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What Is the Future of Circulating Tumor Cells in Colorectal Cancer?

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Abstract

Purpose of Review To introduce recent progress on circulating tumor cell (CTC) research in colorectal cancer (CRC) and to highlight clinical application of CTCs from detection to assessment of treatment response.

Recent Findings CTC biological characteristics in CRC play an important role in the detection of CTCs. The in vitro culture of CTCs from CRC patients (cell lines and organoids) can potentially facilitate rapid drug testing and treatment prediction. CTC detection should be standardized with improved detection rate in CRC; further, clinical investigation is still needed to clarify its potential as a tool for early CRC detection, a predictive and prognostic marker to ultimately guide treatment.

Summary Gaining an improved understanding of CTCs characteristics and ultimately, integration of CTC detection and utilization of CTCs may lead to optimized tumor treatment and facilitate precision medicine.

Keywords Circulating tumor cells · Colorectal cancer · Detection · Clinical marker

Introduction

CTCs were first described in 1869 [1]; however, CTCs did not draw attention either in the laboratory or clinic for a long period. Incorporation of CTCs was hindered by its low abundance in the blood and the lack of qualified detection technology compared to other liquid biopsies. CTCs are extremely rare in circulating blood (less than 100 CTCs in a million blood cells), especially in colorectal cancer. However, significant progress related to detection technology has been made in the last decade. CTCs have been approved by the FDA in 2007 as a prognostic marker in the metastatic colorectal cancer.

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The rarity and heterogeneity of CTCs in colorectal cancer require detection techniques with high specificity and high sensitivity to distinguish them from epithelial non-tumor cells and leukocytes in blood. In comparison analysis of CTCs, comparing the primary colorectal cancer site and metastatic sites, there was both similarity and disparity based on the protein expression levels or the gene mutation status [2, 3•], which demonstrated that CTCs could be a good representation of intra-tumor heterogeneity. Although the CTC detection rate in CRC is low, its potential clinical significance triggers the importance of further investigation. Therefore, there is much interest in developing CTC-related analysis, including CTC counts, CTC dynamics, and CTC-related markers to further establish the clinical utility of CTCs.

In this review, the CTC biologic characteristics, detection technology, and clinical application and challenges will be summarized and discussed.

The Biology of CTCs in Colorectal Cancer

CTCs in CRC share many common tumor characteristics with other solid tumors. They are shed from the primary tumor and or metastatic deposits and then enter the blood circulation either as a single cell or cells cluster [4]. The exact process of how CTCs from CRC enter the blood is not clear; however, studying primary colorectal cancer mouse models with labeling may help. CRC tumor cells first enter the vasculature but

then need to change their morphology to enhance their motility in a process called mesenchymal-epithelial transition. Once entering the circulation, CTCs confront blood shear, immune attack, and anoikis. Therefore, the half-life for CTCs is short, ranging from several minutes to hours. While this short half-life allows for CTC counts to be used as a tool for monitoring tumor response to surgery or other modalities, it can also lead to challenges for CTC detection, especially the time points for blood sampling and CTC analysis.

Physically, the size and morphology of CTCs vary a lot. CTC counts in the blood in CRC are low compared to other tumors like breast cancer and prostate cancer [5]. According to FDA approval, the cutoff value for CTCs in metastatic CRC for unfavorable prognosis is 3 in 7.5 ml blood, while in breast cancer and prostate cancer, the cutoff value is 5, which means that the application of CTCs in CRC confronts more challenges compared to other solid tumors. The molecular profiling of CTCs from CRC is limited. The early relevant data was from the experiments in orthotopic mouse models of colorectal cancer, which showed CTCs exhibited stem cell characteristics, with downregulation of epithelial and proliferation markers and upregulation of stem cell markers DLG7 and BMI1 [6•, 7]. Recently, the success of in vitro culture of CTCs from colorectal cancer patients has been inspiring. These studies have demonstrated that CTCs not only have a stem cell-like phenotype with increased capacity of tumor formation and self-renewal but also provide an invaluable tool of rapid treatment testing and prediction for individual patients [8••]. In comparison analysis of CTCs and corresponding tumor tissues, the mutational profiles of CTC were similar, but not identical to the corresponding tumor tissues. For example, the mutations in key genes such as KRAS or TP53 or BRAF could be detected in CTCs but not consistently within the tumors [3•, 9]. This mutational heterogeneity between tumor tissue and CTC should be taken into consideration during targeted therapy and tumor response monitoring.

