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Circulating tumor cells: Biological features and survival mechanisms

Xiaofeng LI[1]; Jinyang ZHENG[2]; Jinfeng ZHU[3]; Xin HUANG[4]; Huanhuan ZHU[5]; Bingdi CHEN[6,*]

[1] Department of Oncology, Affiliated Quanzhou First Hospital of Fujian Medical University, Quanzhou, China
[2] Department of Pathology, Affiliated Quanzhou First Hospital of Fujian Medical University, Quanzhou, China
[3] Department of Medical Oncology, Affiliated Quanzhou First Hospital of Fujian Medical University, Quanzhou, China
[4] Department of Dermatology, School of Medicine, Tongji Hospital, Tongji University, Shanghai, China
[5] Department of Central Laboratory, Shanghai Tenth People's Hospital, Shanghai, China
[6] Shanghai East Hospital, The Institute for Biomedical Engineering & Nano Science, Tongji University School of Medicine, Shanghai, China

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Abstract: Circulating tumor cells (CTCs) are neoplastic cells that are detached from primary tumors and enter circulation. Enumeration and characterization of CTCs are of significance in cancer diagnosis, prognosis, and treatment monitoring. CTC survival in the bloodstream is a limiting step for the development of metastases in distant organs. Recent technological advances, especially in single-cell molecular analyses have uncovered heterogeneous CTC survival mechanisms. Undergoing epithelial-to-mesenchymal transition (EMT), increasing stem cell-like properties, and forming cell clusters enable CTCs to adapt to the harsh microenvironment of the circulation. Expressing and releasing several immunosuppressive molecules help CTCs escape from anti-cancer immune mechanisms. This review article summarizes the biological characteristics of CTCs and focuses on the recent understanding of the mechanisms by which CTCs survive in circulation. Additionally, the clinical and therapeutic implications of CTCs are discussed.

Abbreviations

CAF	Cancer-associated fibroblast
CDX	CTC-derived xenograft
CTC	Circulating tumor cells
EMT	Epithelial-to-mesenchymal transition
EpCAM	Epithelial cellular adhesion molecule
MDSC	Myeloid derived suppressor cell

Introduction

Circulating tumor cells (CTCs) are a group of extraordinarily rare cancer cells that shed from solid tumors and enter the bloodstream. It is estimated that cancer patients have only 1 CTC per 1–10 million white blood cells (WBCs) (Dive and Brady, 2017). Compared to WBCs, CTCs possess unique biophysical and immunophenotypic characteristics. CTCs tend to be larger and express epithelial cell markers such as the epithelial cellular adhesion molecule (EpCAM) and cytokeratins (Micalizzi *et al.*, 2017). These features enable the enrichment of CTCs from a vast number of blood cells. Recently, a variety of enrichment approaches, although technically challenging, have been developed to detect CTCs (Deng *et al.*, 2022). Enumeration and phenotyping of CTCs are valuable for cancer diagnosis and prognosis. For example, a large, multicenter, prospective trial demonstrated that the presence of ≥3 CTCs at baseline predicted shorter survival in patients with metastatic renal cell carcinoma (Basso *et al.*, 2021). CTC enumeration could be used to distinguish patients with colorectal cancer from non-cancerous populations, with a sensitivity and specificity of 85.3% and 78.5%, respectively (Tsai *et al.*, 2021). Hence, CTC detection and characterization provide important biomarkers for screening and monitoring malignant diseases.

CTCs are considered to be an intermediate stage of metastasis, which involves a complex multistep process including cell detachment from the primary tumor, entry into vessels, extravasation, and colonization to distant tissues (Jerabkova-Roda *et al.*, 2022; Stock, 2022). Once shed into the circulation, CTCs face a harsh niche that is completely different from the tumor microenvironment established at the primary site. Most cancer cells die in the vascular system where there are significant shear forces, robust innate immunity, and oxidative stress (Massagué and Obenauf, 2016). Only a few CTCs can adapt to the

