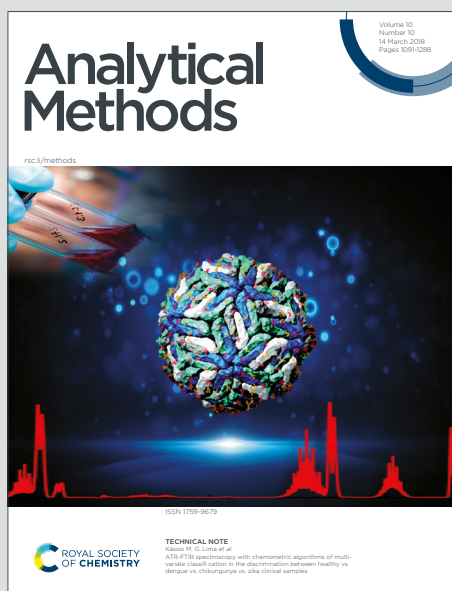


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Detection and Indications of Circulating Tumor Cells in Hepatocellular Carcinoma

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Competing Interests

The authors declare that they have no competing interests.



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Abstract

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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 recurrence within one year, and they account for most mortalities. It is urgent to develop powerful tools for HCC early diagnosis and real-time monitoring. Currently, detection of circulating tumor cells (CTCs) shows great potential in HCC early 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 CTCs detection and application, with a particular focus on clinical implications in HCC.

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 death¹. The high mortality rate is caused by recurrence and metastasis². The bleak prognosis of HCC is primary 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 significant correlation with the prognosis of HCC, and 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 metastasis³. 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 metastasis⁴. As a typical liquid form of biopsy, monitoring the molecular alterations of CTCs holds great

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promise for precise prognosis and personalized treatment decisions for HCC⁵. Studies have shown that survivin-positive CTCs are significantly associated with the TNM tumor stage, BCLC stage, and degree of differentiation⁶. 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 CTCs isolation^{7, 8} were introduced into CTCs 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 cells¹¹. Another challenge is the heterogeneity of tumor cells¹², 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 hampers 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.

2. CTCs 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 assays¹³ 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 Cellsearch¹⁶. 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 platforms²⁰.

Multiple antigens are employed to improve sensitivity 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 al²². (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. Cellcollector inserts a needle coated with anti-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% .

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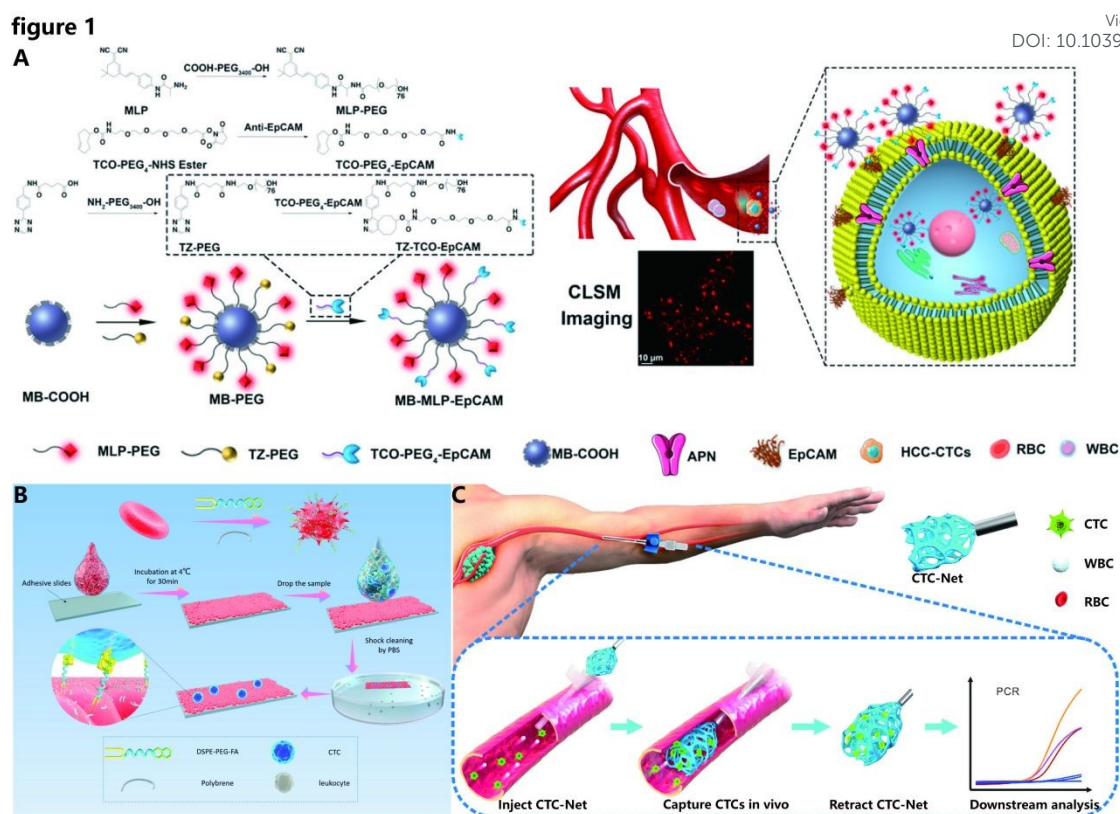


Figure 1. Mechanisms of novel functionalized materials for CTCs capture. (A) Synthetic route and construction of the MB-MLP-EpCAM probe for targeted HCC-CTCs detection. (B) Biomimetic capture using an RBC monolayer: DSPE-PEG-FA-modified RBCs target tumor cells, with subsequent polybrene treatment enhancing 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 CTCs capture.

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 purity²⁸. 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 the 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). 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 resistance³¹. 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 purity³². Furthermore, the increased fluid pressure inside the filters can damage captured cells³³.

2.3. Micro devices for CTC isolation and analysis

Microfluidic technology involves systems with micrometer-scale flow paths and tiny vessels in which chemical and biological experiments can be conducted precisely and efficiently. These systems have been widely used for CTC detection in the past decade (Fig. 2C,D)^{34–37}.

