Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

CRITICAL REVIEW

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2025, **17**, 8865

Detection and indications of circulating tumor cells in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death due to late diagnosis, high recurrence rate and poor response to systemic therapy. Although surgery is still the optimal therapy, only a small number of HCC patients are eligible for radical resection at the time of diagnosis. Even those receiving liver resection are likely to suffer from recurrence within one year, and they account for most mortalities. It is urgent to develop powerful tools for early HCC diagnosis and real-time monitoring. Currently, detection of circulating tumor cells (CTCs) shows great potential in early HCC detection and treatment response, both for initial diagnosis and recurrences. Because detection is non-invasive, CTCs can present real-time monitoring of tumor progress. At the same time, as intact tumor cells in circulation, detection of CTCs may lead to an understanding of the mechanisms of HCC recurrence and metastasis. In this review, we discuss the developments in CTC detection and application, with a particular focus on clinical implications in HCC.

Received 11th August 2025
Accepted 13th October 2025

DOI: 10.1039/d5ay01320h

rsc.li/methods

1. Introduction

Liver cancers, 75–85% of which are hepatocellular carcinoma (HCC), rank as the sixth most commonly diagnosed cancer and the third leading cause of cancer-related death.[1] The high mortality rate is caused by recurrence and metastasis.[2] The bleak prognosis of HCC is primarily due to limitations of current methods for early diagnosis and dynamic monitoring. We have investigated the mechanism of HCC and identified biomarkers for HCC prognosis and treatment, such as STYK1, KLF4 and HNF-6. Recently we found that circulating tumor cells (CTCs) have a significant correlation with the prognosis of HCC, may serve as a biomarker for monitoring progress and guiding therapy, and could greatly improve HCC outcomes.

CTCs, tumor cells that shed from the primary or metastatic tumor and intravasate into the circulation system, are responsible for metastasis.[3] As "seeds" of metastasis, CTCs offer an opportunity to interrogate the most aggressive cancer clones, providing privileged insight into the biology and vulnerabilities of blood-borne metastasis.[4] As a typical liquid form of biopsy, monitoring the molecular alterations of CTCs holds great promise for precise prognosis and personalized treatment decisions for HCC.[5] Studies have shown that survivin-positive CTCs are significantly associated with the TNM tumor stage, BCLC stage, and degree of differentiation.[6] In the last decade, researchers have intensely developed devices and assays for CTC isolation and analysis. In particular, methods relying on negative cancer cell surface charges to realize CTC isolation[7,8] were introduced into CTC investigation. Along with the improvements of detection technology, studies of CTCs' genomics, transcriptomics and proteomics have become increasingly intensive. As a result, the importance of CTCs as "liquid biopsy" for scientific research and clinical indication has attracted growing interest.[9,10]

Even though they have shown great potential in HCC, there are still challenges to the clinical application of CTCs. The primary issue is the limited count of CTCs, with only a few CTCs per milliliter of blood, while there are millions of other blood cells.[11] Another challenge is the heterogeneity of tumor cells,[12] which makes the use of biomarkers more complex and limits the effectiveness of biomarker-based capture. Furthermore, the differences in CTCs from different circulatory locations greatly hamper CTC isolation and identification. In this review, we will discuss the developments in isolation and downstream analysis of CTCs, with a particular focus on the application of CTCs in HCC.

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2. CTC isolation

As a bridge for tumor metastasis and latency in circulation, CTCs hold great potential for cancer investigation. However, major challenges are the extremely low number and obvious heterogeneity of the isolated CTCs. In the last decade, considerable effort has been devoted to improving detection of CTCs. The commonly used isolation methods are immunoaffinity assays[13] and physical property-based assays.[14,15]

2.1. Immunoaffinity assays

Immunoaffinity assays capture CTCs either by targeting tumor-specific antigens or by depleting blood cells through white blood cell (WBC) biomarkers and red blood cell (RBC) lysis. Epithelial adhesion molecules (EpCAM) are commonly used as sorting antigens for CTC capture. For example, the well-known CellSearch protocol, which uses anti-EpCAM antibodies for positive CTC capture, is the only procedure approved by the US Food and Drug Administration (FDA) for use in breast, prostate, and colorectal cancers. However, the epithelial–mesenchymal transition (EMT) process, which leads to low expression of EpCAM, poses a great challenge to CTC capture by CellSearch.[16] In fact, CellSearch is often used as a standard to assess other methods.[17,18] Similarly, other commercial devices for CTC isolation employ immunobeads, such as magnetic-activated cell sorting (MACS)[19] and surface-enhanced Raman scattering (SERS)-based platforms.[20]

