New advances in circulating tumor cell-mediated metastasis of breast cancer (Review)

JIANG-SHAN ZHOU[1], ZI-NING LIU[1], YUAN-YUAN CHEN[1], YU-XI LIU[1], HUA SHEN[2], LI-JUN HOU[1,3] and YI DING[1,3]

[1]Laboratory of Pathophysiology, Weifang Medical University, Weifang, Shandong 261053, P.R. China; [2]Department of Mathematics and Statistics, University of Calgary, Alberta T2N 1N4, Canada; [3]Key Laboratory of Applied Pharmacology, Weifang Medical University, Weifang, Shandong 261053, P.R. China

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Abstract. Breast cancer stands as the most prevalent form of cancer affecting women, with metastasis serving as a leading cause of mortality among patients with breast cancer. Gaining a comprehensive understanding of the metastatic mechanism in breast cancer is essential for early detection and precision treatment of the disease. Circulating tumor cells (CTCs) play a vital role in this context, representing cancer cells that detach from tumor tissues and enter the bloodstream of cancer patients. These cells travel in the blood circulation as single cells or clusters. Recent research has shed light on the enhanced metastatic potential of CTC clusters compared to single CTCs, despite their limited occurrence. The aim of the present review was to explore recent findings on CTCs with a particular focus on the clustering phenomenon of CTCs observed in breast cancer. Additionally, the present review delved into the comparison between single CTCs and CTC clusters regarding their implications for the treatment and prognosis of patients diagnosed with metastatic breast cancer. By examining the role and mechanisms of CTCs in breast cancer metastasis, the present review provided an improved understanding of CTCs and their significance in early detection of breast cancer metastasis through peripheral blood analysis. Moreover, it contributed to the comprehension of cancer prognosis and prediction by highlighting the implications of CTCs in these aspects. Ultimately, the present study seeks to advance knowledge in the field and pave the way for improved approaches to breast cancer management.

Correspondence to: Professor Yi Ding, Laboratory of Pathophysiology, Weifang Medical University, 7166 Bao Tong West Street, Weifang, Shandong 261053, P.R. China
E-mail: dingyi6767@163.com

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Contents

1. Introduction

Breast cancer has been a focus of attention for numerous years due to its high incidence and high mortality rate. According to Global Cancer Statistics 2020, in that year, the newly diagnosed cases of breast cancer accounted for 11.7% of all newly diagnosed cases of cancer worldwide, and breast cancer-related deaths accounted for 6.9% of all new cancer-related deaths (1). Despite the growing popularity of early screening for breast cancer and the development of increasingly diverse and precise diagnostic and treatment methods, numerous patients with breast cancer are still diagnosed after metastases (2). In recent years, the incidence and mortality rates of breast cancer have remained high, according to survey data (3).

Distant metastasis is considered as a significant cause of mortality in patients with breast cancer. Existing studies have shown that 20-30% of patients with breast cancer may develop metastasis after diagnosis and treatment of the primary tumor, and ~90% of cancer-related deaths are also due to metastasis (4). A previous study has revealed that breast cancer metastases exhibit significant heterogeneity, with lung, bone, and liver being the most common metastatic targets, and the sites of metastasis significantly affecting patient prognosis (5). Bone is a predilection site for distant metastasis of breast cancer. Data indicate that 62.5% of patients with initial metastatic breast cancer are diagnosed with bone metastases, and the proportion of bone metastases among patients with advanced breast cancer is as high as 75%. The occurrence of bone metastases significantly affects the quality of life of patients, and their life expectancy is only two to three years after the diagnosis of bone metastases (6-8). Patients with liver metastases are traditionally considered to have a poor prognosis. Survey and

follow-up statistics reveal that the median overall survival of patients with liver metastases is 16.3 months, and the estimated five-year survival rate is only 8.5% (9).

Lung metastases often occur in patients with basal-like breast cancer, and lead to a short life expectancy, with a median survival period of only 22 months after treatment. What is more concerning is that lung metastases develop in 60-70% of patients who succumb to breast cancer (10,11). Due to the complexity of the metastasis process, there is currently a lack of effective treatment for metastatic breast cancer. Therefore, it is highly necessary and beneficial to conduct in-depth research on the mechanism of breast cancer metastasis, promote accurate diagnosis and prevent metastasis as early as possible.