*Address correspondence to: Bingdi Chen, inanochen@tongji.edu.cn
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hostile conditions in the circulation. Several such mechanisms of CTC adaptation have been identified (Schuster *et al.*, 2021; Pereira-Veiga *et al.*, 2022). CTC aggregates have been frequently detected in the blood of patients with epithelial tumors (Cho *et al.*, 2012). The formation of such CTC aggregates offered a survival advantage and increased cancer stemness, leading to a 20- to 100-fold greater metastatic potential than single CTCs (Schuster *et al.*, 2021). CTCs from patients with melanoma exhibited the activation of lipogenic and iron homeostatic pathways, which conferred resistance to oxidative stress (Hong *et al.*, 2021). In another finding, the supplementation of N-acetyl-L-cysteine (NAC) and other antioxidants could increase the *ex vivo* survival and expansion of CTCs (Teng *et al.*, 2021). These studies suggest that the antioxidant defense system is important for the maintenance of CTC viability in blood circulation. CTCs typically undergo epithelial-to-mesenchymal transition (EMT), a reversible cellular process involving loss of cell-cell adhesion, epithelial polarization, and acquisition of mesenchymal cell features (Sun *et al.*, 2018; Xin *et al.*, 2020). The phenotypic plasticity allows CTCs to withstand the mechanical stress of the bloodstream (Xin *et al.*, 2020).

In the past two decades, single-cell profiling approaches have gained increasing attention in cancer studies (Ho *et al.*, 2019; Wu *et al.*, 2021). These techniques enable the investigation of intra-tumoral heterogeneity at single-cell resolution. A variety of single-cell multi-omics studies have indicated that CTCs are highly heterogeneous (Sun *et al.*, 2021b; Barnett *et al.*, 2022; Khan *et al.*, 2022). For example, Suvilesh *et al.* (2022) established liquid biopsy CTC-derived xenograft (CDX) tumor models in immunodeficient mice and found different drug responses and cell heterogeneity in CDX tumors. Using a single-cell immunoblotting chip technology, Abdulla *et al.* (2022) identified a novel CTC subpopulation that lacks the expression of Bax, a proapoptotic member of the Bcl-2 protein family that also displays chemoresistance. In another study, Chen *et al.* (2022) reported that the expression levels of epithelial and stem cell-like markers vary in two distinct groups of CTCs.

In this review article, we summarize recent advances in understanding the biological characteristics of CTCs. Given the importance of CTC survival in cancer dissemination, we focus on the mechanisms by which CTCs survive in blood circulation. Additionally, we discuss the clinical and therapeutic implications of CTCs.

Biological Features of CTCs

CTC heterogeneity

In the ongoing single-cell analysis era, the vast heterogeneity of CTCs is being uncovered. CTCs are known to comprise a heterogeneous population of malignant cells. Cho *et al.* (2021) reported that CTCs from the same patient with metastatic gastric cancer are heterogeneous in terms of morphology and marker expression, indicating an intrapatient heterogeneity. Chen *et al.* (2021b) noted that CTCs isolated from gastric cancer patients vary greatly in size. Single cell-based DNA sequencing analysis revealed that small and large CTCs have distinct genomic mutations. While the former have an enrichment in the Kirsten rat sarcoma viral oncogene homolog (KRAS) and Rap1 pathways, the latter is enriched in the phosphoinositide-3-kinase–protein kinase B/Akt or MET/PI3K/AKT pathway. Tumor heterogeneity has been partly ascribed to genetic instability and somatic mutations (Knoche *et al.*, 2021). Genomic profiling analysis has shown that CTCs share similar mutational signatures with the matched primary tumor samples (Alves *et al.*, 2022). Su *et al.* (2019) performed genomic analyses of tumor and CTC samples obtained from small-cell lung cancer patients and found that the majority of mutations in primary tumors are retained in CTCs. Kondo *et al.* (2017) analyzed the KRAS genotype in CTCs from 11 patients with metastatic colorectal cancer and found that 10 of 11 patients had the same KRAS status between the primary tumors and CTCs. Paoletti *et al.* (2018) performed next-generation sequencing (NGS) of CTCs and paired tissue metastases from patients with breast cancer and observed 85% concordance in somatic mutation and copy-number profiles. They also found a small set of CTC subclones with unique somatic mutations, confirming the heterogeneity of CTCs. However, Kim *et al.* (2020) reported conflicting findings on bladder cancer samples. They performed whole exome sequencing of 20 matched pairs of primary bladder cancers and CTCs and demonstrated that their mutation concordance is only 3%–24%. Despite such a report, somatic mutation represents an important cause of CTC heterogeneity.