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figure 2

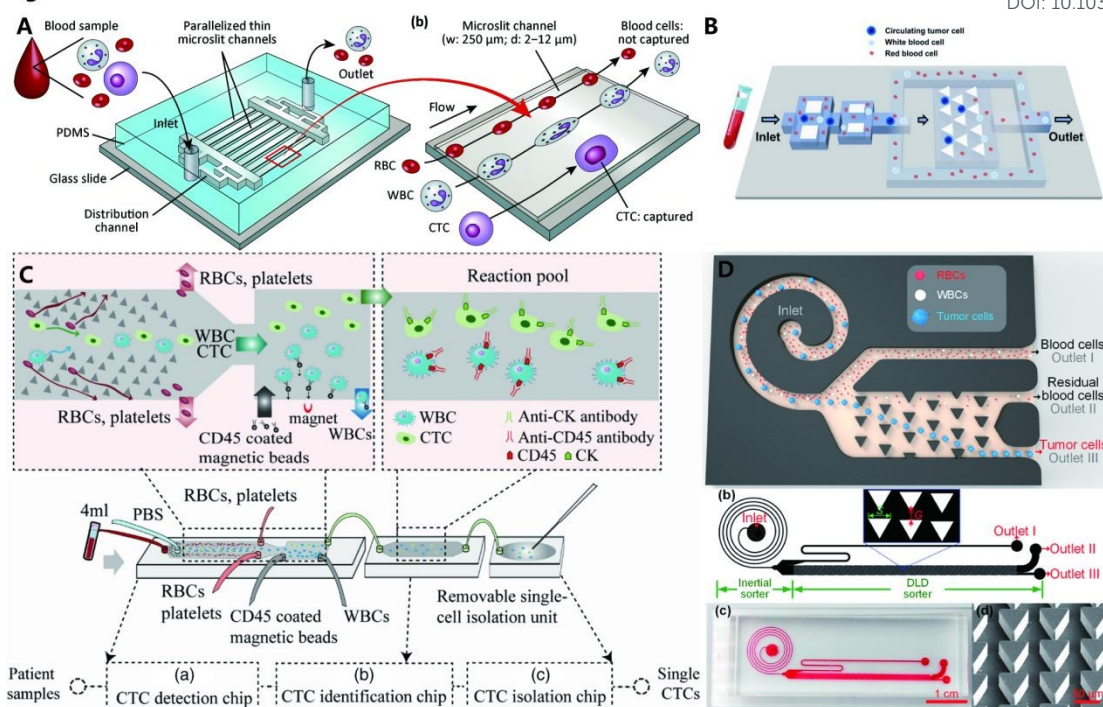


Figure 2. Schematics of microfluidic strategies for circulating tumor cell (CTC) isolation. (A) Affinity-based capture: CTCs are selectively bound to antibodies in a functionalized channel while blood cells flow through. (B) Size-based filtration: CTCs are physically retained by a microfilter, allowing 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 CTCs sorting, shown with a device photograph and SEM image of the pillars.

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 contact⁴⁰⁻⁴². Similar devices that increase the contact area by microstructure include fish herringbone structures⁴³ and nano-ciliated structures,⁴⁴ which allow CTCs 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 MnO₂/TiO₂/FTO substrates also exhibited good performance for CTCs 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 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 D-Chip, based on the weak deformability of CTCs. The key design of D-Chip includes slanted weirs with a weir gap of 7μm, 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 channel⁵³. 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 minutes) by hydrodynamic forces present in curvilinear microchannels to allow size-based isolation of viable CTCs⁵⁴. Miao Sun et al. incorporated elastic materials in microfluidic chips to focus cells into traps according to hydrodynamic trapping⁵⁵. 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 preprocessing by methods such as leukocytes depletion and RBC lysis⁵⁶.

When blood flows through the vortices-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 main stream⁵⁷. Amir et al. found that increasing the height of the reservoir provides more space for the particles’ orbits and reduces particle-particle collisions, thereby

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increasing the separation efficiency⁵⁸. Furthermore, Camille Raillon and colleagues integrated vortex device and an impedance chip into a single detection system, enabling label-free isolation and efficient subsequent analysis of CTCs⁵⁹.

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 clusters⁶². However, further validation is needed to evaluate the 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 analysis⁶⁵. 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 expansion⁶⁶. 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 efficiency⁶⁷.

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 technique⁶⁸. 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



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contamination. And last, the high portability and low equipment requirements make it easy to apply. 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 world¹. 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 advanced stage and suffer from poor outcomes⁷¹. Recent studies showed that CTCs hold huge potential for revealing the mechanism of tumor metastasis, monitoring recurrence and guiding individualized treatment for HCC⁷².

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 year⁷⁵.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 try 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 HCC⁷⁸.

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 Can-patrol technique⁷⁶. 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.

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Epithelial-Mesenchymal Transition (EMT) has become prominently implicated as a means by which transformed epithelial cells acquire the abilities to invade, resist apoptosis, and disseminate⁸¹. 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, as well as migration, invasion and promotion of apoptosis by inhibiting EMT⁸².

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 therapies⁸³⁻⁸⁶. The CSCs are always identified as being CD44+/CD24-, CD133+, or ALDH1+⁸⁷. Through a qRT-PCR CTCs detection platform, Wei Guo et al. screened the expression patterns of nine putative CSC biomarkers systematically and constructed a HCC CTCs detection panel, including EpCAM, CD90, CD133, 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 group⁸⁸.

In addition to statically analyzing CTCs molecular characteristics, researchers can dynamically observe pathophysiological changes at the cellular level by in vitro culturing, as well as at the systemic level by xenograft models⁸⁹⁻⁹¹. 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 patients⁹⁰. Another team created a device that efficiently captures CTCs while inducing in-situ chemotherapy⁹². 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 the GV-Lipo/SF/DT increased CTC elimination efficiency in vivo, thus effectively preventing metastasis in orthotopic HCC models⁹³.

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 system⁹⁴. 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 protection⁹⁵. 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 for 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 metastasis⁹⁷. YZ 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 patients⁹⁸. The absence of CTCs 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 those without metastasis¹⁰⁰. 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 CTC number¹⁰¹. After liver transplantation, another cure for HCC in which both the tumor and tumor associated environment are removed, CTCs are the important for detection of HCC relapse. Hwang et al. sorted CTCs from HCC patients undergoing living donor liver transplantation(LT) as EPCAM(+), CD90(+) and EpCAM+/CD90+ CTCs, and they found that the detection of EpCAM+ CTCs or EpCAM+/CD90+ CTCs before and on

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the first day after surgery was significantly associated with HCC recurrence after LT¹⁰².
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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 the group with CTCs ≥ 16 and M-CTCs percentage $\geq 2\%$ before surgery held a significantly higher risk of early recurrence, multi-intrahepatic recurrence and lung metastasis. Moreover, they observed postoperative increase of CTCs 1 to 2 months before detection of recurrent or metastatic lesions⁹⁶. Y. Lei et al. enriched CTCs by the Can-Patrol CTCs 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 promotes entry of CTCs into the bloodstream. This result indicated a potential drawback of laparoscopic liver resection in facilitating the release of CTCs¹⁰³. 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 on 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, constant/increased number of CTCs after liver resection or ablation suggest rapid tumor progression and poor prognosis¹⁰⁴⁻¹⁰⁶. 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 CTCs count showed decreased OS and shorter recurrence free survival¹⁰⁷.

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, used a randomized trial to clarify CTCs' role in reflecting the effect of TACE upon

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 patients¹⁰⁹. 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 3cm or less HCC⁹⁸.

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 response¹¹¹. 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 group¹¹². 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, as well as 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, both their genetic and phenotypic characteristics, are needed, and may lead to development of postoperative individualized therapy.

4. Conclusion and Future Perspectives

HCC CTCs detection offers significant advantages over conventional biopsy in terms of low cost, minimal invasion, high precision, and patient compliance. Most

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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 future⁷².

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, 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.

1. Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F., Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* **2021**, 71 (3), 209-249.
2. Siegel, R. L.; Miller, K. D.; Fuchs, H. E.; Jemal, A., Cancer statistics, 2022. *CA Cancer J Clin* **2022**, 72 (1), 7-33.
3. Deng, Z.; Wu, S.; Wang, Y.; Shi, D., Circulating tumor cell isolation for cancer diagnosis and prognosis. *EBioMedicine* **2022**, 83, 104237.
4. Ring, A.; Nguyen-Sträuli, B. D.; Wicki, A.; Aceto, N., Biology, vulnerabilities and clinical applications of circulating tumour cells. *Nat Rev Cancer* **2023**, 23 (2), 95-111.
5. Salehi, M.; Lavasani, Z. M.; Keshavarz Alikhani, H.; Shokouhian, B.; Hassan, M.; Najimi, M.; Vosough, M., Circulating Tumor Cells as a Promising Tool for Early Detection of Hepatocellular Carcinoma. *Cells* **2023**, 12 (18).
6. Yu, J.; Wang, Z.; Zhang, H.; Wang, Y.; Li, D. Q., Survivin-positive circulating tumor cells as a marker for metastasis of hepatocellular carcinoma. *World J Gastroenterol* **2021**, 27 (43), 7546-7562.
7. Wu, S.; Gu, L.; Qin, J.; Zhang, L.; Sun, F.; Liu, Z.; Wang, Y.; Shi, D., Rapid Label-Free Isolation of Circulating Tumor Cells from Patients' Peripheral Blood Using Electrically Charged Fe(3)O(4) Nanoparticles. *ACS Appl Mater Interfaces* **2020**, 12 (4), 4193-4203.
8. Chen, J.; Li, D.; Zhou, C.; Zhu, Y.; Lin, C.; Guo, L.; Le, W.; Gu, Z.; Chen, B., Principle Superiority and Clinical Extensibility of 2D and 3D Charged Nanoprobe Detection Platform Based on Electrophysiological Characteristics of Circulating Tumor Cells. *Cells* **2023**, 12 (2).
9. Markou, A.; Tzanikou, E.; Lianidou, E., The potential of liquid biopsy in the management of cancer patients. *Semin Cancer Biol* **2022**, 84, 69-79.

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Analytical Methods Accepted Manuscript

10. Nikanjam, M.; Kato, S.; Kurzrock, R., Liquid biopsy: current technology and clinical applications. *Hematol Oncol* **2022**, *15* (1), 131. View Article Online
DOI: 10.1039/D5AY01320H
11. Lim, S. B.; Di Lee, W.; Vasudevan, J.; Lim, W. T.; Lim, C. T., Liquid biopsy: one cell at a time. *NPJ Precis Oncol* **2019**, *3*, 23.
12. Zhang, Q.; Lou, Y.; Yang, J.; Wang, J.; Feng, J.; Zhao, Y.; Wang, L.; Huang, X.; Fu, Q.; Ye, M.; Zhang, X.; Chen, Y.; Ma, C.; Ge, H.; Wang, J.; Wu, J.; Wei, T.; Chen, Q.; Wu, J.; Yu, C.; Xiao, Y.; Feng, X.; Guo, G.; Liang, T.; Bai, X., Integrated multiomic analysis reveals comprehensive tumour heterogeneity and novel immunophenotypic classification in hepatocellular carcinomas. *Gut* **2019**, *68* (11), 2019-2031.
13. Chu, Q.; Mu, W.; Lan, C.; Liu, Y.; Gao, T.; Guan, L.; Fang, Y.; Zhang, Z.; Liu, Y.; Liu, Y.; Zhang, N., High-Specific Isolation and Instant Observation of Circulating Tumour Cell from HCC Patients via Glypican-3 Immunomagnetic Fluorescent Nanodevice. *Int J Nanomedicine* **2021**, *16*, 4161-4173.
14. Zhao, K.; Zhao, P.; Dong, J.; Wei, Y.; Chen, B.; Wang, Y.; Pan, X.; Wang, J., Implementation of an Integrated Dielectrophoretic and Magnetophoretic Microfluidic Chip for CTC Isolation. *Biosensors (Basel)* **2022**, *12* (9).
15. Geng, W.; Liu, Y.; Yu, N.; Qiao, X.; Ji, M.; Niu, Y.; Niu, L.; Fu, W.; Zhang, H.; Bi, K.; Chou, X., An ultra-compact acoustofluidic device based on the narrow-path travelling surface acoustic wave (np-TSAW) for label-free isolation of living circulating tumor cells. *Anal Chim Acta* **2023**, *1255*, 341138.
16. Sun, Y. F.; Guo, W.; Xu, Y.; Shi, Y. H.; Gong, Z. J.; Ji, Y.; Du, M.; Zhang, X.; Hu, B.; Huang, A.; Chen, G. G.; Lai, P. B. S.; Cao, Y.; Qiu, S. J.; Zhou, J.; Yang, X. R.; Fan, J., Circulating Tumor Cells from Different Vascular Sites Exhibit Spatial Heterogeneity in Epithelial and Mesenchymal Composition and Distinct Clinical Significance in Hepatocellular Carcinoma. *Clin Cancer Res* **2018**, *24* (3), 547-559.
17. Gruijs, M.; Zeelen, C.; Hellingman, T.; Smit, J.; Borm, F. J.; Kazemier, G.; Dickhoff, C.; Bahce, I.; de Langen, J.; Smit, E. F.; Hartemink, K. J.; van Egmond, M., Detection of Circulating Tumor Cells Using the Attune NxT. *Int J Mol Sci* **2022**, *24* (1).
18. Wan, S.; Kim, T. H.; Smith, K. J.; Delaney, R.; Park, G. S.; Guo, H.; Lin, E.; Plegue, T.; Kuo, N.; Steffes, J.; Leu, C.; Simeone, D. M.; Razimulava, N.; Parikh, N. D.; Nagrath, S.; Welling, T. H., New Labyrinth Microfluidic Device Detects Circulating Tumor Cells Expressing Cancer Stem Cell Marker and Circulating Tumor Microemboli in Hepatocellular Carcinoma. *Sci Rep* **2019**, *9* (1), 18575.
19. Wang, X.; Sun, L.; Zhang, H.; Wei, L.; Qu, W.; Zeng, Z.; Liu, Y.; Zhu, Z., Microfluidic chip combined with magnetic-activated cell sorting technology for tumor antigen-independent sorting of circulating hepatocellular carcinoma cells. *PeerJ* **2019**, *7*, e6681.
20. Pang, Y.; Wang, C.; Xiao, R.; Sun, Z., Dual-Selective and Dual-Enhanced SERS Nanoprobes Strategy for Circulating Hepatocellular Carcinoma Cells Detection. *Chemistry* **2018**, *24* (27), 7060-7067.
21. Xia, W.; Li, H.; Li, Y.; Li, M.; Fan, J.; Sun, W.; Li, N.; Li, R.; Shao, K.; Peng, X., In Vivo Coinstantaneous Identification of Hepatocellular Carcinoma Circulating Tumor Cells by Dual-Targeting Magnetic-Fluorescent Nanobeads. *Nano Lett* **2021**, *21* (1), 634-641.
22. Zhang, T.; Peng, W.; Jiang, W.; Gao, K.; Liu, W., Ultradense Erythrocyte Bionic Layer Used to Capture Circulating Tumor Cells and Plasma-Assisted High-Purity Release. *ACS Appl Mater Interfaces* **2021**, *13* (21), 24543-24552.
23. Cheng, S. B.; Wang, M.; Zhang, C.; Chen, M. M.; Wang, Y. K.; Tian, S.; Zhan, N.; Dong, W. G.; Xie, M.; Huang, W. H., Flexible Three-Dimensional Net for Intravascular Fishing of Circulating Tumor Cells. *Anal Chem* **2020**, *92* (7), 5447-5455.
24. Hong, B.; Zu, Y., Detecting circulating tumor cells: current challenges and new trends. *Theranostics*

2013, 3 (6), 377-94.