Biologically, most CTCs in CRC have an epithelial trait with the expression of anti-epithelial cell adhesion molecule (EpCAM). EpCAM is utilized for CTC detection along with cytokeratins 8, 18, and 19 (CK8, 18, and 19) in the cellSearch system in metastatic CRC. While EpCAM is an epithelial marker which is not exclusive in CRC and always fluctuates in blood in solid tumors, based on the tumor metastasis theory, tumor cells initiate metastasis through intravasation-translocation-extravasation-colonization process, so some tumor cells may lose the expression of EpCAM. To improve the sensitivity and specificity of CTC detection in CRC, exploitation of other colorectal cancer markers specific for CRC is needed.

Comprehensive understanding of the biologic properties of CTCs could improve its detection, assist in the understanding of the tumor metastasis, and potentially, help guide tumor treatment in the era of precision medicine.

The Markers for CTCs in Colorectal Cancer

Specified CTC markers in CRC are very important for CTC detection. However, up until now, no novel markers have been identified for CTC detection in CRC. Recently, Plastin3 has been reported to be a potential CTC marker with prognostic value in CRC patients, including among early-stage patients without lymph node metastasis [10•, 11]. While comparison studies about the efficiency of Plastin3 in improving CTC detection in CRC are lacking currently, a functional study has recently demonstrated that Plastin3 could induce tumor EMT by upregulating F-actin and microfilament expression in Lovo cells [11]. These finding suggest that Plastin3 CTCs may exhibit high potential of metastasis. One limitation, however, is that Plastin3 is not a universal colorectal cancer marker.

The CTC origin in CRC shows CTCs are good representation of primary and metastatic CRC tumor cells, including their inherent heterogeneity and evolving traits during tumor development and treatments. Therefore, the different subpopulations of CTCs in CRC may stand for their individual corresponding tumor colonies. This may explain why specific CTCs may only partially correlate with tumor response and also inform us that a single marker for CTC detection in CRC may not be enough.

Current Technologies Used for CTC Isolation and Detection in Colorectal Cancer

The rarity and heterogeneity of CTCs in colorectal cancer limit its detection and present methods applied are based on the physical properties and biological characteristics of CTCs in the colorectal cancer. The former mainly utilize tumor size, deformability and density of CTCs to discriminate tumor cells from blood cells. The most commonly used technique is the filtration methods based on cancer cells size, like ISET and MetaCell® technologies. These methods have a higher recovery rate but exclude the small tumor cells whose sizes are similar to leukocytes. The overlap in size and density between CTCs and leukocytes degrades the purity of these methods; therefore, further steps are needed to stratify and specify the tumor cells. RT-PCR, immunohistochemistry staining, and flow cytometry can also be used. The methods based on the CTC biological characteristics are mainly dependent on the epitope and epithelial markers on tumor cells, which are barely expressed on the leukocytes. Among these methods, the cellSearch system are the most known in colorectal cancer, and this platform has been approved by FDA to detect CTCs as prognostic marker in metastatic colorectal cancer with the baseline ≥ 3 in 7.5 ml peripheral blood. However, this method has a very low detection rate in colorectal cancer from 10 to 30%, as the epitope EpCAM, CKs, and other proteins like

vimentin used for CTC detection are not expressed in tumor cells all the time [12]. The other commercial device that has already been approved by the European Union for separation and enumeration of CTCs is GILUPI CellCollector™. This device first uses a medical wire coated with EpCAM antibodies for direct in vivo CTCs separation via insertion in the cubital vein for 30 min; then, ex vivo post-capture analysis including antibodies staining and screened for CTCs under the microscope is performed [13, 14]. This method is mostly used in thoracic tumors, and the comparison of CTC counts through cellSearch and medical wire showed that the latter technique had a higher sensitivity (≥ 60%) in capturing CTCs from lung cancer patients. Additionally, GILUPI CellCollector also detected a significant median decrease of CTCs after initial first-line therapy, which was not observed through the cellSearch result [13].