Multiple antigens are employed to improve sensitivity and to improve the affinity for CTCs. For instance, Xia *et al.* (Fig. 1A) [21] demonstrated that the combination of EpCAM and aminopeptidase N (APN) as two specific targets could greatly improve the capture efficiency and purity of HCC-CTCs. They also synthesized a dual-targeting magnetic-fluorescent nanobead to accurately detect HCC-CTCs in one step. Rather than immunomagnetic beads, Zhang *et al.*[22] (Fig. 1B) creatively engineered RBCs to capture CTCs and then released them by adding plasma. Because RBCs, as normal blood cells, can avoid absorption by leukocytes, this method exhibited high capture efficiency and purities of 80% and 95%, respectively. Moreover, materials used for CTC capture and release were mainly obtained from blood, thereby minimizing foreign disruption and creating a familiar circulatory environment. As a result, the cell survival rate exceeded 95%. Also, increasing the sample volume is another strategy to improve the chances of capturing CTCs. The CellCollector involves inserting a needle coated with anti-

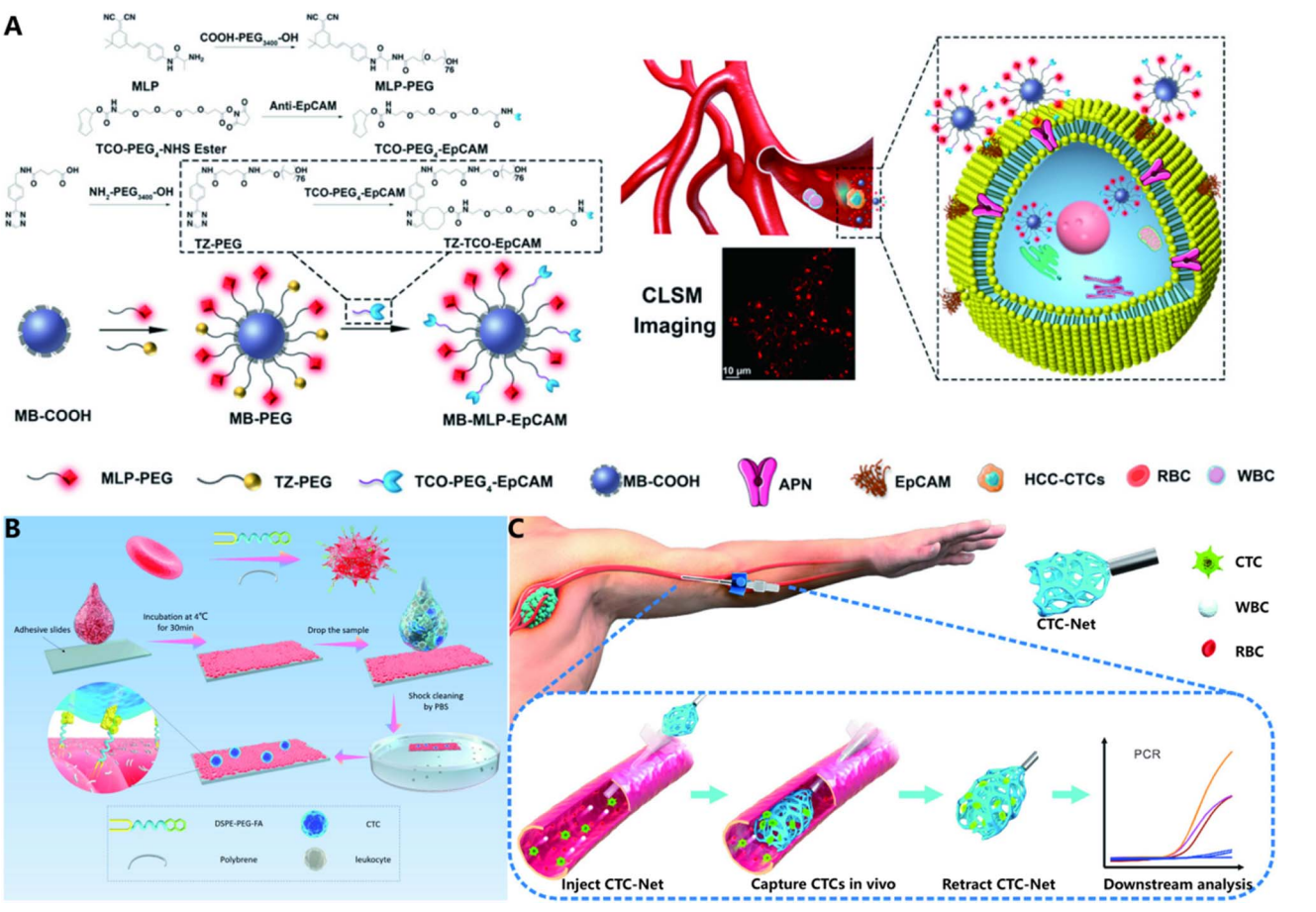

Fig. 1 Mechanisms of novel functionalized materials for CTC capture. (A) Synthetic route and construction of an MB-MLP-EpCAM probe for targeted HCC-CTC detection. (B) Biomimetic capture using an RBC monolayer: DSPE-PEG-FA-modified RBCs target tumor cells, with subsequent polybrene treatment enhancing deformability and forming a dense layer that isolates CTCs with high purity by resisting leukocyte adhesion. (C) Schematic of an injectable and retractable 3D scaffold for *in vivo* CTC capture.

EpCAM into the elbow vein (Fig. 1C).[23] Because blood flows continuously through the needle at 5 cm per second, the capture efficiency of CTCs could reach up to 40%.

These positive isolation methods achieve high purity. However, the processes of incubation and magnetic sorting not only reduce efficiency but also increase the risk of specimen contamination and cell lysis.[24,25] In addition, these methods depend on the antigen expressed on CTCs' surfaces, so that the sensitivity is poor due to the heterogeneity. For example, EMT cells will be lost by these EpCAM-based positive isolations. It has been widely accepted that malignant cells undergoing EMT exhibit low expression or no expression of EpCAM and are more invasive.[26,27]