25. Zhang, Z.; Ramnath, N.; Nagrath, S., Current Status of CTCs as Liquid Biopsy in Lung Cancer and Future Directions. *Front Oncol* **2015**, 5, 209.

26. Cheng, Y.; Luo, L.; Zhang, J.; Zhou, M.; Tang, Y.; He, G.; Lu, Y.; Wang, Z.; Pan, M., Diagnostic Value of Different Phenotype Circulating Tumor Cells in Hepatocellular Carcinoma. *J Gastrointest Surg* **2019**, 23 (12), 2354-2361.

27. Chen, M.; Xu, R.; Wu, L.; Chen, X., Relationship between circulating tumor cells undergoing EMT and short-term efficacy following interventional treatment in patients with hepatocellular carcinoma. *J Interv Med* **2020**, 3 (3), 146-150.

28. Low, W. S.; Wan Abas, W. A., Benchtop technologies for circulating tumor cells separation based on biophysical properties. *Biomed Res Int* **2015**, 2015, 239362.

29. Shimmyo, N.; Furuhashi, M.; Yamada, M.; Utoh, R.; Seki, M., Process simplification and structure design of parallelized microslit isolator for physical property-based capture of tumor cells. *Analyst* **2022**, 147 (8), 1622-1630.

30. Lu, C.; Xu, J.; Han, J.; Li, X.; Xue, N.; Li, J.; Wu, W.; Sun, X.; Wang, Y.; Ouyang, Q.; Yang, G.; Luo, C., A novel microfluidic device integrating focus-separation speed reduction design and trap arrays for high-throughput capture of circulating tumor cells. *Lab Chip* **2020**, 20 (22), 4094-4105.

31. Hao, S. J.; Wan, Y.; Xia, Y. Q.; Zou, X.; Zheng, S. Y., Size-based separation methods of circulating tumor cells. *Adv Drug Deliv Rev* **2018**, 125, 3-20.

32. Guglielmi, R.; Lai, Z.; Raba, K.; van Dalum, G.; Wu, J.; Behrens, B.; Bhagat, A. A. S.; Knoefel, W. T.; Neves, R. P. L.; Stoecklein, N. H., Technical validation of a new microfluidic device for enrichment of CTCs from large volumes of blood by using buffy coats to mimic diagnostic leukapheresis products. *Sci Rep* **2020**, 10 (1), 20312.

33. Hvieh, G. E.; Parveen, Z.; Wagner, C.; Janning, M.; Quidde, J.; Stein, A.; Müller, V.; Loges, S.; Neves, R. P.; Stoecklein, N. H.; Wikman, H.; Riethdorf, S.; Pantel, K.; Gorges, T. M., A novel microfluidic platform for size and deformability based separation and the subsequent molecular characterization of viable circulating tumor cells. *Int J Cancer* **2016**, 138 (12), 2894-904.

34. Xu, M.; Zhao, H.; Chen, J.; Liu, W.; Li, E.; Wang, Q.; Zhang, L., An Integrated Microfluidic Chip and Its Clinical Application for Circulating Tumor Cell Isolation and Single-Cell Analysis. *Cytometry A* **2020**, 97 (1), 46-53.

35. Xiang, N.; Wang, J.; Li, Q.; Han, Y.; Huang, D.; Ni, Z., Precise Size-Based Cell Separation via the Coupling of Inertial Microfluidics and Deterministic Lateral Displacement. *Anal Chem* **2019**, 91 (15), 10328-10334.

36. Wang, K.; Zhou, L.; Zhao, S.; Cheng, Z.; Qiu, S.; Lu, Y.; Wu, Z.; Abdel Wahab, A. H. A.; Mao, H.; Zhao, J., A microfluidic platform for high-purity separating circulating tumor cells at the single-cell level. *Talanta* **2019**, 200, 169-176.

37. Wang, J.; Li, Y.; Wang, R.; Han, C.; Xu, S.; You, T.; Li, Y.; Xia, J.; Xu, X.; Wang, D.; Tang, H.; Yang, C.; Chen, X.; Peng, Z., A Fully Automated and Integrated Microfluidic System for Efficient CTC Detection and Its Application in Hepatocellular Carcinoma Screening and Prognosis. *ACS Appl Mater Interfaces* **2021**, 13 (25), 30174-30186.

38. Yoon, H. J.; Shanker, A.; Wang, Y.; Kozminsky, M.; Jin, Q.; Palanisamy, N.; Burness, M. L.; Azizi, E.; Simeone, D. M.; Wicha, M. S.; Kim, J.; Nagrath, S., Tunable Thermal-Sensitive Polymer-Graphene Oxide Composite for Efficient Capture and Release of Viable Circulating Tumor Cells. *Adv Mater* **2016**, 28 (24), 4891-7.

