequently with a small number of CTCs in peripheral blood. Furthermore, in these patients with localized PCa, CTC numbers were not associated with tumor volume, pathological stage and Gleason score, indicating that CTCs are more likely to originate from metastasis sites instead of primary lesions (91,92).

Notably, a number of men have low CTCs despite widespread disease, indicating heterogeneity in CTC phenotype or detection (93,94). Chen *et al* (95) suggested that an incremental expression of EMT-associated genes in CTCs is associated with mCRPC. It was found that genes that promote mesenchymal transitioning into a more malignant state, including insulin-like growth factor (IGF)1, IGF2, epidermal growth factor receptor, forkhead box p3 and transforming growth factor β3, were commonly observed in these cells. In addition, Osmulski *et al* (96) found that CTCs from patients with CRPC are three times softer, three times more deformable and seven times more adhesive than counterparts from patients with castration-sensitive PCa. The present study indicates that nanomechanical phenotypes of CTCs may serve as novel and effective biomarkers for mCRPC. More recently, the detection of small nuclear CTCs was found to be associated with the presence of visceral metastases and should be formally explored as a putative blood-borne biomarker to identify patients at risk of developing this clinical evolution of PCa (24).

Overall, enumeration and characterization of CTCs has shown a strong potential in the prognosis and prediction of patients with metastatic and advanced PCa, whereas CTC detection is unreliable in the early and localized disease state.

Response-indicator and efficacy-response biomarker. An increasing number of systemic therapies with life extending capacity have become available in mCRPC, including abiraterone acetate (AA), enzalutamide, sipuleucel-T, docetaxel, cabazitaxel and radium-223 (97). More compounds are currently being evaluated in promising pivotal trials, such as Tasquinimod, ARN-509 and ODM-201 (97). Limitations of the currently available biomarkers make treatment decisions challenging (97). Considering the increasing complexity of treatment algorithms in mCRPC, the current demand of research is to identify and characterize biomarkers with prognostic, predictive and surrogate quality, allowing for information on clinically meaningful outcomes and on which therapy to offer patients in different and complex scenarios. Currently, the selection of a targeted therapy for an individual patient is based on analysis of the primary tumor for the expression and/or genomic status of a specific molecular target (98,99). However, primary carcinomas, in particular PCa, show a marked intrapatient heterogeneity in regard to genotypic and phenotypic characteristics, and tumor cells may transform dynamically during the extended time period between primary tumor resection and metastatic relapse due to parallel progression and/or natural selection of the fittest clones (100). Metastases from different sites are genetically heterogeneous (29), and CTCs have the advantage that they may represent the entire spectrum of distant metastases (101). Thus, CTC detection and characterization may become a valuable tool to refine prognosis and serve as a real-time biopsy, and has the potential to monitor cancer treatment, provide useful predictive information for the selection of the most appropriate treatment and guide precision cancer therapies (100).

A growing number of studies have demonstrated that the dynamic change of CTC count is associated with a significant curative effect (102,103). Patients with CRPC received AA 1,000 mg daily and continuously, a post-therapy CTC count of <5 per 7.5 ml was prognostic for longer survival relative to a CTC count of ≥5, which indicated that lower CTC enumeration was positively associated with AA therapeutic effect (104). Furthermore, the change of CTC counts was used as an endpoint in patients with mCRPC to determine the efficacy and tolerability of cabozantinib at lower starting doses (36,105). For the first time, Dorff *et al* (25) detected CTCs in men with biochemical recurrence PCa to monitor the therapeutic effect of a combination herbal supplement, Prostate Health Cocktail.

In addition, molecular profile may be characterized in CTC as a potential predictive value of tumor sensitivity to a therapeutic modality or not (Table III), potential clinical uses of CTCs in determining prognosis and monitoring treatment effects, and as a source of tissue to identify predictive markers of drug sensitivity to guide treatment selection. Data from quantitative RT-PCR to detect circulating prostate-derived PSA and PSMA mRNA pre- and post- radical prostatectomy improves the accuracy of the Kattan nomogram to predict biochemical recurrence (72). In addition, biomarker response to docetaxel treatment was detected in 20 patients with CRPC (75). In response to docetaxel treatment, KLK3 levels decreased in 80% (95% CI, 60-100%), PCA3 in 89% (95% CI, 68-100%) and TMPRSS2-ERG in 86% (95% CI, 60-100%) of patients. By contrast, the blood samples from all 32 healthy volunteers were reproducibly negative for all three markers. A longitudinal study of a single patient with PCa who received

Table III. molecular profiling in prostate cancer circulating tumor cells as potential predictive value of tumor sensitivity to therapeutic modalities.