Circulating tumor cells (CTCs) are cancer cells that have shed off from developed tumor tissues and entered the vasculature or lymphatics, and are circulating in peripheral blood vessels. CTCs are very rare in the blood, with only 1-10 CTCs per milliliter of blood, compared with billions of blood cells (12). However, they can now be detected and counted by separating them from blood cells using various enrichment methods (13,14). CTC counts identify and quantify the epithelial cell adhesion molecule (EpCAM) protein produced by epithelial tumor cells, making it a useful diagnostic tool that may also aid in determining prognosis and assessing treatment efficacy. It is currently recognized that having ≥5 CTCs per 7.5 ml of blood is associated with poorer overall survival and progression-free survival (15). A previous study has demonstrated that the number of CTCs is an independent predictor of progression-free survival and overall survival in patients with metastatic breast cancer prior to the initiation of treatment (16). The CTC detection system CellSearch (17) has been approved by the Food and Drug Administration (FDA) for therapeutic monitoring of metastatic breast cancer. In short, CTCs are considered as the precursor cells for the formation of distant metastasis, which promotes the occurrence of cancer metastasis. The metastatic process experienced by CTCs generally includes detachment from the primary tumor tissue, invasion into the blood circulation, extravasation from the circulatory system, and finally colonization in distant organs (18).

A recent study demonstrated that CTCs in the circulating blood of patients with cancer exist not only in the form of single cells, but also clusters. The size of CTC clusters can range from two tumor cells to >100 cells. CTC clusters are also known by various names such as circulating tumor microemboli, circulating micrometastases, circulating tumor aggregates or tumor cell clumps (19). CTC clusters are rarer than single CTCs but may have a 50-fold higher potential for distant metastasis than single CTCs (20).

Previous research on CTC has been limited by the lack of CTC isolation technology. Due to their rarity in the blood compared to blood cells, it has been difficult to isolate them alive (21,22). However, with the development of CTC detection and capture technology, the presence of CTC can be detected in the blood of various patients with cancer (23-25). Although the detection rate of CTC clusters is lower than that of single CTCs, at least one CTC cluster can be detected in >50% of the patients with cancer (26), which suggests that most patients have a high risk of metastasis. The formation of CTCs is the initiation of tumor metastasis, and studying CTCs can provide a good understanding of the early dynamics of breast cancer metastasis. Identifying CTCs in patients at an early stage, and taking timely measures to inhibit their generation and eliminate them, may have significant benefits in suppressing breast cancer.

2. Formation of circulating tumor cell clusters

As early as the 19th century, the Australian pathologist John Ashworth introduced the concept of CTCs (27). In the 1970s, Nowell (28) revised the definition of CTCs stating that they are cancer cells that originate from primary tumors or metastatic tumors, acquire the ability to break away from the basement membrane, invade the tissue matrix, and enter the blood vessels.

With the advancement of CTC separation and detection technology, the understanding of CTC is gradually improving. Using multichromatographic lineage tracing, researchers have observed that polyclonal dissemination from cell clusters account for >90% of metastases in common mouse breast cancer models (29). Although this cell mass in animal models may not have come from the clonal growth of single cells, it fully shows that metastasis through cell clusters is a common means of cancer metastasis. CTC clusters have become the focus of researchers due to their stronger ability to promote metastasis than single CTCs. The source of CTC clusters has aroused the interest of researchers. Currently, there are two main views on the source of CTC clusters. The first supports that CTC clusters are directly shed from the primary tumor tissue, and the second suggests that they originate from the accumulation of single CTCs in blood vessels (30,31). Currently, researchers are more inclined to consider that the aggregation effect occurs after tumor cells fall off, and various signaling pathways and related molecules have been identified to be associated with the aggregation of CTCs. Most of these related pathways and molecules causing CTC aggregation are also related to breast cancer metastasis. This suggests that the formation of CTC clusters is strongly associated with breast cancer metastasis (19).