2.2. Physical property-based assays

It is generally acknowledged that cancer cells are larger and less deformable than normal blood cells. This principle is the theoretical basis for most physical property-based isolation methods. Density gradient centrifugation remains the simplest method for sample pretreatment, albeit with its limitations in efficiency and purity.[28] In contrast, filtration has emerged as a powerful and efficient alternative for enriching CTCs, offering both high throughput and minimal cell damage.

Shimmyo *et al.* (Fig. 2A)[29] created a CTC isolator equipped with an array of thin microslit channels with a depth of 2 to 12 μm and a length of 4,8, or 12 mm. They tested various input flow rates and depths and lengths of microslit channels and determined that high capture efficiency and purity could be achieved when the microchannel depth is 3.3 mm, the flow rate is 60 μm per minute, and the channel length is 8 or 12 mm. Lu C. *et al.* (Fig. 2B)[30] created a novel microfluidic chip to separate CTCs by streamline-based focused separation and filtration. The capture efficiency of this chip was stable (up to 94.8%) over a large range of flow rates (5–40 mL h^{-1}). Also, this chip achieved efficient release and high activity of the captured CTCs because of the weak interaction between the cell and the chip.

Unlike antibody-based methods, physical property-based isolation better maintains the integrity of biological information and intactness of CTCs. Thus, they are more conducive to precise observation of molecular characteristics and drug resistance.[31] However, CTCs may be lost in the processes of dilution, centrifugation, container conversion, *etc.* At the same time, contamination by WBCs would lead to poor purity.[32] Furthermore, the increased fluid pressure inside the filters can damage captured cells.[33]

2.3. Microdevices for CTC isolation and analysis

Microfluidic technology involves systems with micrometer-scale flow paths and tiny vessels in which chemical and biological