39. Yan, S.; Chen, P.; Zeng, X.; Zhang, X.; Li, Y.; Xia, Y.; Wang, J.; Dai, X.; Feng, X.; Du, W.; Liu, B. F.; Liu, B. F., Integrated Multifunctional Electrochemistry Microchip for Highly Efficient Capture, Release, Lysis, and Analysis of Circulating Tumor Cells. *Anal Chem* **2017**, *89* (22), 12039-12044. DOI: 10.1039/D5AY01320H
40. Stott, S. L.; Hsu, C. H.; Tsukrov, D. I.; Yu, M.; Miyamoto, D. T.; Waltman, B. A.; Rothenberg, S. M.; Shah, A. M.; Smas, M. E.; Korir, G. K.; Floyd, F. P., Jr.; Gilman, A. J.; Lord, J. B.; Winokur, D.; Springer, S.; Irimia, D.; Nagrath, S.; Sequist, L. V.; Lee, R. J.; Isselbacher, K. J.; Maheswaran, S.; Haber, D. A.; Toner, M., Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. *Proc Natl Acad Sci U S A* **2010**, *107* (43), 18392-7.
41. Chen, J.; Liu, C. Y.; Wang, X.; Sweet, E.; Liu, N.; Gong, X.; Lin, L., 3D printed microfluidic devices for circulating tumor cells (CTCs) isolation. *Biosens Bioelectron* **2020**, *150*, 111900.
42. Chu, C. H.; Liu, R.; Ozkaya-Ahmadov, T.; Swain, B. E.; Boya, M.; El-Rayes, B.; Akce, M.; Bilen, M. A.; Kucuk, O.; Sarioglu, A. F., Negative enrichment of circulating tumor cells from unmanipulated whole blood with a 3D printed device. *Sci Rep* **2021**, *11* (1), 20583.
43. Huang, X.; Tang, J.; Hu, L.; Bian, R.; Liu, M.; Cao, W.; Zhang, H., Arrayed microfluidic chip for detection of circulating tumor cells and evaluation of drug potency. *Anal Biochem* **2019**, *564-565*, 64-71.
44. Jan, Y. J.; Chen, J. F.; Zhu, Y.; Lu, Y. T.; Chen, S. H.; Chung, H.; Smalley, M.; Huang, Y. W.; Dong, J.; Chen, L. C.; Yu, H. H.; Tomlinson, J. S.; Hou, S.; Agopian, V. G.; Posadas, E. M.; Tseng, H. R., NanoVelcro rare-cell assays for detection and characterization of circulating tumor cells. *Adv Drug Deliv Rev* **2018**, *125*, 78-93.
45. Cui, H.; Liu, Q.; Li, R.; Wei, X.; Sun, Y.; Wang, Z.; Zhang, L.; Zhao, X. Z.; Hua, B.; Guo, S. S., ZnO nanowire-integrated bio-microchips for specific capture and non-destructive release of circulating tumor cells. *Nanoscale* **2020**, *12* (3), 1455-1463.
46. Li, R.; Chen, F. F.; Liu, H. Q.; Wang, Z. X.; Zhang, Z. T.; Wang, Y.; Cui, H.; Liu, W.; Zhao, X. Z.; Sun, Z. J.; Guo, S. S., Efficient Capture and High Activity Release of Circulating Tumor Cells by Using TiO₂ Nanorod Arrays Coated with Soluble MnO₂ Nanoparticles. *ACS Appl Mater Interfaces* **2018**, *10* (19), 16327-16334.
47. Yi, B.; Wu, T.; Zhu, N.; Huang, Y.; Yang, X.; Yuan, L.; Wu, Y.; Liang, X.; Jiang, X., The clinical significance of CTC enrichment by GPC3-IML and its genetic analysis in hepatocellular carcinoma. *J Nanobiotechnology* **2021**, *19* (1), 74.
48. Wang, Y.; Li, J.; Pei, Z.; Pei, Y., A glutathione activatable bioprobe for detection of hepatocellular carcinoma cells in peripheral blood via carbohydrate-protein interaction. *Anal Chim Acta* **2022**, *1221*, 340106.
49. Qin, L.; Zhou, W.; Zhang, S.; Cheng, B.; Wang, S.; Li, S.; Yang, Y.; Wang, S.; Liu, K.; Zhang, N., Highly Efficient Isolation of Circulating Tumor Cells Using a Simple Wedge-Shaped Microfluidic Device. *IEEE Trans Biomed Eng* **2019**, *66* (6), 1536-1541.
50. Lee, Y.; Guan, G.; Bhagat, A. A., ClearCell® FX, a label-free microfluidics technology for enrichment of viable circulating tumor cells. *Cytometry A* **2018**, *93* (12), 1251-1254.
51. Rahmanian, M.; Sartipzadeh Hematabad, O.; Askari, E.; Shokati, F.; Bakhshi, A.; Moghadam, S.; Olfatbakhsh, A.; Al Sadat Hashemi, E.; Khorsand Ahmadi, M.; Morteza Naghib, S.; Sinha, N.; Tel, J.; Eslami Amirabadi, H.; den Toonder, J. M. J.; Majidzadeh, A. K., A micropillar array-based microfluidic chip for label-free separation of circulating tumor cells: The best micropillar geometry? *J Adv Res* **2023**, *47*, 105-121.
52. Hakim, M.; Khorasheh, F.; Alemzadeh, I.; Vossoughi, M., A new insight to deformability

correlation of circulating tumor cells with metastatic behavior by application of a new deformability based microfluidic chip. *Anal Chim Acta* **2021**, *1186*, 339115.

53. Shepherd, S. J.; Issadore, D.; Mitchell, M. J., Microfluidic formulation of nanoparticles for biomedical applications. *Biomaterials* **2021**, *274*, 120826.

54. Smith, K. J.; Jana, J. A.; Kaehr, A.; Purcell, E.; Opdycke, T.; Paoletti, C.; Cooling, L.; Thamm, D. H.; Hayes, D. F.; Nagrath, S., Inertial focusing of circulating tumor cells in whole blood at high flow rates using the microfluidic CTCKey™ device for CTC enrichment. *Lab Chip* **2021**, *21* (18), 3559-3572.

55. Sun, M.; Zhou, X.; Quan, Y.; Zhang, L.; Xie, Y., Highly flexible elastomer microfluidic chip for single cell manipulation. *Biomicrofluidics* **2022**, *16* (2), 024104.

56. Li, M.; Ge, C.; Yang, Y.; Gan, M.; Xu, Y.; Chen, L.; Li, S., Direct separation and enumeration of CTCs in viscous blood based on co-flow microchannel with tunable shear rate: a proof-of-principle study. *Anal Bioanal Chem* **2022**, *414* (26), 7683-7694.

57. Renier, C.; Pao, E.; Che, J.; Liu, H. E.; Lemaire, C. A.; Matsumoto, M.; Triboulet, M.; Srivas, S.; Jeffrey, S. S.; Rettig, M.; Kulkarni, R. P.; Di Carlo, D.; Sollier-Christen, E., Label-free isolation of prostate circulating tumor cells using Vortex microfluidic technology. *NPJ Precis Oncol* **2017**, *1* (1), 15.

58. Mohamadsharifi, A.; Hajghassem, H.; Kalantar, M.; Karimi, A.; Tabatabaei Asl, M.; Hosseini, S.; Badieirostami, M., High-Efficiency Inertial Separation of Microparticles Using Elevated Columned Reservoirs and Vortex Technique for Lab-on-a-Chip Applications. *ACS Omega* **2023**, *8* (31), 28628-28639.

59. Raillon, C.; Che, J.; Thill, S.; Duchamp, M.; Desbiolles, B. X. E.; Millet, A.; Sollier, E.; Renaud, P., Toward Microfluidic Label-Free Isolation and Enumeration of Circulating Tumor Cells from Blood Samples. *Cytometry A* **2019**, *95* (10), 1085-1095.

60. Dashzeveg, N. K.; Jia, Y.; Zhang, Y.; Gerratana, L.; Patel, P.; Shajahan, A.; Dandar, T.; Ramos, E. K.; Almubarak, H. F.; Adorno-Cruz, V.; Taftaf, R.; Schuster, E. J.; Scholten, D.; Sokolowski, M. T.; Reduzzi, C.; El-Shennawy, L.; Hoffmann, A. D.; Manai, M.; Zhang, Q.; D'Amico, P.; Azadi, P.; Colley, K. J.; Platani, L. C.; Shah, A. N.; Gradishar, W. J.; Cristofanilli, M.; Muller, W. A.; Cobb, B. A.; Liu, H., Dynamic Glycoprotein Hyposialylation Promotes Chemotherapy Evasion and Metastatic Seeding of Quiescent Circulating Tumor Cell Clusters in Breast Cancer. *Cancer Discov* **2023**, *13* (9), 2050-2071.