The high expression of cell-cell adhesion proteins is considered an important factor for the formation of CTC clusters in the blood. Plakoglobin, a key component of cell adhesion, can promote metastasis by stimulating the formation of tumor cell clusters in invasive micropapillary carcinoma of the breast (IMPC) (32). Studies have revealed that plakoglobin activates the PI3K/Akt/Bcl-2 signaling pathways promoting the formation of tumor cell clusters in IMPCs (32). In addition, *in vitro* experiments have shown that the formation of clusters greatly inhibits the loss of cells compared to single cells in both adherence and suspension cultures, making CTC clusters more likely to survive (32). There are numerous studies on how plakoglobin is involved in the signaling pathways related to the prognosis of breast cancer. For example, KRT13 promotes the differentiation and metastasis of breast cancer stem cells through the plakoglobin/c-Myc signaling pathway (33). *In vitro* experiments have confirmed that overexpression of KRT13 can promote the proliferation, migration and invasion ability of MCF7 cells, and after inoculation of MCF7 cells overexpressing KRT13, accelerated tumor growth and increased distant metastasis were observed *in vivo* (33). Additionally, detecting the plakoglobin status *in vivo* can also help to predict the prognosis of patients with metastatic breast

cancer. By counting CTCs and CTC clusters in patients with metastatic breast cancer and analyzing their protein expression, it has been identified that the number of CTC clusters in the bodies of patients with positive plakoglobin is markedly higher than that of patients with negative plakoglobin. The abundant presence of CTC clusters and high expression of plakoglobin, have been thus revealed to be significantly associated with the poor prognosis of patients with cancer (34).

CD44 is a non-kinase cell-surface transmembrane glycoprotein that is involved in cell-cell interactions, cell adhesion and migration. CD44 mediates cell aggregation through affinity between tumor cells of the same type (30,35). Cells that overexpress CD44 have various characteristics of cancer stem cells. The CD44 gene produces multiple isoforms after undergoing complex alternative splicing, and the interaction between these isoforms and different ligands can stimulate numerous cancer-related signals. For example, CD44v8-10, one of the CD44 variant subtypes, can promote MCF-7 cell migration and sphere formation (36). CD44 is therefore widely recognized as a tumor stem cell marker in various cancers (35). A previous study revealed that CD44 can guide the accumulation of CTCs and promote the metastasis of breast cancer (30). CD44 mediates tumor cell aggregation through intercellular homophilic interactions and initiates CD44-PAK2 interaction to further activate focal adhesion kinase (FAK) signal, which is a non-receptor tyrosine kinase. Literature previously reported that FAK can mediate the cell signal transduction by the integrin and growth factor receptors and regulate various cellular functions including adhesion and proliferation after activation (30,37). CD44 can also mediate the aggregation of tumor cells through the epidermal growth factor receptor (EGFR), a tyrosine kinase. The combination of targeting HER2 and EGFR in the treatment of triple-negative breast cancer (TNBC) was reported in a previous study (38). A recent study has demonstrated that EGFR can enhance CD44-mediated tumor cell aggregation during the formation of CTC clusters. CD44 can in turn regulate the stability of EGFR during cell separation and circulation (39). The tumor suppressor microRNA-30c (miR-30c) is transcriptionally regulated by GATA3 in breast tumors and directly targets TWF1 to play a role in drug resistance (40). Another study has confirmed that CD44 is the direct target of miR-30c, while EGFR is the downstream target of the miR-30c-CD44 pathway (39). MiR-30c can become a negative regulator of CTC accumulation and lung metastasis by targeting CD44 and its downstream effector EGFR. Inhibiting EGFR can block the accumulation of tumor stem cells in TNBC and lung metastasis *in vivo* (39,40).