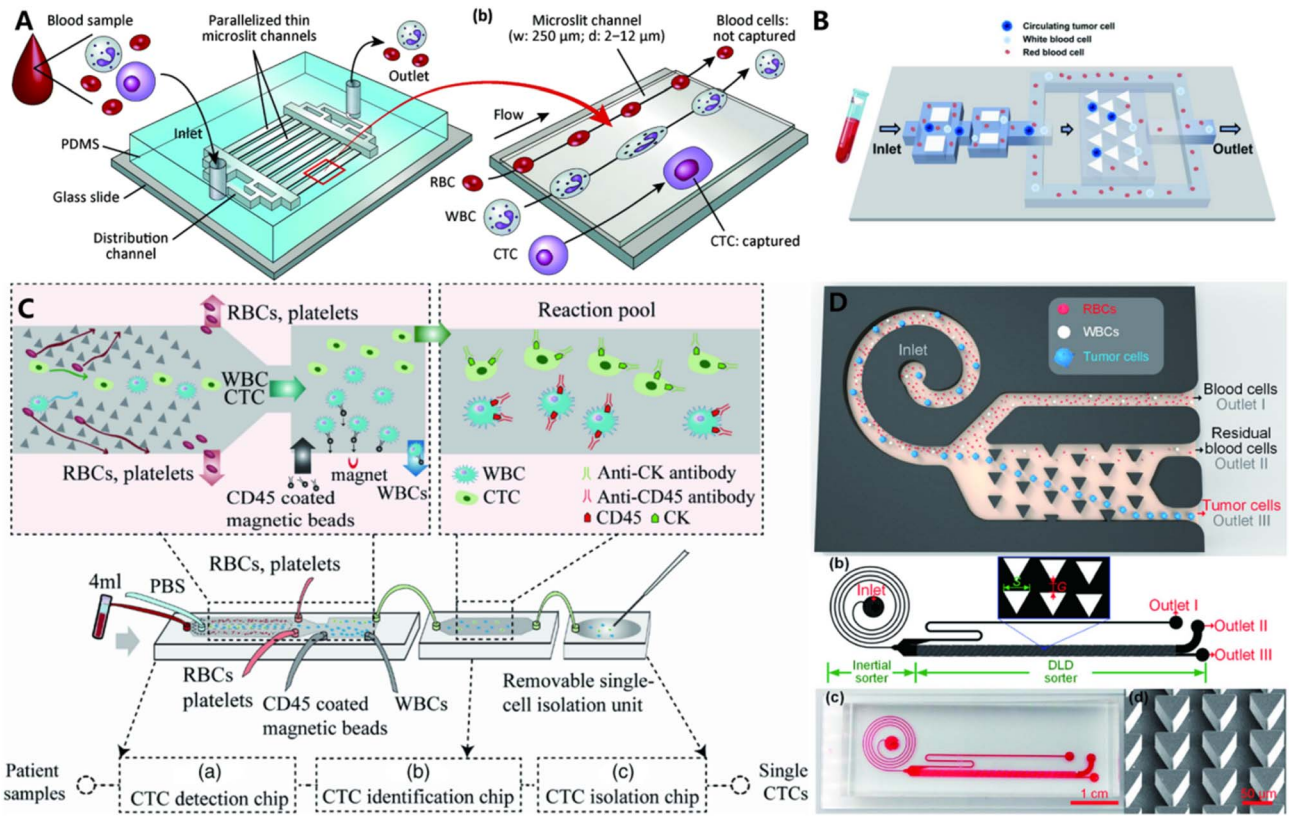

Fig. 2 Schematics of microfluidic strategies for circulating tumor cell (CTC) isolation. (A) Affinity-based capture: CTCs are selectively bound to antibodies in a functionalized channel while blood cells flow through. (B) Size-based filtration: CTCs are physically retained using a microfilter, allowing most blood cells to pass. (C) Integrated multi-chip workflow: blood is processed through sequential chips for separation, immunofluorescence identification, and single-CTC retrieval for genomic analysis. (D) Two-stage label-free separation: design combines an inertial spiral with a deterministic lateral displacement (DLD) array for continuous CTC sorting, shown with a device photograph and SEM image of the pillars.

experiments can be conducted precisely and efficiently. These systems have been widely used for CTC detection in the past decade (Fig. 2C and D).[34–37]

2.3.1. Immunoaffinity assays. In microchannels, cells are in closer proximity to the antibody-coated substrate than in macro-scale systems. Thus, they have a greater chance of being captured by antibodies immobilized on the substrate.[38,39] Micropillars coated with antibodies are often arrayed in a microchannel to enlarge the functional surface and disturb laminar flow, thereby increasing the opportunity of antigen–antibody contact.[40–42] Similar devices that increase the contact area by the microstructure include fish herringbone structures[43] and nano-ciliated structures,[44] which allow CTC capture, lysis, and genetic characterization to be performed on a single chip.

Nanomaterials have been found to increase the contact probability of antibodies and CTCs and are now being applied in CTC capture. Cui *et al.*[45] grew ZnO nanowires on the surfaces of PDMS pillars for CTC attachment and retention. The ZnO-coated microstructure greatly increased the functional surface area and promoted the attachment of CTCs. Since ZnO is sensitive to pH, the captured cells could be detached with minimal damage in mildly acidic solution. In addition, a system built by Li *et al.*[46] using MnO_2/TiO_2/FTO substrates also exhibited good performance for CTC isolation.

However, as with macro-systems, although some new antigens have been employed,[47,48] there is still no ideal tumor-specific antigen to adsorb all CTCs. This has been an insurmountable gap, greatly limiting the development of this isolation method.