Therapeutic modalities	Molecular profiling	Predictive value	(Refs.)
Radical prostatectomy	PSA (-)	Biochemical recurrence (-)	(72)
	PSMA (-)	Biochemical recurrence (-)	(72)
Docetaxel	PCA3 (-)	Sensitivity	(75)
	TMPRSS2-ERG (-)	Sensitivity	(75)
	KLK3 (-)	Sensitivity	(75)
Abiraterone acetate	AR (+)	Resistance	(106)
	MYC (+)	Resistance	(106)
	Ki67 (+)	Resistance	(107)
	AR nuclear translocation (+)	Resistance	(107)
	AR-V7-positive	Resistance	(76,108)
Enzalutamide	AR-V7-positive	Resistance	(76,108)
Taxanes	AR-V7-positive	Sensitivity	(108)
Cabazitaxel	AR-V7-positive	Sensitivity	(109)
ADT	Harboring AR mutation	Resistance	(34)
	Wnt5a (+)	Resistance	(112)

(-), decreased; (+), increased; ADT, androgen deprivation therapy; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; PCA3, prostate cancer antigen 3; TMPRSS2-ERG, transmembrane protease serine 2 and ETS-related fusion gene; KLK, kallikrein-related peptidase; AR, androgen receptor; AR-V7, androgen receptor splice variant 7.

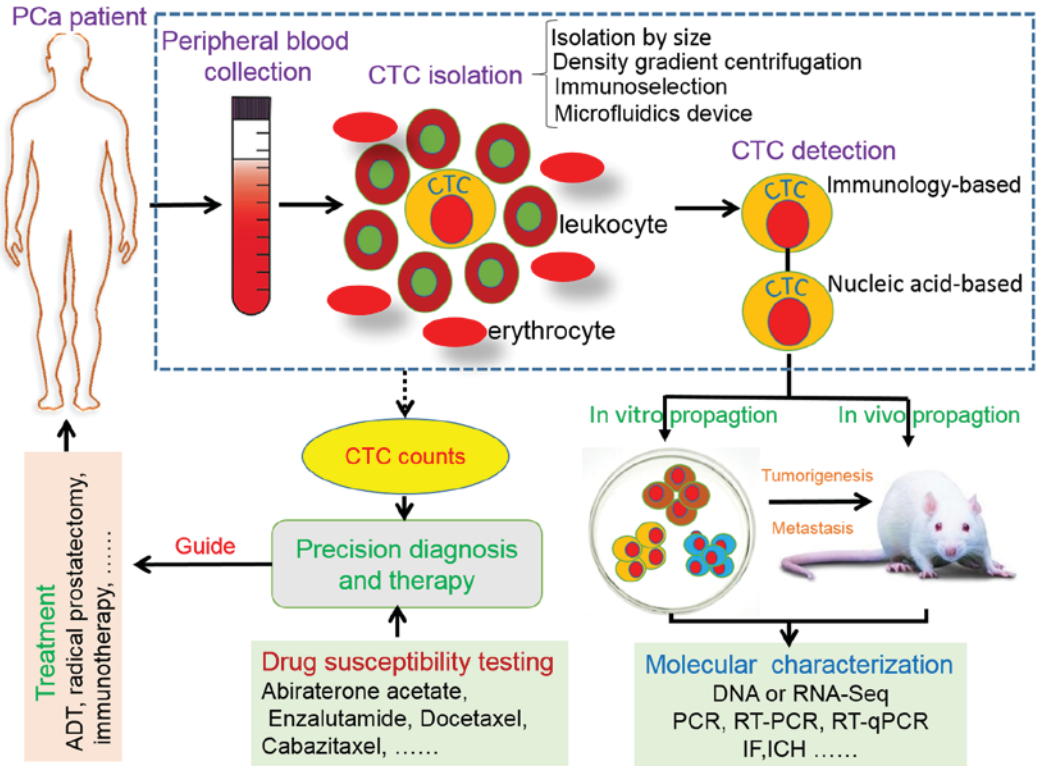

Figure 1. Circulating tumor cell in prostate cancer: Precision diagnosis and therapy.