However, for early diagnosis of colorectal cancer or diagnosis of cancer metastasis, these current technologies cannot meet this need [15]. Research endeavors especially in the fields of chemistry, materials science, and bioengineering should be integrated to overcome the detection hurdles caused by low number of CTCs and heterogeneity in CRC. Approaches utilizing engineering-modified material like three-dimensional micro/nano-structures could enhance the sensitivity of rare cell detection. Additionally, a binary-blend fiber-based capture assay has exhibited lower false positive readings and higher sensitivity and selectivity. With preclinical specimens compared to the commercial IsoFlux CTC detection System, this method uses a fibrous mat named electrospun blend fibrous mats (EBFMs) which could enhance the media's ability of CTCs capture [16•]. Table 1 summarizes the main reported methods for CTC detection in CRC to date. Each method has pros and cons, and only a few CTC detection methods have been approved for routine clinical use. Even the approved cellSearch and medical wire are limited with the detection equipment and inadequate high sensitivity. Integrating both physical tumor and molecular properties could improve the CTC detection rate and facilitate clinical practice. Standardization of the CTC counts method is challenging, as some methods utilize the CTC-associated marker expression levels not CTC quantification for determination of CTCs, and there is no standardization among different methods, nor the cutoff value for baseline among different stages of colorectal cancer. However, moving toward global standardization of the methodology of quantification of CTC is important to optimize interpretation of results.

Clinical Relevance of CTCs in CRC

The clinical roles of CTCs in CRC are a key driver for the improvement of CTC detection. These roles include figuring out the clinical relevance of CTCs in CRC, including the value of CTCs as an early diagnosis marker, predictor of treatment response, and treatment guidance for personalized treatment recommendations. We will try to answer the following question to clarify the CTCs' potential application in CRC.

Can CTCs Be Applied as a CRC Marker in the Early Detection of CRC?

Early detection of tumor is pivotal for tumor treatment and patient overall survival. For colorectal cancer, early screening through colonoscopy and flexible sigmoidoscopy should be routinely applied but the invasiveness of the procedure limits the use and adoption. Other radiographic screening methods, including magnetic resonance imaging and computed tomography, are cost-prohibitiveand expose patients to radiation. The molecular marker, carcinoembryonic antigen (CEA), has high false positive rates. Therefore, there is much interest in evaluating CTCs as a potential screening tool for CRC. CTCs can be released from early tumor lesions and detected at an early stage of disease, so CTCs along with relevant circulating tumor DNA (ctDNA) draws much attention for its convenience and real-time sampling as liquid biopsy [38•]. The CTC assay of EBFM detected individuals with stages II, III, and IV colorectal cancer, and the results perfectly matched the pathological analyses results [16]. Even in benign colorectal disease, CTCs can be detected in peripheral blood with a much lower number compared to non-metastatic cancer of the colon and rectum [39]. Therefore, it is promising that CTCs could be used as cancer marker for early detection and diagnosis in the future.

Although there is great potential for CTCs in detecting CRC in a screening population, right now, single CTC quantification cannot meet clinical needs and alternative methods are being developed to improve the detection rate of CTCs in CRC. Integrating molecular profiling could facilitate the detection of CTC analysis, and the molecular characteristics of gastrointestinal tract are utilized for CTC detection and CRC screening. For example, gastrointestinal tumor-associated antigen 2 (GA733-2) and CEA were used to detect CTCs in CRC in the second step of RT-PCR after the immunomagnetic enrichment [34]. Additionally, Cytokeratin 19 Epispot assay was used to detect CTCs in CRC patients with liver metastasis. After negative selection and deletion of CD45 positive blood cells, enriched viable CTC cells were analyzed as CK-19 releasing cells. It turned out CK19-Epispot assay had a much higher detection rate than the classic cellSearch system both in mesenteric and peripheral blood [40]. Welinder et al. detected the CTCs in 25 colorectal cancer patients through the cellSearch system; however, the difference was that when they added anti-CK20 (a well-established marker for colon epithelium) into the conventional cellSearch antibodies panel (CK8/18/19) for CTC analysis, they found CK20 significantly improved CTC detection [41]. These studies indicated that CTC-