2.3.2. Physical property-based assays. Common physical property-based enrichment methods in microfluidics include using dam structures, columns, side flow, micro-chambers, vortices, *etc.*[49,50] Mehdi Rahmanian *et al.* created a microfluidic chip with micropillars to separate CTCs based on size, providing high capture efficiency (>85%), purity (>90%) and viability (97%).[51] Maziar Hakim *et al.* designed a new micro-device, called the D-chip, based on the weak deformability of CTCs. The key design of the D-chip includes slanted weirs with a weir gap of 7 μm, resulting in capture efficiencies as high as 93%.[52]

In addition, due to the extremely small Reynolds number, liquid flow is always laminar in the microfluidic channel.[53] Therefore, many microfluidic chips have been designed based on these special fluid dynamics principles. A spiral microfluidic device achieved an amazing throughput (2.4 mL per minute) by hydrodynamic forces present in curvilinear microchannels to allow size-based isolation of viable CTCs.[54] Miao Sun *et al.* incorporated elastic materials in microfluidic chips to focus cells into traps according to hydrodynamic trapping.[55] Alternatively, a sandwiched flow microfluidic device utilized shear-induced diffusion to migrate CTCs from the side streams into a cell-free center stream, enabling separation without pre-processing by methods such as leukocyte depletion and RBC lysis.[56]

When blood flows through a vortex chip composed of serial sudden expansion–contraction reservoirs within a microchannel, CTCs are trapped in the centers of the vortices in each reservoir while blood cells undergo side flow back to the main stream.[57] Amir *et al.* found that increasing the height of the reservoir provides more space for the particles' orbits and reduces particle–particle collisions, thereby increasing the separation efficiency.[58] Furthermore, Camille Raillon and colleagues integrated a vortex device and an impedance chip into a single detection system, enabling label-free isolation and efficient subsequent analysis of CTCs.[59]

In addition, according to recent studies, CTC clusters may have much greater metastatic potential.[60,61] Mert Boya *et al.* introduced cluster-wells to selectively detect CTC clusters (ranging from 2 to over 100 cells) in untreated blood samples from prostate cancer patients based on the large size of CTC clusters.[62] However, further validation is needed to evaluate their application in hepatocellular carcinoma.

2.3.3. Single cell capture and release. To better understand tumor progress, researchers hope to comprehensively interpret the genomics, transcriptomics and proteomics information carried by CTCs.[63,64] As a result, there is a growing need for single cell capture and release technology, because effective capture and release of individual CTCs is a prerequisite for such downstream analysis.[65] Reem Khojah *et al.* designed a microstructure for single-cell capture utilizing the converse magnetoelectric effect, and the captured cells could be used for cell culture and expansion.[66] Instead of magnetoelectric manipulation, Rui Li *et al.* designed a single CTC capture and encapsulation platform based on ZnO nanofibers and surface acoustic waves, which significantly improved the capture efficiency.[67]

Beyond capture, characterization technologies have also advanced, allowing interpretation of the mechanism of cancer development. Chang Feng *et al.* developed a facile system for analyzing the molecular phenotype of single CTCs by integrating a single CTC capture microchip with a DNA isothermal amplification technique.[68] They achieved the analysis of membrane protein junction plakoglobin (JUP), which is closely related to cancer metastasis. After phenotypic analysis, CTCs still maintained physiological activity and could be used for drug testing.

In conclusion, microfluidic technology has shown tremendous advantages in CTC detection.[69,70] First of all, it achieves higher sensitivity and specificity than macroscopic operations. Second, it is more efficient and economical because of lower reagent and time consumption. Third, the closed system can effectively avoid sample loss and contamination. And last, the high portability and low equipment requirements make it easy to be applied. Thus microfluidics technology holds great significance for cancer screening.

3. Scientific and clinical applications of CTCs in hepatocellular carcinoma (HCC)

HCC is one of the most common cancers and is recognized as the third leading cause of cancer-related death in the world.[1] HCC is also highly malignant, characterized by insidious onset, rapid and invasive growth, high recurrence rate, and high

fatality. As a result, most patients are diagnosed at the advanced stage and suffer from poor outcomes.[71] Recent studies showed that CTCs hold huge potential for revealing the mechanism of tumor metastasis, monitoring recurrence and guiding individualized treatment for HCC.[72]