Intercellular adhesion molecule 1 (ICAM1) is a cell surface glycoprotein and an adhesion receptor. Similar to CD44, ICAM1 can also guide the accumulation of the same type of tumor cells and promote the formation of CTC clusters (41). A recent study has also determined that ICAM1 not only guides tumor cell aggregation through homophilic ICAM1-ICAM1 interactions, but also promotes homotypic tumor cell clustering to form CTC clusters. Moreover, it can drive tumor-endothelial heterotypic cell adhesion, and knockdown of the expression of ICAM1 significantly inhibits the aggregation of tumor cells (41). ICAM1 also plays a role in recruiting chemotactic leukocytes from circulating sites to inflammatory sites. Additionally, ICAM1 serves as a biosensor to transmit intracellular and extracellular signals, regulating cell functions such as the barrier properties of epithelial cells and endothelial cells and cell migration (42). Heparanase (HPSE) is an endoglycosidase associated with metastasis, which can participate in cell adhesion through its non-enzymatic activity. HPSE promotes the formation of CTC clusters and regulates the FAK-Src-paxillin signaling pathway and the expression of ICAM1 (43). The coordinated effect between these adhesion proteins and enzymes may jointly promote the aggregation of tumor cells. These findings highlight the importance of adhesion proteins and enzymes in the formation of CTC clusters and the promotion of metastasis in breast cancer.

In addition to the aforementioned related molecules that can promote the accumulation of CTCs, changes in the tumor microenvironment can also lead to the accumulation of tumor cells. Studies (31) have shown that hypoxia in the tumor microenvironment is a triggering factor for the upregulation of cell adhesion components and CTC aggregation. To track breast cancer cells involved in breast cancer progression in a mouse model, a research group dynamically tracked spontaneous hypoxic events by expressing the reporter vector, HIF1a-expressing eYFP, which is an expression-enhancing yellow fluorescent protein that can only be generated under the control of hypoxic-response element repeats. They observed that 80.6% of the CTC clusters were positive for HIF1a (31). This indicates that most of the CTC clusters originate from hypoxic regions and are undergoing hypoxic processes, compared with the normal oxygen content of individual CTCs. This study also revealed that hypoxic CTC clusters have higher transfer ability than those with normal oxygen levels (31). Although there are few studies on hypoxia-triggered CTC aggregation, there are numerous studies on hypoxia-mediated angiogenesis and breast cancer metastasis. For example, the interaction of HIF-2, desmoglein2 and other factors with a hypoxic environment promotes angiogenesis and the metastatic process of breast cancer (44,45), including the accumulation of CTCs.

In summary, as revealed in Fig. 1, it is evident that CTCs can produce an aggregation effect under the joint action of different signaling pathways and the tumor microenvironment, leading to a significant enhancement of their metastatic potential. However, further research is required to explore effective strategies to trigger the aggregation of CTCs to inhibit the spread of breast cancer metastasis.

3. Comparison of single CTCs and CTC cluster

It is widely recognized that CTCs in breast cancer, similar to other cancers, exhibit significant heterogeneity (46). This heterogeneity can be observed not only among single CTCs but also between single CTCs and CTC clusters, with differences in both cell structure and function (47). Of particular interest to researchers is the higher metastatic potential of CTC clusters and the differences between individual CTCs and CTC clusters, including their respective methylation levels, stem cell characteristics, survival advantages, and immune escape, which are all closely related to their ultimate metastatic potential.

The DNA of stemness and proliferation-associated transcription factors in CTC clusters exhibits hypomethylation.