Table 1 Summarization of current CTC detection methods in CRC

Methods	Isolation principle	CTC detection characteristics	Sample volume	Reference
Cytotrack	Density isolation and immunological method	Definition of a CTC: > 4 μm diameter, DAPI-positive, CK-positive, CD45-negative. Low recovery in CRC (< 10%)	7.5 ml	[5, 17]
ISET technology	Size-based filtration and hematoxylin and eosin (HE) staining	CTC with CD45 negative hyperchromatic nucleus, irregular shape, high cytoplasm nucleus ratio (> 0.5) and cell size ≥ 12 μm	1–14/ml	[2, 18–21]
VTX Liquid Biopsy System	Microfluidic chip based on cell size, deformability and multivalent binding	Labeling free, viable	7.5 ml	[22]
CellSievo	Funnel filtration based on size-exclusion and immunostaining	EpCAM+/DAPI+ cells	10 ml	[23]
CMx platform	Microfluidic device utilizing antibody conjugated membrane	CK20+/DAPI+/CD45-viable cells	2 ml	[24]
IsoFlux	Microfluidic chip based on flow control and immunomagnetic capture	CK+,CD45-pan-cytokeratin antibody targets multi-cytokeratins	14 ml	[25]
PCR	Tissue-specific and EMT transcript examination	CK20, VIL1, CLU, and TIMP1 expression levels for survival prognosis prediction	7.5–20 ml	[26, 27•, 28]
MetaCell®	Size-based filtration method and cytomorphological evaluation	Nuclear size ≥ 10 μm; irregular nuclear contour; visible cytoplasm, cells size over 15 μm prominent nucleoli; high nuclear-cytoplasmic ratio; (vi) presence of proliferating cell, actively invading cells creating 2D or 3D cell groups.	8 ml	[29, 30]
CellSearch system	Immunomagnetic separation and IHC staining	EpCAM+/CK+/DAPI+/ CD45− cells	7.5–10 ml	[31••, 32]
AdnaTest ColonCancerDetect kit	EpCAM-based immunomagnetic separation and tumor-specific mRNA analysis	detection of tumor-specific mRNA transcripts CEA, EGFR and GA733–2	5 ml	[33••, 34]
Vortex technology	Laminar microvortices for enrichment and downstream molecular analyses	DAPI+/CD45− with both nucleus size ≥ 9 μm and a nucleus-to-cytoplasm (N:C) ratio ≥ 0.6	4–10 ml	[35]
GILUPI CellCollector™	Catheter-based or wire-based enrichment of EpCAM-positive cells	EpCAM+/keratins+/Hoechst33342+/CD45−	In vivo capture of CTCs	[36•]
Parsortix system	Microfluidic based particle separation and IHC staining	CK+/CD45−/DAPI+ morphologically intact nucleus	7.5 ml	[37]

associated marker could efficiently improve the detection rate. Moreover, these markers reflect part of tumor profiling property, which could be used to predict the tumor. For instance, patients with high CTC-CK20 had a significantly worse median OS than those with low expression of C20, with a trend toward inferior OS in the high CTC-survivin group [28].

Therefore, with the advent of multidisciplinary and rapidly developed state-of-art technology, combining the high sensitivity molecular methodologies, there is much room to improve CTC detection. Many attempts are currently still in the animal model stage [42, 43]; however, the appearance of CTCs in benign colorectal disease is a reminder of need for close surveillance for these patients in case of malignant transformation.

Can Circulating Tumor Cells Be Used as Prognostic Marker in CRC?