3.1. Scientific research

Most HCC patients lose their chances for radical resection at the time of diagnosis because of intrahepatic or distant metastasis.[73,74] Even for those receiving surgical resection, recurrence is still a major concern and half of the relapsed patients die within one year.[75] As a real-time monitoring approach, CTCs may offer opportunities for early indication, not only for the primary tumor but also for recurrent cases. Scientists are trying to reveal the mechanism of tumor progress and explore an effective treatment program by molecularly characterizing CTCs.[6,76,77] Heterogeneous biomarker expression within tumors and between patients has led to different outcomes of antigen-dependent CTC isolation in HCC.[78]

From a systematic perspective, CTCs collected from different circulatory sites and at different time points can better present molecular changes during tumor evolution than single-point puncture biopsy.[79,80] FGL1 is a ligand that binds to lymphocyte-activation gene 3 (LAG-3) to inhibit anti-tumor immunity. Q. Yan *et al.* first investigated the FGL1 expression of HCC CTCs by the CanPatrol technique.[76] The results showed that patients with (FGL1+)CTCs were more likely to exhibit distant metastases. Therefore, they inferred that FGL1 may play an important role in CTC dissemination. In addition, they proposed that FGL1 may contribute to PD-1/PD-L1 immunotherapy tolerance.

The Epithelial–Mesenchymal Transition (EMT) has become prominently implicated as a means by which transformed epithelial cells acquire the abilities to invade, resist apoptosis, and disseminate.[81] Some metastasis-related genes and pathways may also contribute to CTC release. A study proposed that downregulation of BCAT1 could suppress proliferation of HCC cells and migration, invasion and promotion of apoptosis by inhibiting the EMT.[82]

Among cells entering circulation, stem cell-like subpopulations termed circulating cancer stem cells (CSCs) are thought to have the capacity to evade immune destruction, thus driving tumor progression, metastasis, and resistance to chemical therapies.[83–86] CSCs are always identified as being CD44+/CD24−, CD133+, or ALDH1+.[87] Through a qRT-PCR CTC detection platform, Wei Guo *et al.* screened the expression patterns of nine putative CSC biomarkers systematically and constructed a HCC CTC detection panel, including EpCAM, CD90, CD133, and CK19. In addition, the high accuracy of this panel for HCC diagnosis, especially in early-stage and in AFP-negative cases, was validated by another independent group.[88]

In addition to statically analyzing CTC molecular characteristics, researchers can dynamically observe pathophysiological changes at the cellular level by *in vitro* culturing and at the systemic level using xenograft models.[89–91] Li Hu *et al.* conducted 3D culture of CTCs isolated from patient blood samples to form globules and found that CTC globules could be used to better predict short-term recurrence in HCC patients.[90] Another team created a device that efficiently captures CTCs while inducing *in situ* chemotherapy.[92] Although validated only *in vitro* with cell lines, this represents an inspiring result for further investigation and drug testing. Mu, W. and colleagues created a multi-point co-attack nanodevice (GV-Lipo/sorafenib (SF)/digitoxin (DT)) to dissociate CTC clusters, block the formation of CTC-neutrophil clusters and finally kill single CTCs. It was successfully verified that GV-Lipo/SF/DT increased CTC elimination efficiency *in vivo*, thus effectively preventing metastasis in orthotopic HCC models.[93]

Cells surrounding CTCs also influence CTC dissemination. Li's study showed that the decreased lymphocyte numbers following percutaneous radiofrequency ablation (RFA) contributed to the increased number of CTCs in HCC. They believed that the decreased number of lymphocytes weakened immune surveillance and the killing function, allowing more tumor cells to survive in the circulatory system.[94] However, Chen *et al.* reported that patients with CTC-WBC clusters were more susceptible to tumor recurrence, suggesting that these clusters may serve as a form of CTC protection.[95] This was an inspiring and revolutionary suggestion that needs further exploration, although it has not yet been deeply investigated by Chen's group.

3.2. Clinical applications of CTCs

3.2.1. Early diagnosis and prognosis. CTCs provide an important approach for early diagnosis of HCC, especially for screening and postoperative follow-up. It is reported that CTCs appear earlier than conventional imaging findings and provide ideal sensitivity and specificity.[26,96]