The heterogeneity of CTCs can be coordinated by epigenetic and microenvironmental factors. Genome-wide analyses of DNA methylation in CTCs from breast cancer patients indicate the heterogeneity of CTCs at the epigenetic level (Cho *et al.*, 2012; Pixberg *et al.*, 2017). Epigenetic modulation of genes involved in proliferation, stemness, and EMT likely contributes to the survival of CTCs in circulation. In addition to genetic and epigenetic heterogeneity, CTCs show spatial heterogeneity. Sun *et al.* (2021a) observed that CTCs from different vascular sites of hepatocellular carcinoma patients displayed distinct transcriptional profiles, which was revealed by single-cell RNA sequencing. The spatial heterogeneity of CTCs has also been detected in lung cancer patients with brain metastasis (Ruan *et al.*, 2020). As CTCs are usually arrested in capillary beds (Perea Paizal *et al.*, 2021), blood flow generates different levels of mechanical forces at these CTC-arrested sites, thereby contributing to spatial heterogeneity (Follain *et al.*, 2018). Additionally, CTC diversity is increased upon anti-cancer treatments. Stewart *et al.* (2020) reported that there is a significant increase in the intratumoral heterogeneity score of CTCs isolated from relapsed small-cell lung cancer patients after chemotherapy. The complex heterogeneity of CTCs makes it necessary to dissect the characteristics of CTC subpopulations at the single-cell level.

Plasticity of CTCs

Epithelial-mesenchymal plasticity is an important feature of CTCs (Burr *et al.*, 2020). During intravasation, circulation,

and extravasation, CTCs typically undergo epithelial-to-mesenchymal or mesenchymal-to-epithelial transitions. These morphological changes involve the dysregulation of many EMT-related genes (Qi *et al.*, 2018; Burr *et al.*, 2020). Yu *et al.* (2013) reported that CTCs from breast cancer patients express transforming growth factor (TGF)-β pathway components and the Forkhead box C1 (FOXC1) transcription factor, which are key regulators of EMT. Similarly, EMT markers were also enriched in CTCs isolated from non-small cell lung cancer patients (Ntzifa *et al.*, 2021). Transcriptomic analysis has demonstrated that surviving CTCs express both epithelial and mesenchymal markers, indicating a hybrid epithelial/mesenchymal state (Genna *et al.*, 2020). Using single-cell sequencing technology, Sun *et al.* (2018) uncovered dynamic changes in CTCs isolated from hepatocellular carcinoma patients. The epithelial phenotype of CTCs switched to the mixed epithelial/mesenchymal phenotype along disease progression, which was causally linked to the activation of Smad2 and β-catenin-related signaling pathways (Sun *et al.*, 2018). Blood flow forces have also been reported to promote EMT in CTCs via c-Jun N-terminal kinase (JNK) signaling (Xin *et al.*, 2020).

In addition to morphological plasticity, CTCs exhibit metabolic plasticity (Chen *et al.*, 2018a; Zafeiriadou *et al.*, 2022). Compelling evidence indicates that metabolic reprogramming augments the adaptation of cancer cells to microenvironmental stress (Martínez-Reyes and Chandel, 2021; Tang *et al.*, 2022). Increased expression of phosphoglycerate kinase (PGK1) and glucose-6-phosphate dehydrogenase (G6PD), the key regulators of glycolysis and the pentose phosphate pathway, respectively, suggests active glucose metabolism of tumor cells (Chen *et al.*, 2020). CTCs from prostate cancer patients (Chen *et al.*, 2018b) and breast cancer (Chen *et al.*, 2020) patients also abundantly expressed PGK1 and G6PD. The CTCs with high expression of PGK1 and G6PD represent an aggressive subpopulation and serve as a potential marker for poor prognosis (Chen *et al.*, 2018b; Chen *et al.*, 2020). CTCs show increased expression of atonal bHLH transcription factor 8 (ATOH8) upon shear stress, which promotes hexokinase 2 (HK2)-mediated glycolysis and survival (Huang *et al.*, 2020). These studies suggest that metabolic plasticity contributes to an aggressive phenotype of CTCs.

Stem-like features of CTCs

Stem-like cancer cell populations have been identified in all solid tumors including brain, liver, lung, breast, stomach, ovary, colon, thyroid, and prostate cancers (Du *et al.*, 2022). They possess self-renewal and tumor-initiating capacities. A large number of studies have reported the link between stem cell signatures and the survival of cancer patients (Ponomarev *et al.*, 2022), highlighting the involvement of cancer stem cells in tumor progression.