It is known that CTC quantification has been used as a prognostic marker in metastatic colorectal cancer since the approval of FDA in 2007. Patients with positive CTC counts have shorter overall survival and PFS irregardless of the treatment modality, compared to those with negative CTC counts. Additionally, CTC positive patients trended to have a higher level of carcinoembryonic antigen and more advanced stage cancer, with CTC counts in stage IV colorectal cancer patients being significantly higher than that in stage I to III patients [18]. A recent prospective study of 140 patients with resectable colorectal liver metastases showed CTC positivity was significantly associated with impaired OS, especially

recurrence-free survival [44]. And also, there were evidence that patients with low baseline CTC counts (< 3) had longer survival than those with high CTCs (≥ 3) [45••]. Another prospective single-center study of 183 patients with newly diagnosed non-disseminated CRC showed patients with CTCs before surgery had a significant decrease in 5-year of recurrence-free survival and colon cancer-related survival (CCRS) compared to those without CTCs. Moreover, in this study, the presence of CTCs immediately after surgery was not significantly associated with RFS and CCRS; however, CTCs 2–3 years after surgery were significant. These findings suggest that the time point for CTC sampling and analysis was important [46, 47]. Kaifi et al. also found high CTC counts correlated significantly with tumor burden, liver metastasis, and high serum CEA in stage IV colorectal cancer [48]. In contrast, the prevalence of CTC positivity did not significantly differ according to age, gender, differentiation, and site of primary tumor or recurrence based on the finding of Romiti et al. [49]. Lalmahomed et al. also pointed out the presence of CTCs in preoperative peripheral blood samples does not identify patients at risk for early disease recurrence after curative resection of colorectal liver metastases and that the disease-free and overall survival were similar between patients with or without CTCs [50]. Another large cohort, multiinstitutional study of 472 patients with stage III CRC found that CTC enumeration was not associated with worse DFS and OS in patients with stage III CRC, no matter the baseline of CTC from CTC ≥ 1, CTC ≥ 2, and to CTC ≥ 3. Additionally, there was no association between the CTC counts and the clinicopathological characteristics, although detection of CTC ≥ 1 was significantly different between the three stages of CRC (IIIA 40%, IIIB 32%, IIIC 47%; $P = 0.016$) [31••]. Likewise, Eliasova et al. also detected CTCs in different stages of colorectal cancer without significant difference [30] and CTC baseline status was not associated with treatment response in CRC [33••]. In conclusion, the role of CTCs in prediction of colorectal cancer prognosis is still controversial based on these studies. At this time, the association between CTC enumerations and colorectal cancer stages still need further investigation and validation.

Can CTCs Be Used to Monitor Disease Progression and Treatment Response?

In CRC, monitoring tumor progression and treatment response requires observation of the tumor-related parameters continuously through either blood measurement like CEA levels or image analysis like computer tomography, MRI. CTCs originate from primary or metastatic tumors; therefore, theoretically, the tumor dynamic could be reflected by the CTC fluctuation. CTC dynamics in the peripheral blood may be significant allowing for reflection of the tumor response and guide tumor treatment. Technologically, lack of standardization and consensus for CTC cutoff value in baseline across different CTC detection methods in CRC worldwide makes it hard to interpret results across institutions. However, longitudinal tracking of CTC enumeration can be independent of the variation of methods. Tan et al. traced CTC dynamics from nine metastatic colorectal cancer patients, which measured CTCs prior to chemotherapy and at every other chemotherapy session during the course of treatment. They found that CTCs count trends generally correlated with radiological results, and even changed before the CT image, especially before the dynamics of CEA levels [23]. This indicated that CTC dynamics was more sensitive compared to other methods, suggesting that analysis of CTCs changes during the course of treatment may be useful in monitoring response to therapy in metastatic colorectal cancer. Virgílio et al. also evaluated the CTC kinetics and found that patients with CTC counts down after treatment had a favorable progression-free survival compared to the patients with CTC counts up after treatment [19]. The early CTC status and CTC status changes during treatment, but not the baseline of CTCs before treatment, were significantly associated with tumor response. CTC status change profile during treatment was an independent predictor of both progression-free survival and overall-survival in RAS-BRAF wild-type colorectal cancer. Moreover, CTC status assessed early during treatment with anti-EGFR monoclonal antibodies may predict treatment failure earlier than imaging-based tools [33••]. The dynamics of CTCs in colorectal cancer draws more attention and is more practical than single CTC detection.