Serum alpha-fetoprotein (AFP) is a clinically recommended biomarker for HCC diagnosis and prognosis. However, most HCC continues to be diagnosed beyond an early stage due to insufficient sensitivity and specificity of AFP. Takahashi K. *et al.* found that AFP mRNA-positive CTCs emerged earlier and were more indicative of HCC diagnosis than serum AFP. They also found that AFP mRNA (+) CTCs were sources of HCC metastasis.[97] Y. Z. *et al.* found that CTCs and AFP were independent risk factors affecting HCC recurrence in patients undergoing percutaneous RFA. In addition, they proposed a scoring system according to CTCs and other factors to predict the prognosis of these patients.[98] The absence of CTC clusters was found to be an independent predictor of poor response to transcatheter arterial chemoembolization (TACE).[99] X. Zhao *et al.* collected portal vein blood samples of 104 HCC patients and found that the number of preoperative CTCs was higher in patients with postoperative metastasis than in those without metastasis.[100] Lina Zhao *et al.* used Ki67, a proliferation index of malignant tumors, as a biomarker for activity of HCC CTCs. They concluded that Ki67-positive CTCs were better predictors of HCC recurrence than the CTC number.[101] Liver transplantation is another curative approach for HCC that removes both the tumor and its associated microenvironment. Circulating tumor cells (CTCs) are important for detecting HCC relapse after liver transplantation. Hwang *et al.* sorted CTCs from HCC patients undergoing living

donor liver transplantation (LT) into EPCAM(+), CD90(+), and EpCAM+/CD90+ CTCs, and found that the detection of EpCAM+ CTCs or EpCAM+/CD90+ CTCs before surgery and on the first day after surgery was significantly associated with HCC recurrence after LT.[102]

Epithelial tumor cells undergo progressive loss of adhesive properties through EpCAM downregulation, while acquiring mesenchymal features that potentiate cellular motility and invasiveness. Qi *et al.* classified CTCs into three groups as epithelial, mesenchymal, and hybrids and found that the group with CTCs ≥16 and M-CTCs percentage ≥2% before surgery held a significantly higher risk of early recurrence, multi-intrahepatic recurrence and lung metastasis. Moreover, they observed a postoperative increase of CTCs 1 to 2 months before detection of recurrent or metastatic lesions.[96] Y. Lei *et al.* enriched CTCs by the CanPatrol CTC enrichment technique and found that laparoscopic liver resection augmented the quantity of CTCs. They speculated that the possible cause was the intra-abdominal pressure needed for laparoscopic liver resection that promotes entry of CTCs into the bloodstream. This result indicated a potential drawback of laparoscopic liver resection in facilitating the release of CTCs.[103] As a liquid biopsy, CTCs seem to be more sensitive and precise for recurrence screening than other common methods like ultrasound, CT or enhanced CT.

3.2.2. Therapeutic options. Compared to traditional observations of tumor characteristics by biopsies from resections or punctures, CTCs sampled at multiple time points are more powerful indicators for treatment decisions and response evaluation. Decreasing CTC counts correlate with longer overall survival (OS) and recurrence-free survival. On the other hand, a constant/increased number of CTCs after liver resection or ablation suggests rapid tumor progression and poor prognosis.[104–106] In a study of 105 early-stage HCC patients who underwent R0 resection, 76.5% patients exhibited a significant decrease in CTC numbers one month after surgery. However, patients exhibiting increased postoperative CTC counts showed decreased OS and shorter recurrence free survival.[107]

Since CTCs derived from peripheral blood are amenable to repeated sampling, oncologists can perform real-time evaluation for tumor progress and therapy response. CTCs are vital for guiding treatment in biopsy-ineligible patients with unresectable disease receiving palliative therapy.[108] In a single-center retrospective clinical study, a randomized trial was used to clarify CTCs' role in reflecting the effect of TACE on HCC patients. They proposed that CTCs could be used as a measure of TACE in tumor progress monitoring. They also found that preoperative TACE reduced early recurrence and long-term prognosis in CTC-positive patients.[109] Another retrospective study based on 162 HCC patients who underwent RFA concluded that CTC-positive (>2/3.2 mL) is an independent risk factor for tumor recurrence after RFA for 3 cm or less HCC.[98]

As many cancer patients may develop resistance and/or progressive disease, biomarker-directed therapy and predictive testing of drug responses are key to effective treatment options for an individual patient.[110,111] Xie *et al.* isolated CTCs from 31 patients with advanced HCC who were treated with either cytotoxic chemotherapy or sorafenib. They found that CTCs from the former were more likely to develop resistance than those from the latter. This information could help predict treatment response.[111] On the other hand, Zhang *et al.* cultured CTCs from HCC patients in 3D and tested sorafenib and oxaliplatin sensitivity *via* spheroid formation assay. Results showed that CTCs cultured with either sorafenib or oxaliplatin formed fewer spheroids than the control group.[112] This information is valuable for guiding drug options in the hope of improving therapeutic outcomes.