61. Taftaf, R.; Liu, X.; Singh, S.; Jia, Y.; Dashzeveg, N. K.; Hoffmann, A. D.; El-Shennawy, L.; Ramos, E. K.; Adorno-Cruz, V.; Schuster, E. J.; Scholten, D.; Patel, D.; Zhang, Y.; Davis, A. A.; Reduzzi, C.; Cao, Y.; D'Amico, P.; Shen, Y.; Cristofanilli, M.; Muller, W. A.; Varadan, V.; Liu, H., ICAM1 initiates CTC cluster formation and trans-endothelial migration in lung metastasis of breast cancer. *Nat Commun* **2021**, *12* (1), 4867.

62. Boya, M.; Ozkaya-Ahmadov, T.; Swain, B. E.; Chu, C. H.; Asmare, N.; Civelekoglu, O.; Liu, R.; Lee, D.; Tobia, S.; Biliya, S.; McDonald, L. D.; Nazha, B.; Kucuk, O.; Sanda, M. G.; Benigno, B. B.; Moreno, C. S.; Bilen, M. A.; McDonald, J. F.; Sarioglu, A. F., High throughput, label-free isolation of circulating tumor cell clusters in meshed microwells. *Nat Commun* **2022**, *13* (1), 3385.

63. Agashe, R.; Kurzrock, R., Circulating Tumor Cells: From the Laboratory to the Cancer Clinic. *Cancers (Basel)* **2020**, *12* (9).

64. Castro-Giner, F.; Aceto, N., Tracking cancer progression: from circulating tumor cells to metastasis. *Genome Med* **2020**, *12* (1), 31.

65. Xu, J.; Liao, K.; Yang, X.; Wu, C.; Wu, W., Using single-cell sequencing technology to detect circulating tumor cells in solid tumors. *Mol Cancer* **2021**, *20* (1), 104.

66. Khojah, R.; Xiao, Z.; Panduranga, M. K.; Bogumil, M.; Wang, Y.; Goiriena-Goikoetxea, M.; Chopdekar, R. V.; Bokor, J.; Carman, G. P.; Candler, R. N.; Di Carlo, D., Single-Domain Multiferroic

Array-Addressable Terfenol-D (SMaRT) Micromagnets for Programmable Single-Cell Capture and Release. *Adv Mater* **2021**, 33 (20), e2006651.

67. Li, R.; Gong, Z.; Yi, K.; Li, W.; Liu, Y.; Wang, F.; Guo, S. S., Efficient Detection and Single-Cell Extraction of Circulating Tumor Cells in Peripheral Blood. *ACS Appl Bio Mater* **2020**, 3 (9), 6521-6528.

68. Feng, C.; Mao, D.; Lu, C.; Zhang, Q.; Liu, X.; Wu, Q.; Gong, X.; Chen, G.; Zhu, X., Single-Cell Analysis of Highly Metastatic Circulating Tumor Cells by Combining a Self-Folding Induced Release Reaction with a Cell Capture Microchip. *Anal Chem* **2021**, 93 (2), 1110-1119.

69. Sun, D.; Ma, Y.; Wu, M.; Chen, Z.; Zhang, L.; Lu, J., Recent progress in aptamer-based microfluidics for the detection of circulating tumor cells and extracellular vesicles. *J Pharm Anal* **2023**, 13 (4), 340-354.

70. Cao, R.; Zhang, M.; Yu, H.; Qin, J., [Recent advances in isolation and detection of circulating tumor cells with a microfluidic system]. *Se Pu* **2022**, 40 (3), 213-223.

71. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* **2018**, 69 (1), 182-236.

72. Ahn, J. C.; Teng, P. C.; Chen, P. J.; Posadas, E.; Tseng, H. R.; Lu, S. C.; Yang, J. D., Detection of Circulating Tumor Cells and Their Implications as a Biomarker for Diagnosis, Prognostication, and Therapeutic Monitoring in Hepatocellular Carcinoma. *Hepatology* **2021**, 73 (1), 422-436.

73. Huang, Z. L.; Zhang, P. B.; Zhang, J. T.; Li, F.; Li, T. T.; Huang, X. Y., Comprehensive Genomic Profiling Identifies FAT1 as a Negative Regulator of EMT, CTCs, and Metastasis of Hepatocellular Carcinoma. *J Hepatocell Carcinoma* **2023**, 10, 369-382.

74. Sun, B.; Ji, W.; Liu, C.; Lin, X.; Chen, L.; Qian, H.; Su, C., miR-2392 functions as tumour suppressor and inhibits malignant progression of hepatocellular carcinoma via directly targeting JAG2. *Liver Int* **2022**, 42 (7), 1658-1673.

75. Nevola, R.; Ruocco, R.; Criscuolo, L.; Villani, A.; Alfano, M.; Beccia, D.; Imbriani, S.; Claar, E.; Cozzolino, D.; Sasso, F. C.; Marrone, A.; Adinolfi, L. E.; Rinaldi, L., Predictors of early and late hepatocellular carcinoma recurrence. *World J Gastroenterol* **2023**, 29 (8), 1243-1260.

76. Yan, Q.; Lin, H. M.; Zhu, K.; Cao, Y.; Xu, X. L.; Zhou, Z. Y.; Xu, L. B.; Liu, C.; Zhang, R., Immune Checkpoint FGL1 Expression of Circulating Tumor Cells Is Associated With Poor Survival in Curatively Resected Hepatocellular Carcinoma. *Front Oncol* **2022**, 12, 810269.

77. Lei, Y.; Wang, X.; Sun, H.; Fu, Y.; Tian, Y.; Yang, L.; Wang, J.; Xia, F., Association of Preoperative NANOG-Positive Circulating Tumor Cell Levels With Recurrence of Hepatocellular Carcinoma. *Front Oncol* **2021**, 11, 601668.

78. Ogle, L. F.; Orr, J. G.; Willoughby, C. E.; Hutton, C.; McPherson, S.; Plummer, R.; Boddy, A. V.; Curtin, N. J.; Jamieson, D.; Reeves, H. L., Imagestream detection and characterisation of circulating tumour cells - A liquid biopsy for hepatocellular carcinoma? *J Hepatol* **2016**, 65 (2), 305-13.

79. Cui, K.; Ou, Y.; Shen, Y.; Li, S.; Sun, Z., Clinical value of circulating tumor cells for the diagnosis and prognosis of hepatocellular carcinoma (HCC): A systematic review and meta-analysis. *Medicine (Baltimore)* **2020**, 99 (40), e22242.

80. Prasoppokakorn, T.; Buntho, A.; Ingrungruenglert, P.; Tiyyarattanachai, T.; Jaihan, T.; Kulkraisri, K.; Ariyaskul, D.; Phathong, C.; Israsena, N.; Rerknimitr, R.; Treeprasertsuk, S.; Chaiteerakij, R., Circulating tumor cells as a prognostic biomarker in patients with hepatocellular carcinoma. *Sci Rep* **2022**, 12 (1), 18686.

81. Lu, J.; Kornmann, M.; Traub, B., Role of Epithelial to Mesenchymal Transition in Colorectal Cancer. *Int J Mol Sci* **2023**, 24 (19).

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43
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45
46
47
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51
52
53
54
55
56
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60

82. Ding, Y.; Wang, X.; Lu, S.; Lai, A.; Xie, B.; He, X.; Liu, Q., BCAT1, as a prognostic factor for HCC, can promote the development of liver cancer through activation of the AKT signaling pathway and EMT. *J Mol Histol* **2023**, *54* (1), 25-39.