The CTC-related markers are the proteins highly expressed on CTC cells. They not only embody the tumor characteristics, a cluster of CTCs populations with specified characteristics, but also are easier to be detected and analyzed than direct CTC enumeration. So analysis and monitoring of CTC-related markers during treatment can aid in surveillance, predict tumor response, and guide tumor treatment [28, 51]. For example, a panel of tissue-specific and epithelial-to-mesenchymal transition transcripts (GAPDH, VIL1, CLU, TIMP1, LOXL3, ZEB2) in isolated CTCs was tested to evaluate the prognostic and predictive value in patients with metastatic colorectal cancer. At baseline, patients with high CTC markers had a shorter median PFS and OS than patients with low CTC markers; PFS and OS for patients with increased CTC markers were also shorter than those of patients with CTC markers that decreased in response to treatment. These therapy-refractory patients identified by CTC markers were not detected by standard image techniques computed tomography [27•]. As mentioned before, Plastin3-positive CTCs potentially represent a population of CTCs which have undergone EMT and have high metastatic potential with downregulated EpCAM and upregulated stem cell markers like CD133 and CD44. The association between PLS3-positive CTC and prognosis was particularly strong in patients with Dukes B and Dukes C

[10•]. CTCs with elevated expression of proteins involved in tumor metastasis, including TGF-β, CXCL1, S100A4, and MACC1, especially non-receptor guanine nucleotide exchange factors (GEFs), tend to be associated with a shorter PFS. Expression of these proteins can fuel metastatic progression in a variety of cancers, and these CTC-related markers are strong prognostic for metastatic colorectal cancer with reduced overall survival [4, 52]. Such lineage of CTCs may imply tumor response with high risk of tumor resistance and recurrence. For instance, the expression of CD44 variant exon 9 in the CTCs is strongly correlated to treatment refractoriness, recurrence, and prognosis of human colorectal cancer [7, 8••, 53]. CK20 was also used to assist the detection of CTCs in colon cancer patients through RT-PCR [26, 54, 55], and CTCs with CK20 RT-PCR were identified as an independent negative prognostic marker in colon cancer patients [26].

For advanced colorectal cancer, 5-fluorouracil-based chemotherapy and neoadjuvant therapy are canonical, but only some patients derive benefit, making it necessary and urgent to explore proper parameters for treatment determination and response prediction. While just a few studies investigated the impact of CTCs in CRC on the tumor treatment response prediction, a single-arm phase II trial with four-drug regimen irinotecan and oxaliplatin plus tegafur-uracil and cetuximab showed patients with low CTC counts had a longer median OS [56•]. However, in rectal cancer treated with short course RT, Nesteruk et al. found CTCs detected at 7 days after surgery was a meaningful prognostic marker for the local recurrence, CTC detected preoperatively and at 24 h after resection had no such prognostic value [54]. The CTC-related marker analysis also obtained meaningful results. For example, Abdallah et al. evaluated drug resistance-related proteins in CTCs and sought to predict patients' response who received 5-fluorouracil-based FOLFIRI or FOLFOX chemotherapy in two cohorts of metastatic colorectal cancer patients using ISET technology and immunocytochemistry staining. They found thymidylate synthase (TYMS) staining in CTCs correlated with disease progression, while not in the primary and metastatic sites, patients who had CTC count ≥ 2 demonstrated more TYMS expression, which correlated with worse prognosis [1, 45••]. Additionally, multidrug resistance-associated protein 1 (MRP1) positive CTCs also showed a worse progression free survival (PFS) in comparison to those with MRP1 negative CTCs in patients with FOLFOX [2, 20]. Up until now, the evidence about the role of CTCs in rectal cancer patients received neoadjuvant chemoradiation (RCTX) is not convincing enough. Hinz et al. found cytokeratin 20-positive circulating tumor cells were a marker for response after RCTX but not for prognosis in a cohort of 63 rectal cancer patients with RCTX treatment [42]. Responders after RCTX had a lower incidence of CTC compared to non-responders [55]. It is possible that radiotherapy and systemic anti-tumor therapies might mobilize CTCs [57], especially radiotherapy, and potentially accelerate tumor migration. This would lead to treatment-related increases in the release of CTCs. This speculation needs confirmation in the future studies and could explain why CTC detection at some time points has no clinical relevance.