During the latent phase of malignant tumors and the process of hematogenous metastasis, circulating tumor cells (CTCs)—as intact, viable cells—detach from the solid tumor, enter the bloodstream, and subsequently settle in a new metastatic microenvironment. There, they serve as a bridge connecting the primary tumor to the metastatic site. Detection of CTCs provides opportunities for early diagnosis and prognosis and a real-time approach for progress monitoring and treatment response evaluation. However, the mechanism of how CTCs survive in circulation and finally establish distant lesions is still poorly understood. More studies on CTCs, on both their genetic and phenotypic characteristics, are needed and may lead to development of postoperative individualized therapy.

4. Conclusion and future perspectives

HCC CTC detection offers significant advantages over conventional biopsy in terms of low cost, minimal invasion, high precision, and patient compliance. Most importantly, as a real-time monitoring tool, it provides critical guidance for precise treatment strategies. Therefore, CTC detection serves as a valuable tool for screening high-risk populations, conducting postoperative follow-up, and monitoring recurrence through periodic testing. CTCs have broad application prospects in HCC clinical practice, such as tumor screening in patients with cirrhosis and monitoring for relapse after liver resection or transplantation. Given its minimal invasiveness, CTC-based liquid biopsy holds the potential to eventually replace conventional puncture biopsies, which carry a risk of tumor seeding, in the diagnosis and management of HCC in the near future.[72].

However, significant challenges remain due to the heterogeneity of tumor cells and the diversity of treatment regimens. Further exploration is needed to improve the identification and detection of HCC CTCs and to advance their clinical application as biomarkers. Future developments in this field may focus on the following directions:

(1) Enhancing detection technologies: to overcome the limitation of low EpCAM expression in HCC cells, signal amplification strategies, such as the utilization of gold nanoparticles, could be employed to augment recognition efficiency. Furthermore, apheresis technology, commonly used in hematological diseases, shows promise for future application in HCC CTC isolation, potentially increasing both capture efficiency and the detection positivity rate by processing larger blood volumes.

(2) Advancing functional analysis platforms: microfluidics provides a powerful platform for rare cell capture and analysis. Future studies could integrate CTC capture with microfluidic-based 3D culturing and organoid generation. This integration would facilitate drug sensitivity testing and enable a more profound analysis of the interplay between tumor cells and their microenvironment, which is crucial for addressing the challenge of drug resistance, a major contributor to the high mortality rate of HCC.

(3) Expanding clinical utility as biomarkers: while the significance of CTCs in early diagnosis and prognosis is established, their utility in guiding systemic therapy requires further clinical validation. Given that systemic therapies often exhibit limited efficacy against intrahepatic tumors, the presence of CTCs may indicate potential extrahepatic metastases, suggesting that such patients might be more suitable for systemic treatments. Notably, in liver transplantation, current selection criteria (*e.g.*, Milan and UCSF criteria) rely on radiographic metrics but fall short in accurately reflecting metastatic potential. Therefore, leveraging advances in CTC detection, future research should focus on developing CTC-based criteria for selecting liver transplant candidates, providing a more objective and scientific framework for prognostic evaluation.

(4) Integration with complementary liquid biopsy biomarkers: circulating tumor DNA (ctDNA), cell-free RNA (cfRNA), and extracellular vesicles (EVs) serve as liquid biopsy biomarkers complementary to CTCs.[113–115] The integration of these multi-analyte biomarkers is poised to significantly enhance the overall landscape of HCC diagnosis, monitoring, and therapeutic management.

Altogether, serving as latent seeds in circulation, HCC CTCs hold huge potential, not only in clinical practice but also in biological research aimed at revealing the mechanisms of tumor relapse and metastasis. The future of CTC applications lies in technological refinement, functional analysis, and their integrated use with other biomarkers to achieve truly personalized medicine for HCC patients.

Author contributions

Longtao Liu, Lingling Qu and Xia Wu conceived the review scope, conducted literature analysis, drafted the manuscript, and critically revised its scientific content. Zhihao Wang, Shiyan He, Zhenyu Liu and Tong Zhang offered the source and software. Corresponding authors Jing Lin, Jie Wang and Shouye Zhao supervised the project, secured funding, and finalized the manuscript. All authors reviewed and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

There is no data associated with this perspectives paper.

Acknowledgements

This work was supported by the Guiding Project of Fujian Provincial Department of Science and Technology (2023D034), the Natural Science Foundation of Xiamen (3502Z202373106), and the Scientific Research Foundation of State Key Laboratory of Vaccines for Infectious Diseases, Xiang An Biomedicine Laboratory (2024XAKJ0102013).

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