In recent years, the stem-like properties of CTCs have attracted increasing attention (Chen *et al.*, 2021a; Lee *et al.*, 2022). CTCs can express a variety of stem cell markers. Wang *et al.* (2022) reported that the stem cell marker CD44 was detected in CTCs isolated from gastric cancer patients. The presence of CD44-positive CTCs was associated with recurrent disease and poor survival of patients. CTCs from melanoma patients had a high aldehyde dehydrogenase 1 activity and the expression of multiple stemness transcription factors such as OCT3/4, NANOG, and SOX2 (Wang *et al.*, 2022). When injected into immunodeficient mice, melanoma CTCs showed tumorigenic and metastatic potentials. CTCs from patients with glioblastoma showed a robust expression of stemness-associated genes including SOX2, OCT4, and NANOG (Felici *et al.*, 2022). Cancer stemness is suggested to also drive therapeutic resistance (Najafi *et al.*, 2019). Consistently, circulating glioma cells exhibited resistance to radiotherapy and chemotherapy (Liu *et al.*, 2018). Zhang *et al.* (2020) demonstrated that CTCs from bladder cancer patients coexpressed both stem cell and EMT markers, suggesting the relationship between EMT and stemness. Leucine-rich G-protein-coupled receptor 5 (LGR5) is an important driver of cancer stemness (Zhang *et al.*, 2017). A study showed that the high LGR5 expression in CTCs was associated with colorectal cancer metastasis (Wang *et al.*, 2018). These observations show that stem-like CTCs may contribute to metastatic lesion formation.

Survival Mechanisms of CTCs in the Circulation

When cancer cells are shed from primary tumors enter the circulation, they have to overcome multiple challenges that impair cell survival (Fig. 1). Most CTCs lack the ability to survive in circulation, with an estimated half-life of 1–2.4 h (Meng *et al.*, 2004). However, a small minority of CTCs can survive and then seed metastases. Scrutiny of their survival strategies is of significance in eliminating persistent CTCs in therapy.

Clustering enhances CTC survival

The clustering of CTCs has been observed in many cancer types (Schuster *et al.*, 2021). For example, Chen *et al.* (2022) identified CTC clusters in 6 of the 8 hepatocellular carcinoma patients tested. Francescangeli *et al.* (2021) isolated large CTC clusters from metastatic colorectal cancer patients. There is evidence that such CTC clustering enhances the metastatic potential of CTCs (Piñeiro *et al.*, 2020). Aceto *et al.* (2014) reported that CTC clusters have 23-50-fold higher metastatic activity than single CTCs. Aggregation of CTCs was found to induce anoikis resistance in the suspension culture with fluid shear stress (Maeshiro *et al.*, 2021).

In addition to the formation of homo-clusters, CTCs can interact with immune cells such as neutrophils and macrophages to form hetero-clusters (Chen *et al.*, 2018b; Osmulski *et al.*, 2021). Osmulski *et al.* (2021) reported that the inclusion of macrophages in CTC clusters facilitated survival-promoting mechanical fitness through the induction of epithelial-mesenchymal plasticity. CTCs could also recruit and form heterotypic clusters with inflamed neutrophils, which then enhanced CTC extravasation (Chen *et al.*, 2018b). Myeloid-derived suppressor cells (MDSCs) clustered with CTCs have been shown to promote tumor cell proliferation (Sprouse *et al.*, 2019). Additionally, CTCs could interact with endothelial cells and cancer-associated fibroblasts (CAFs) (Sharma *et al.*, 2021). Such cellular