The Future Perspectives of CTCs in CRC

CTCs in CRC have great potential in the clinical utility; however, the uncertainties confronted in the CTC detection and clinical relevance need to be resolved before universal adoption in clinical practice. The following summarization could be the future perspectives for CTCs investigation in CRC.

The Standardization of CTC Detection

CTC detection and analysis contain multiple steps, including sampling, storing, handling, analysis, and result interpretation. Each step can influence the final results; however, right now, there are no unified criteria for CTC detection and all the steps therein. Therefore, these factors should be taken into consideration when clinical trials are planned. The preanalytical condition of blood sample is the easiest step to standardize, as qualified pretreatment of blood sample could prevent the degradation or loss of CTCs and maximize the integrity of CTCs for further analysis [58•]. Automation is another trend of CTC detection for auxiliary diagnosis, especially in the era of artificial intelligence. For instance, microfluidic innovation in the lab to a commercial automated system like VTX liquid biopsy system is a good example [22, 59] and is helpful for the achievement of CTC detection standardization in clinic.

The methods for evaluating the CTCs along with the properly defined cutoff value in a specified tumor stage in CRC should be recommended as well for standardization. Meanwhile, the time points for CTC detection in CRC cannot be ignored either. Cancer treatment could cause CTCs mobilization, thereby leading to more CTCs entering the blood. While present investigations have not fully explored these hypotheses, this speculation may explain the inconsistency of CTC clinical relevance among different investigations.

The Profiling of CTCs in CRC

Another important factor in CTC detection as CRC marker is its false positivity, as it was reported that CTCs could be detected even in benign disease like colonic or rectal polyps and healthy donors [5, 39]. Patients with colorectal cancer, however, generally do have higher CTC counts than patients with colorectal polyps [39], reminding us the importance of establishing a positive cutoff value for selection of CTC. Comprehensive profiling of CTCs in CRC may effectively solve such problems. Deep understanding of CTCs

characteristics could also help identify potential targeted therapeutic agents which target specific driver mutations in CRC. For example, research found RhoA signaling was necessary for the intra-capillary morphology switch (ICMS) and the arrest of CTCs, targeting RhoA using a clinically approved RhoA/ROCK inhibitor, could efficiently inhibit the initial arrest of individual CTCs and reduce the incidence of tumor metastasis in animal models [60]. This finding is very important for stages II, III, and IV colorectal cancer with high risk of metastasis. Profiling CTCs could provide more information about CTCs characteristics, and in vitro cultivation of viable CTCs, including the cell lines and organoid culture, ensures enough sample for next-generation sequencing and single cell sequencing [61, 62•, 63•]. As organoid systems have been show to predict response in patients with gastrointestinal cancers, completely profiling the organoids derived from CTCs will exploit some novel CTC-related markers specific to CRC for disease detection and treatment evaluation, and unveil new perspectives for target treatment [54]. As there are both concordance and disparity between liquid biopsy and tumor tissues, the cultured organoids derived from CTCs will help magnify the disparity and expose the acquired treatment resistance target for treatment [38•, 64, 65], which is a huge step for precision medicine.