83. Mok, E. H. K.; Leung, C. O. N.; Zhou, L.; Lei, M. M. L.; Leung, H. W.; Tong, M.; Wong, T. L.; Lau, E. Y. T.; Ng, I. O. L.; Ding, J.; Yun, J. P.; Yu, J.; Zhu, H. L.; Lin, C. H.; Lindholm, D.; Leung, K. S.; Cybulski, J. D.; Baker, D. M.; Ma, S.; Lee, T. K. W., Caspase-3-Induced Activation of SREBP2 Drives Drug Resistance via Promotion of Cholesterol Biosynthesis in Hepatocellular Carcinoma. *Cancer Res* **2022**, *82* (17), 3102-3115.

84. Wang, J.; Yu, H.; Dong, W.; Zhang, C.; Hu, M.; Ma, W.; Jiang, X.; Li, H.; Yang, P.; Xiang, D., N6-Methyladenosine-Mediated Up-Regulation of FZD10 Regulates Liver Cancer Stem Cells' Properties and Lenvatinib Resistance Through WNT/ β -Catenin and Hippo Signaling Pathways. *Gastroenterology* **2023**, *164* (6), 990-1005.

85. Chiang, J.; Chen, P. C.; Pham, J.; Nguyen, C. Q.; Kaur, K.; Raman, S. S.; Jewett, A., Characterizing hepatocellular carcinoma stem markers and their corresponding susceptibility to NK-cell based immunotherapy. *Front Immunol* **2023**, *14*, 1284669.

86. Ren, Z.; Chen, Y.; Shi, L.; Shao, F.; Sun, Y.; Ge, J.; Zhang, J.; Zang, Y., Sox9/CXCL5 axis facilitates tumour cell growth and invasion in hepatocellular carcinoma. *Febs j* **2022**, *289* (12), 3535-3549.

87. Zeng, Z.; Fu, M.; Hu, Y.; Wei, Y.; Wei, X.; Luo, M., Regulation and signaling pathways in cancer stem cells: implications for targeted therapy for cancer. *Mol Cancer* **2023**, *22* (1), 172.

88. Guo, W.; Sun, Y. F.; Shen, M. N.; Ma, X. L.; Wu, J.; Zhang, C. Y.; Zhou, Y.; Xu, Y.; Hu, B.; Zhang, M.; Wang, G.; Chen, W. Q.; Guo, L.; Lu, R. Q.; Zhou, C. H.; Zhang, X.; Shi, Y. H.; Qiu, S. J.; Pan, B. S.; Cao, Y.; Zhou, J.; Yang, X. R.; Fan, J., Circulating Tumor Cells with Stem-Like Phenotypes for Diagnosis, Prognosis, and Therapeutic Response Evaluation in Hepatocellular Carcinoma. *Clin Cancer Res* **2018**, *24* (9), 2203-2213.

89. Zhuang, B.; Zhu, X.; Lin, J.; Zhang, F.; Qiao, B.; Kang, J.; Xie, X.; Wei, X.; Xie, X., Radiofrequency ablation induces tumor cell dissemination in a mouse model of hepatocellular carcinoma. *Eur Radiol Exp* **2023**, *7* (1), 74.

90. Hu, C. L.; Zhang, Y. J.; Zhang, X. F.; Fei, X.; Zhang, H.; Li, C. G.; Sun, B., 3D Culture of Circulating Tumor Cells for Evaluating Early Recurrence and Metastasis in Patients with Hepatocellular Carcinoma. *Onco Targets Ther* **2021**, *14*, 2673-2688.

91. Tang, Y.; Lu, Y.; Chen, Y.; Luo, L.; Cai, L.; Peng, B.; Huang, W.; Liao, H.; Zhao, L.; Pan, M., Pre-metastatic niche triggers SDF-1/CXCR4 axis and promotes organ colonisation by hepatocellular circulating tumour cells via downregulation of Prrx1. *J Exp Clin Cancer Res* **2019**, *38* (1), 473.

92. Liu, C.; Yang, B.; Chen, X.; Hu, Z.; Dai, Z.; Yang, D.; Zheng, X.; She, X.; Liu, Q., Capture and separation of circulating tumor cells using functionalized magnetic nanocomposites with simultaneous in situ chemotherapy. *Nanotechnology* **2019**, *30* (28), 285706.

93. Mu, W.; Chu, Q.; Yang, H.; Guan, L.; Fu, S.; Gao, T.; Sang, X.; Zhang, Z.; Liang, S.; Liu, Y.; Zhang, N., Multipoint Costriking Nanodevice Eliminates Primary Tumor Cells and Associated-Circulating Tumor Cells for Enhancing Metastasis Inhibition and Therapeutic Effect on HCC. *Adv Sci (Weinh)* **2022**, *9* (9), 2101472.

94. Li, Y.; Huang, N.; Wang, C.; Ma, H.; Zhou, M.; Lin, L.; Huang, Z.; Sun, L.; Shi, M.; Liao, W., Impact of liver tumor percutaneous radiofrequency ablation on circulating tumor cells. *Oncol Lett* **2018**, *16* (3), 2839-2850.

95. Chen, J.; Luo, Y.; Xi, X.; Li, H.; Li, S.; Zheng, L.; Yang, D.; Cai, Z., Circulating tumor cell associated

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white blood cell cluster as a biomarker for metastasis and recurrence in hepatocellular carcinoma. *Front Oncol* **2022**, *12*, 931140. View Article Online
DOI: 10.1039/D5AY01320H

96. Qi, L. N.; Xiang, B. D.; Wu, F. X.; Ye, J. Z.; Zhong, J. H.; Wang, Y. Y.; Chen, Y. Y.; Chen, Z. S.; Ma, L.; Chen, J.; Gong, W. F.; Han, Z. G.; Lu, Y.; Shang, J. J.; Li, L. Q., Circulating Tumor Cells Undergoing EMT Provide a Metric for Diagnosis and Prognosis of Patients with Hepatocellular Carcinoma. *Cancer Res* **2018**, *78* (16), 4731-4744.

97. Takahashi, K.; Ofuji, K.; Hiramatsu, K.; Nosaka, T.; Naito, T.; Matsuda, H.; Endo, K.; Higuchi, M.; Ohtani, M.; Nemoto, T.; Nakamoto, Y., Circulating tumor cells detected with a microcavity array predict clinical outcome in hepatocellular carcinoma. *Cancer Med* **2021**, *10* (7), 2300-2309.

98. He, Y. Z.; He, K.; Huang, R. Q.; Liu, L. W.; Ye, S. W.; Qian, J. L.; Peng, P.; Luo, Q. J.; Wang, Z. L.; Hu, Z. M., A clinical scoring system for predicting tumor recurrence after percutaneous radiofrequency ablation for 3 cm or less hepatocellular carcinoma. *Sci Rep* **2021**, *11* (1), 8275.