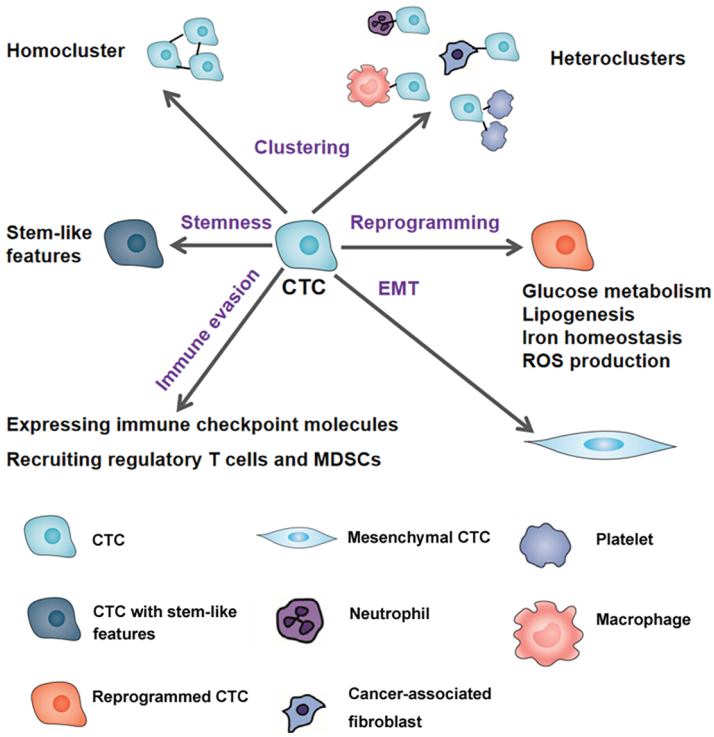

FIGURE 1. The mechanisms linked to circulating tumor cell (CTC) survival. To survive in circulation, CTCs can form homoclusters or heteroclusters with neutrophils, macrophages, cancer-associated fibroblasts, and platelets. Undergoing metabolic reprogramming and epithelial-to-mesenchymal transition (EMT) and acquiring stem-like properties can all enhance CTC survival. In addition, CTCs can express multiple immune checkpoint molecules and release cytokines and chemokines to recruit regulatory T cells and myeloid-derived suppressor cells (MDSCs), which help CTCs escape from the immune attack.

aggregation is believed to play an essential role in the maintenance of CTC survival in blood (Pereira-Veiga *et al.*, 2022). Arnoletti *et al.* (2018) demonstrated that CTCs from patients with pancreatic ductal adenocarcinoma (PDAC), ampullary adenocarcinoma (AA), and distal cholangiocarcinoma (CC) could recruit multiple immune cell types to generate spheroid-like clusters, consequently promoting CTC survival and growth. Consistent with their biological roles, CTC clusters have important clinical values in cancer management. The detection of CTC clusters was of diagnostic and prognostic significance in patients with metastatic cancer (Manjunath *et al.*, 2019; Costa *et al.*, 2020; Lim *et al.*, 2021; Wang *et al.*, 2021a). In one study, CTC clusters could be used as a specific liquid biomarker to distinguish non-small cell lung cancer from high-risk subjects with benign nodules (Manjunath *et al.*, 2019). In a real-world cohort of metastatic breast cancer patients, the presence of CTC clusters at baseline was significantly associated with reduced survival (Costa *et al.*, 2020).

Most CTC clusters (about 80%) are associated with platelets (Lim *et al.*, 2021). Heat shock protein 47 (Hsp47) was found to be involved in platelet recruitment and mediated CTC-platelet interaction (Xiong *et al.*, 2020). Transcriptomic analysis of CTC samples isolated from breast cancer patients revealed an increased expression of Hsp47 in CTC clusters compared to single CTCs (Chen *et al.*, 2020), suggesting the role of Hsp47 in CTC clustering. Cytoskeletal elements including α-tubulin, detyrosinated α-tubulin, and vimentin contribute to the interaction between CTCs and blood cells (Kallergi *et al.*, 2018). Intercellular adhesion molecule 1 (ICAM1) was shown to be involved in homotypic breast cancer cell clustering, and its depletion impaired CTC cluster formation (Taftaf *et al.*, 2021). Upregulation of desmocollin-2 (DSC2) and plakophilin-1 (PKP1) in CTCs led to increased formation of clusters and activation of PI3K/AKT/Bcl-2–mediated pathways, consequently promoting CTC survival in the circulation (Li *et al.*, 2021). In one study, intercellular CD44-CD44 homophilic interactions could facilitate circulating breast cancer cell aggregation through the activation of focal adhesion kinase (FAK) signaling (Liu *et al.*, 2019). Further, knockdown of plakoglobin resulted in the suppression of CTC cluster formation (Aceto *et al.*, 2014). Fibronectin was also shown to participate in cell aggregate formation, and its knockdown blocked cancer cell clustering and reversed anoikis resistance (Han *et al.*, 2021). Collectively, a number of genes contribute to the formation of homotypic or heterotypic CTC clusters, thus promoting CTC survival in circulation. Prevention of CTC clustering may thus represent a promising approach to treating metastatic cancers.