The Integration of CTC Analysis and Other Detections

To improve the CTC detection, we suggest multicenter cooperation from multiple regions for the establishment of a consensus CTC detection workflow. Likewise, to maximize the clinical implication of CTCs in CRC, the multimodality detections are also in need. Here we proposed our own scheme of CTC analysis with other for CRC study (Fig. 1). Integrating CTC analysis and other methodology could be complimentary and mitigate each other's deficiency in the present investigation. ctDNA is another form of liquid biopsy as DNA fragments from necrotic and apoptotic tumor cells. The detection of ctDNA can be done using blood plasma or serum and can show the efficiency of targeted drugs or chemotherapeutics [66]. Importantly, ctDNA has been found to be detectable in all metastatic colorectal cancer cases, whereas circulating tumor cells were detectable only in one-third of cases [38•]. Moreover, when evaluating ctDNA as an alternative screening for CRC, Church et al. assessed the accuracy of circulating methylated SEPT9 DNA (mSEPT9) for detecting CRC in a screening population. The results based on mSEPT9 test showed that CRC signal could be detected in asymptomatic average risk individuals with about 70% sensitivity and 90% specificity in retrospective case control studies [67]. CTCs in the peripheral blood can provide more comprehensive characterization [22] including mRNA, DNA, and protein information, especially histocytology and the in vitro cultured cell lines and organoids derived from CTCs which can be used to test therapeutic agents. However, partnering with ctDNA examination for CRC detection with CTCs for treatment prediction and guidance will maximize the clinic practice of these two liquid biopsies.

Conclusions

CTC is as an important liquid biopsy which provides comprehensive information during colorectal cancer progression. The low invasion and real-time convenience facilitate the capture and investigation, while much more efforts are needed to improve the detection rate and to determine its clinical relevance in CRC, especially the role in the CRC treatment response prediction including neoadjuvant therapy. Up to now, we cannot draw a unified conclusion about the CTCs' treatment response prediction, which we ascribe to current limited detection technology, lack of related clinical studies, and insufficient knowledge about CTCs. On the other hand, the in vitro culture of CTCs from CRC brings a new light for CTCs application in clinic, which provides a good preclinical drug

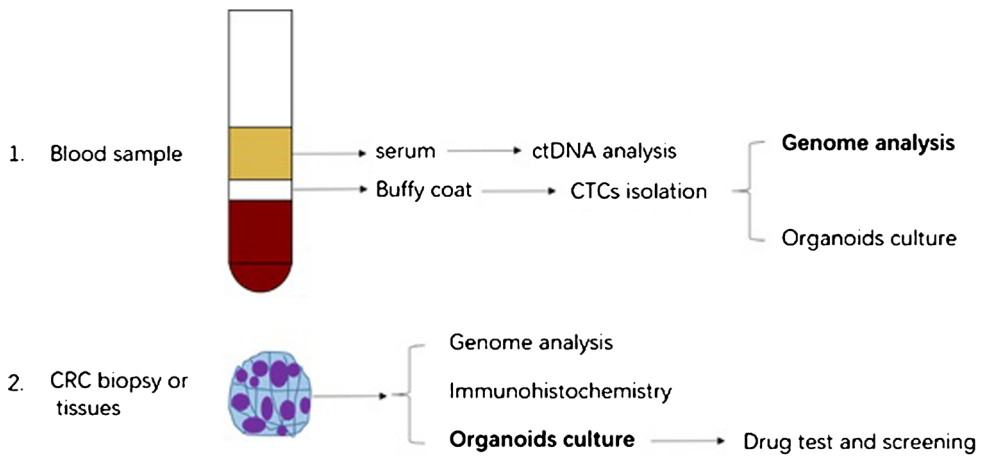

Fig. 1 Schematic illustration of CTCs and tissue sample study workflow. First, the blood sample was collected prior to treatment, during each cycle of treatment, and after treatment, and 6 months after treatment, the sample was analyzed as the circuit of blood sample treatment. Second, the corresponding biopsy or CRC surgery samples were collected and treated as the circuit of tissue samples. The bold text shows the priority of analysis

screen platform [68]. In the future, with technologic developments and multidisciplinary and multiinstitutional cooperation, there will be a full understanding of CTCs in CRC, and CTCs will maximize its clinical role in the personal treatment of CRC.

To better understand the clinical relevance and significance of CTCs in CRC, the investigations should carefully consider the methods adopted for evaluating the CTCs along with the properly defined cutoff value in a specified tumor stage, which may reduce the discordance among different studies.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

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