99. Espejo-Cruz, M. L.; González-Rubio, S.; Espejo, J. J.; Zamora-Olaya, J. M.; Alejandre-Altamirano, R. M.; Prieto-Torre, M.; Linares, C. I.; Guerrero-Misas, M.; Barrera-Baena, P.; Poyato-González, A.; Sánchez-Frías, M.; Ayllón, M. D.; Rodríguez-Perálvarez, M. L.; de la Mata, M.; Ferrín, G., Enumeration and Characterization of Circulating Tumor Cells in Patients with Hepatocellular Carcinoma Undergoing Transarterial Chemoembolization. *Int J Mol Sci* **2023**, *24* (3).

100. Zhao, X.; Zhao, J.; Tao, L.; Pan, Y.; Yang, L.; Zhang, X.; Yuan, J.; Zhu, H., Significance of circulating tumor cells in the portal vein regarding metastases and vascular invasion in hepatocellular carcinoma patients. *J Gastrointest Oncol* **2021**, *12* (6), 3050-3060.

101. Zhao, L.; Song, J.; Sun, Y.; Ju, Q.; Mu, H.; Dong, X.; Ding, J.; Liu, Y.; Wang, X.; Sun, L.; Wu, J.; Jiao, Y.; Lu, S.; Zhao, X., Tumor-derived proliferative CTCs and CTC clusters predict aggressiveness and early recurrence in hepatocellular carcinoma patients. *Cancer Med* **2023**, *12* (13), 13912-13927.

102. Hwang, H. S.; Yoo, J. E.; Han, D. H.; Choi, J. S.; Lee, J. G.; Joo, D. J.; Kim, M. S.; Kim, S. I.; Choi, G. H.; Park, Y. N., Circulating Cancer Stem Cells Expressing EpCAM/CD90 in Hepatocellular Carcinoma: A Pilot Study for Predicting Tumor Recurrence after Living Donor Liver Transplantation. *Gut Liver* **2022**, *16* (3), 443-455.

103. Lei, Y.; Wang, X.; Tian, Y.; Xu, R.; Pei, J.; Fu, Y.; Sun, H.; Wang, Y.; Zheng, P.; Xia, F.; Wang, J., Effect of various hepatectomy procedures on circulating tumor cells in postoperative patients: a case-matched comparative study. *Front Med (Lausanne)* **2023**, *10*, 1209403.

104. Zhou, J.; Zhang, Z.; Zhou, H.; Leng, C.; Hou, B.; Zhou, C.; Hu, X.; Wang, J.; Chen, X., Preoperative circulating tumor cells to predict microvascular invasion and dynamical detection indicate the prognosis of hepatocellular carcinoma. *BMC Cancer* **2020**, *20* (1), 1047.

105. Wang, P. X.; Xu, Y.; Sun, Y. F.; Cheng, J. W.; Zhou, K. Q.; Wu, S. Y.; Hu, B.; Zhang, Z. F.; Guo, W.; Cao, Y.; Huang, X. W.; Zhou, J.; Fan, J.; Yang, X. R., Detection of circulating tumour cells enables early recurrence prediction in hepatocellular carcinoma patients undergoing liver transplantation. *Liver Int* **2021**, *41* (3), 562-573.

106. Zhao, L.; Zheng, Z.; Liu, Y.; Liu, F.; Li, X.; Wu, Z., The mesenchymal circulating tumor cells as biomarker for prognosis prediction and supervision in hepatocellular carcinoma. *J Cancer Res Clin Oncol* **2023**, *149* (9), 6035-6048.

107. Ha, Y.; Kim, T. H.; Shim, J. E.; Yoon, S.; Jun, M. J.; Cho, Y. H.; Lee, H. C., Circulating tumor cells are associated with poor outcomes in early-stage hepatocellular carcinoma: a prospective study. *Hepatol Int* **2019**, *13* (6), 726-735.

108. Chen, Z.; Wang, T.; Chen, C.; Hong, X.; Yu, J.; Ma, Y.; Guo, Y.; Huang, C.; He, X.; Ju, W.; Chen,

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60

M., Circulating Tumor Cell Is a Clinical Indicator of Pretransplant Radiofrequency Ablation for Patients with Hepatocellular Carcinoma. *J Oncol* **2021**, 2021, 7776389. View Article Online
DOI: 10.1039/D5AY01320H

109. Zhang, Q.; Xia, F.; Mo, A.; He, W.; Chen, J.; Zhang, W.; Chen, W., Guiding Value of Circulating Tumor Cells for Preoperative Transcatheter Arterial Embolization in Solitary Large Hepatocellular Carcinoma: A Single-Center Retrospective Clinical Study. *Front Oncol* **2022**, 12, 839597.

110. Su, K.; Guo, L.; He, K.; Rao, M.; Zhang, J.; Yang, X.; Huang, W.; Gu, T.; Xu, K.; Liu, Y.; Wang, J.; Chen, J.; Wu, Z.; Hu, L.; Zeng, H.; Li, H.; Tong, J.; Li, X.; Yang, Y.; Liu, H.; Xu, Y.; Tan, Z.; Tang, X.; Feng, X.; Chen, S.; Yang, B.; Jin, H.; Zhu, L.; Li, B.; Han, Y., PD-L1 expression on circulating tumor cells can be a predictive biomarker to PD-1 inhibitors combined with radiotherapy and antiangiogenic therapy in advanced hepatocellular carcinoma. *Front Oncol* **2022**, 12, 873830.

111. Hsieh, C. H.; Yeh, C. T.; Huang, Y. H.; Lai, M. W., Circulating Tumor Cells Derived from Advanced Hepatocellular Carcinoma Rapidly Develop Resistance to Cytotoxic Chemotherapy. *Anticancer Res* **2022**, 42 (5), 2479-2486.

112. Zhang, Y.; Zhang, X.; Zhang, J.; Sun, B.; Zheng, L.; Li, J.; Liu, S.; Sui, G.; Yin, Z., Microfluidic chip for isolation of viable circulating tumor cells of hepatocellular carcinoma for their culture and drug sensitivity assay. *Cancer Biol Ther* **2016**, 17 (11), 1177-1187.

113. Li, X.; Wang, H.; Li, T.; Wang, L.; Wu, X.; Liu, J.; Xu, Y.; Wei, W., Circulating tumor DNA/circulating tumor cells and the applicability in different causes induced hepatocellular carcinoma. *Curr Probl Cancer* **2020**, 44 (2), 100516.

114. Son, J. A.; Weon, J. H.; Baek, G. O.; Ahn, H. R.; Choi, J. Y.; Yoon, M. G.; Cho, H. J.; Cheong, J. Y.; Eun, J. W.; Kim, S. S., Circulating small extracellular vesicle-derived splicing factor 3b subunit 4 as a non-invasive diagnostic biomarker of early hepatocellular carcinoma. *J Exp Clin Cancer Res* **2023**, 42 (1), 288.

115. Ning, C.; Cai, P.; Liu, X.; Li, G.; Bao, P.; Yan, L.; Ning, M.; Tang, K.; Luo, Y.; Guo, H.; Wang, Y.; Wang, Z.; Chen, L.; Lu, Z. J.; Yin, J., A comprehensive evaluation of full-spectrum cell-free RNAs highlights cell-free RNA fragments for early-stage hepatocellular carcinoma detection. *EBioMedicine* **2023**, 93, 104645.



Data availability

There is no data associated with this perspectives paper.