AA treatment demonstrated that CTCs that acquired AR and MYC gene amplification represented a novel lineage, apparently resistant to AA, and possibly generated from a single resistant cell (106). In addition, despite intrapatient heterogeneity, CTCs from patients with prior exposure to abiraterone had increased the proliferation marker Ki67 expression, and qualitatively, the majority of AR staining within CTCs was intranuclear in subcellular localization (107).

Several studies have demonstrated that detection of AR-V7 in circulating tumor cells from patients with mCRPC

may be associated with resistance to enzalutamide and abiraterone (76,108). However, in AR-V7-positive men, taxanes appear to be more efficacious than enzalutamide or abiraterone therapy, whereas in AR-V7-negative men, taxanes and enzalutamide or abiraterone may have comparable efficacy (108). In addition, Onstenk *et al* (109) recently demonstrated that the response to cabazitaxel appeared to be independent of the AR-V7 status of CTCs from patients with mCRPC. Consequently, cabazitaxel may be a valid treatment option for patients with AR-V7-positive CTCs. In addition, patients with PCa harbored AR point mutations (a p.T878A point mutation and a p.H875Y mutation), which was another common AR modification mediating resistance to androgen deprivation therapy (34).

Furthermore, CTCs may be used as a viable pharmacodynamic marker to provide additional information on the safety profile and efficacy of agents. A phase I study determined the maximum tolerated dose of AEZS-108 in men with taxane- and CRPC, which was partly based on AEZS-108 internalization in CTCs using its autofluorescence (35).

In conclusion, CTC detection and characterization have shown potential to monitor cancer treatment and guide precision cancer therapies; however, the molecular profile of CTCs requires additional study and exploration, and more rigorous large clinical trials are required to confirm its clinical utility.

4. CTC culture

Stable long-term *in vitro* and *in vivo* CTC cultures may provide an opportunity for high-throughput preclinical testing of therapeutic regimens; however, until recently this has had limited success (110). In 2014, Yu *et al* (30) reported a landmark study, in which they established CTC cultures from 6 patients with estrogen receptor-positive breast cancer. Of the 5 CTC lines tested, 3 were tumorigenic in mice. Drug sensitivity testing of CTC lines with multiple mutations revealed potential new therapeutic targets, which could aid the identification of the best therapies for individual cancer patients over the course of their disease. Bichsel *et al* (110) demonstrated the feasibility of merging CTC capture with three-dimensional tumor cell culture by extracting PC3 cells with the microchip and culturing them on-chip for 6 days. Furthermore, CTCs isolated from patients with mPCa could survive and grow in xenotransplants to retain a long-survive capacity (26). Gao *et al* (111) successfully established a prostate CTC line from >100 CTCs, followed by culture *in vitro* for >9 months using organoid conditions modified to support the growth of prostate tumor cells. In summary, with the ceaseless optimization and development of CTC culture techniques, prediction of treatment responses and disease progression should ultimately be improved.

5. Conclusion

The present study mainly described current advances in CTC enrichment and detection strategies, and reviewed how CTCs could move toward precision diagnosis and therapy in PCa (Fig. 1). Recently, Miyamoto *et al* reported a landmark study on RNA-sequencing (RNA-Seq) of single prostate CTCs (112). This study established single-cell RNA-Seq profiles of 77 intact CTCs isolated from 13 patients (mean, 6 CTCs per patient), by using microfluidic enrichment. It was found that single-cell analysis of prostate CTCs revealed heterogeneity in signaling pathways, most importantly, ectopic expression of Wnt5a in PCa cells could attenuate the antiproliferative effect of AR inhibition, whereas its suppression in drug-resistant cells restored partial sensitivity (112). Beyond that, circulating tumor cells may be used as potential therapeutic targets for PCa treatment; killing and eliminating circulating tumor cells has become a novel and effective strategy for PCa therapy (113,114), and surrogate predictors of survival could shorten drug trials for PCa treatment (115). In conclusion, there is great promise in utilizing CTCs as a platform for precision diagnosis and therapy in PCa. Although the future is bright, there is also a huge challenge. More sensitive and effective devices for CTC enrichment and detection are urgently required to be developed. More importantly, CTC assays require validation in clinical trials to achieve clinical validity and clinical utility.

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