Induction of EMT and stemness contributes to CTC survival

Induction of EMT- and stemness-related genes has been shown to enhance anoikis resistance of cancer cells (Wang *et al.*, 2021b; Shait Mohammed *et al.*, 2022; Wang *et al.*, 2022). Interestingly, CTCs can express multiple EMT and cancer stem cell markers (Chen *et al.*, 2013; Werner *et al.*, 2017). Pixberg *et al.* (2017) reported that aggregated CTCs have a global change in DNA methylation, leading to increased expression of genes involved in cell survival, proliferation, and stemness. Single-cell analysis of CTCs from patients with metastatic prostate cancer revealed the abundant expression of EMT-related genes including insulin like growth factor 1 (IGF1), insulin like growth factor 2 (IGF2), epidermal growth factor (EGFR), forkhead box P3 (FOXP3), and transforming growth factor beta 3 (TGFB3) (Chen *et al.*, 2013; Werner *et al.*, 2017). Further, CTCs with stem cell-like features documented overexpression of the pluripotency marker Krüppel-like factor 4 (Klf4) and activation of β-catenin signaling (Zhu *et al.*, 2020).

Clinically, the presence of CTCs expressing EMT markers (phosphatidylinositol-3-kinase α [PI3Kα] and Akt-2) and stem cell markers (aldehyde dehydrogenase 1 [ALDH1]) was associated with reduced survival in patients with metastatic colorectal cancer (Ning *et al.*, 2018). Papadaki *et al.* (2019) reported that a subset of CTCs isolated from metastatic breast cancer patients expressed high levels of ALDH1, TWIST1, and cytokeratin and showed chemoresistant properties. These results suggest that the upregulation of EMT- and stemness-related genes confers an aggressive phenotype to CTCs.

Metabolic reprogramming supports CTC survival

Cells surviving in an anchorage-independent condition are characterized by metabolic reprogramming (Shi *et al.*, 2014). For example, Dihydroceramide desaturase 1 acts as a mediator of HER2-driven glucose metabolic signals and enhances anchorage-independent survival (Linzer *et al.*, 2022). Serum and glucocorticoid kinase-1 plays an essential role in ATP generation during the loss of extracellular matrix attachment, which is associated with the elevation of glucose uptake (Mason *et al.*, 2021). Analysis of CTCs from patients with non-small cell lung cancer also reveals overexpression of multiple metabolism-related genes (Zafeiriadou *et al.*, 2022). For example, an increased number of CTCs with aberrant expression of glucose metabolic genes (PGK1 and G6PD) was associated with advanced tumor stage and metastasis in prostate cancer patients (Chen *et al.*, 2018b). These findings suggest the involvement of metabolic reprogramming in the regulation of CTC behaviors. In another study, single melanoma CTCs showed upregulation of lipogenesis and iron homeostasis pathways, which reduced reactive oxygen species and lipid peroxidation and thereby promoted resistance to ferroptosis (Hong *et al.*, 2021). The PI3K/AKT pathway plays a pivotal role in cellular metabolism via either direct regulation of metabolic enzymes or modulation of metabolism-related transcription factors (Hoxhaj and Manning, 2020). In an anchorage-independent condition, AKT activation leads to the enhancement of cell survival through the induction of glycolysis (Joo *et al.*, 2022). Moreover, the activation of the PI3K/AKT pathway promoted CTC survival in a study (Li *et al.*, 2021). Therefore, the PI3K/AKT pathway may mediate the link between metabolism and survival of CTCs.

Immune evasion by CTCs

Escape from immune attack is important for CTC survival in circulation. It has been documented that CTCs can express multiple immune checkpoint proteins including CD47 and programmed death ligand 1 (PD-L1) (Chikamatsu *et al.*, 2019; Papadaki *et al.*, 2020; Zhang *et al.*, 2021). The transmembrane protein, CD47, can interact with the myeloid inhibitory immunoreceptor signal-regulatory protein-α (SIRPα) to inhibit the phagocytic activity of activated immune cells (Barclay and van den Berg, 2014). Steinert *et al.* (2014) reported that CD47 was upregulated in CTCs from colorectal cancer patients compared to matched tumor tissues and contributed to CTC immune escape. The presence of CD47-positive CTCs has also been suggested to be responsible for tumor relapse in breast cancer patients (Nagahara *et al.*, 2010). The PD-1/PD-L1 axis is another immune checkpoint regulator. The interaction between PD-L1 and PD-1 can halt T cell-mediated immune responses and help tumor cells escape from the surveillance of the immune system (Pantazaka *et al.*, 2022). PD-L1 can also promote the expansion of regulatory T cells, thus enhancing immunosuppression (Dong *et al.*, 2020). The PD-L1/PD-1 interaction is believed to enable CTCs to survive in circulation (Chikamatsu *et al.*, 2019; Papadaki *et al.*, 2020). For example, baseline PD-L1-positive CTCs were detected in 36.1% of 56 patients with metastatic breast cancer that correlated with patient survival (Jacot *et al.*, 2020). In another study, Nicolazzo *et al.* (2016) monitored the PD-L1 expression status in CTCs from non-small lung cancer patients after treatment with the PD-1 inhibitor Nivolumab. They found that patients with PD-L1-positive CTCs developed progressive disease, suggesting that PD-L1-positive CTCs can escape from anti-cancer immunity. In a cohort of patients with advanced non-small cell lung cancer, survival was worse in the group with PD-L1-positive CTCs than in the group with PD-L1-negative CTCs (Sinoquet *et al.*, 2021). In an animal model study, dual inhibition of both PD-L1 and CD47 led to a significant reduction of metastatic formation by CTCs (Lian *et al.*, 2019).

The release of a variety of cytokines and chemokines by CTCs to counteract immune responses is documented (Sun *et al.*, 2021a). For example, Sun *et al.* (2021a) reported that the chemokine C-C motif chemokine ligand 5 (CCL5) was abundantly produced by hepatocellular carcinoma CTCs, which consequently recruited regulatory T cells to suppress cytotoxic T cell immunity. Further, PDAC CTCs produced colony-stimulating factor 1 (CSF1), Interleukin (IL) 34, and IL8 that induced MDSC differentiation (Arnoletti *et al.*, 2022). MDSCs are characterized by their T-cell immunosuppressive functions (Hegde *et al.*, 2021). The induction of MDSCs has also been shown to inhibit anti-cancer immunity (Li *et al.*, 2018). Hence, such interactions with MDSCs support CTC survival and immune evasion.

Clinical Applications of CTCs

The enumeration and characterization of CTCs provide valuable information for cancer detection, prognostic evaluation, and treatment guidance. An example is the CellSearch system, which is designed for the enumeration of CTCs, has been approved by the Food and Drug Administration (FDA) for prognostic analysis in patients with breast, colorectal, and prostate cancers (Deng *et al.*, 2022). A large number of clinical studies have indicated the prognostic value of CTC enumeration in cancer patients (Basso *et al.*, 2021; Deng *et al.*, 2022). In addition to enumeration, molecular phenotyping of CTCs provides an additional clinical relationship with the disease state and patient survival (Sun *et al.*, 2021a; Abdulla *et al.*, 2022; Khan *et al.*, 2022). For example, PD-L1 expression in CTCs was significantly associated with progression-free survival of patients with triple-negative breast cancer (Jacot *et al.*, 2020). Single-cell technologies have enabled the dynamic monitoring of heterogenous CTCs. The reduction in the percentage of PD-L1-positive CTCs reflects a beneficial

response to PD-1/PD-L1 inhibitors in patients with advanced cancers (Tan *et al.*, 2021), suggesting the significance of monitoring CTC dynamic changes. Recently, multiple microfluidic platforms have been developed to integrate CTC enrichment, identification, and molecular analysis, which strengthen the significance of CTCs as clinical biomarkers in cancer detection and treatment (Abdulla *et al.*, 2022; Sajdik *et al.*, 2022).

The enumeration of CTCs also serves as a promising blood test to screen for cancers. For example, the enumeration of EpCAM-positive CTCs yielded a sensitivity of 85.3% and specificity of 78.5% in differentiating colorectal cancers from the non-cancerous controls (Tsai *et al.*, 2021). When combined with serum carcinoembryonic antigen (CEA) assay, CTC testing significantly improved colorectal cancer screening. Total CTC counting provided a potential diagnostic value in hepatocellular carcinoma (Cheng *et al.*, 2019). A meta-analysis proposed CTCs to be used as a biomarker for lung cancer diagnosis given a sensitivity and specificity of 75% and 92%, respectively. The diagnostic potential of CTCs has also been observed in pancreatic cancer, with a sensitivity and specificity of 75.0% and 96.4%, respectively. Moreover, a cut-off of $\geq$3 CTCs in 4 ml venous blood could discriminate between local/regional and metastatic PDACs (Ankeny *et al.*, 2016).

The detection of CTCs also provides prognostic information in many cancers (Deng *et al.*, 2022). For example, a systematic literature review on CTCs in pancreatic cancer suggested that CTCs are present in the peripheral blood of most patients with pancreatic cancer and predict shorter overall survival (Pang *et al.*, 2021b). In patients with metastatic melanoma, CTC detection was associated with worse overall survival and progression-free survival (Aya-Bonilla *et al.*, 2020). Total CTCs and CTC-white blood cell clusters have been identified as prognostic factors for the metastasis-free survival of renal cell carcinoma patients (Guan *et al.*, 2021). Further, combining CTC cluster counts and cell-free DNA levels exhibited the potential to improve the prediction of progression-free survival in non-small cell lung cancer patients (Kapeleris *et al.*, 2022). Taking heterogeneity into consideration, CTCs can be classified into distinct sub-populations, such as epithelial, mesenchymal, partial EMT, and stem cell-like CTCs. Although there is no significant correlation between the total CTC count and clinicopathological variables, the partial EMT sub-population of CTCs showed significant correlations with advanced disease and poor prognosis in pancreatic cancer patients (Semaan *et al.*, 2021). These studies highlight the prognostic value of CTC biomarkers in cancers.

The counts of CTCs have been shown to change during anticancer treatment (Rossi *et al.*, 2012; Pernot *et al.*, 2017; Pang *et al.*, 2021a). This provides a rationale for monitoring therapeutic response based on CTC analysis. CTC counts at baseline served as an independent predictor of treatment response in patients with head and neck cancer (Zhang *et al.*, 2022b). For example, Chong *et al.* (2012) reported that CTCs were detected in 53 out of 94 breast cancer patients before chemotherapy. However, after 3 cycles of chemotherapy, CTC numbers were reduced to an undetectable level. Li *et al.* (2016) quantified CTCs in patients with advanced gastric cancer before and after chemotherapy. They found that elevated CTC numbers during therapy predict a poor prognosis. In contrast, conversion to a favorable CTC level after chemotherapy was associated with a good prognosis. Further, Wang *et al.* (2017) suggested that the assessment of CTC clusters provides additional prognostic values to CTC enumeration alone in metastatic breast cancer patients.

The molecular phenotypes of CTCs may change perceptibly in response to anticancer treatment. For example, CTCs from patients with $ER^+/HER2^-$ (ER: estrogen receptor; HER: human epidermal growth factor receptor 2) breast cancer acquired a subpopulation of $HER2^+$ CTCs after several courses of therapy, which is explained by the reciprocal transformation between $HER2^+$ and $HER2^-$ CTCs (Jordan *et al.*, 2016). Further, the reduced expression of Schlafen 11 in CTCs was associated with therapeutic response in small cell lung cancer patients receiving platinum treatment (Zhang *et al.*, 2022a). The proportion of PD-L1-positive CTCs was reduced in cancer patients who benefited from PD-1/PD-L1 blockade therapies (Tan *et al.*, 2021), suggesting that the PD-L1 level on CTCs is useful in monitoring response to PD-1/PD-L1 inhibitors. Additionally, Spiliotaki *et al.* (2022) examined the expression of PD-L1 in longitudinal CTC samples isolated from 47 advanced NSCLC patients receiving pembrolizumab. They found that the patients with decreased total and $PD\text{-}L1^{low}$ CTCs after first cycle treatment have significantly longer progression-free survival than those with increased CTCs. These observations necessitate that the dynamic analysis of CTC counts and phenotypes is important to monitor therapeutic response in cancer patients.

Conclusions

CTCs have the potential to seed metastases in distant organs. Their survival in circulation is indispensable for successful metastasis. High heterogeneity, phenotypic plasticity, stem-like properties, and cell-cell cross-talk all contribute to CTC survival in the bloodstream microenvironment. Advances in single-cell technologies have enabled the scrutiny of individual CTCs and shed light on the complex mechanisms by which CTCs escape from anoikis and immune attack. An understanding of the molecular characteristics of CTCs will further promote their clinical implications in cancer diagnosis, prognosis, and therapy monitoring.

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Availability of Data and Materials: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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