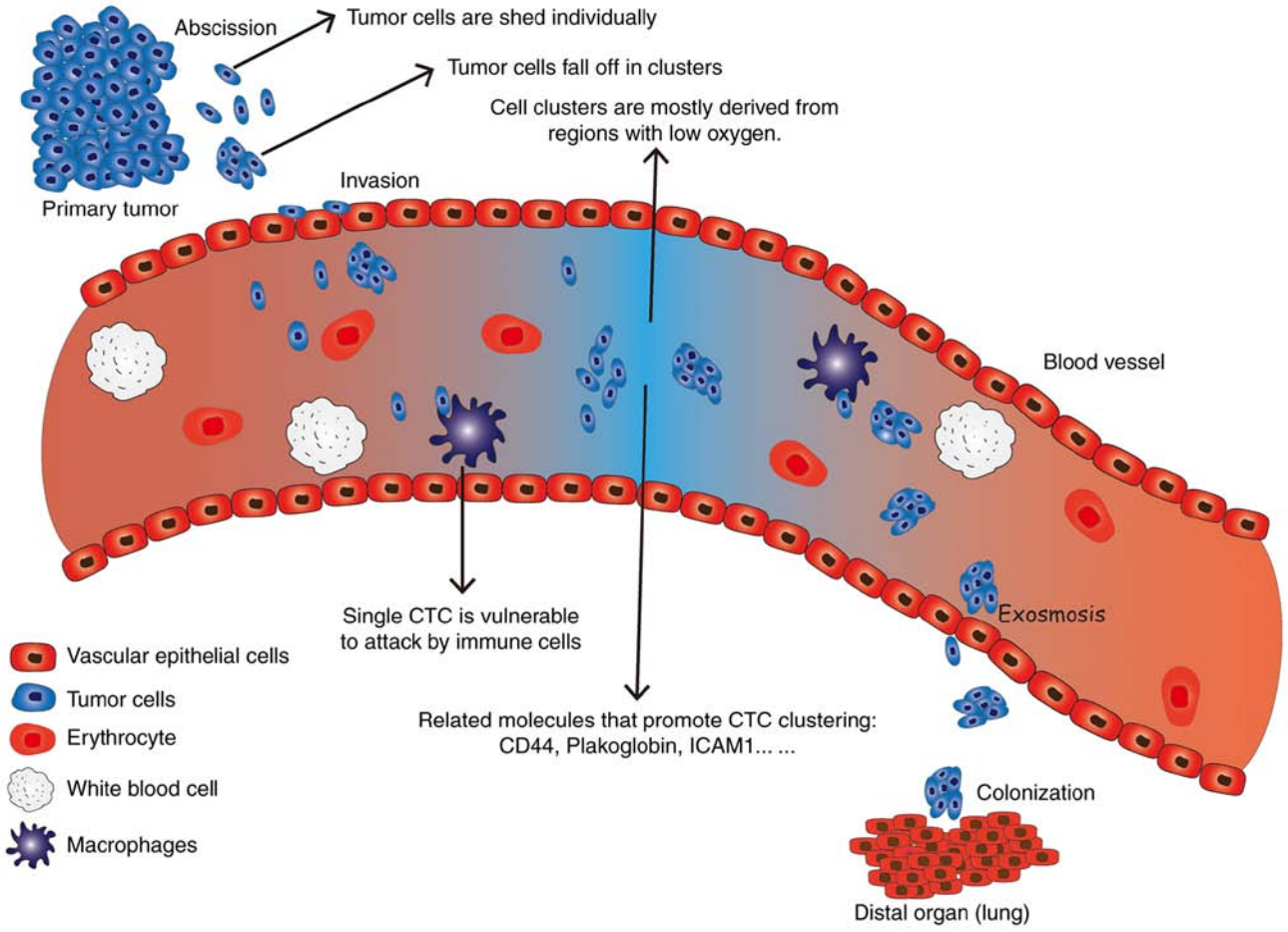

Figure 1. Formation of circulating tumor cell clusters.

DNA methylation is an important epigenetic factor. By isolating CTCs and CTC clusters from the blood of patients with breast cancer and mouse xenograft models, researchers conducted whole-genome sulfite sequencing at single-cell resolution, to analyze genome-wide DNA methylation of individual CTCs and CTC clusters (48). The results revealed that, transcription factors associated with stemness and proliferation, including OCT4, NANOG, SOX2, and SIN3A, were specifically hypomethylated in CTC clusters (48), which is suggestive of a connection between CTC aggregation and DNA methylation that promotes stemness and metastasis. A previous study has revealed that genome-wide hypomethylation is associated with the expression of proto-oncogenes and malignant transformation of tumors (49). In breast cancer, the promoters of certain tumor suppressor genes are hypermethylated. For example, ADAM family member ADAM23, which can participate in neuronal differentiation, is usually downregulated in various types of cancer and is considered to be a cancer suppressor gene. It is observed to express epigenetic silencing by promoting hypermethylation in breast cancer (50). The hypomethylation status of transcription factors associated with stemness and proliferation in CTC clusters increases the expression of proteins related to cell stemness and proliferation, thus, CTC clusters have strong proliferation and differentiation capabilities. This can promote the overall process of metastasis mediated by CTC clusters macroscopically, thereby rendering it a crucial area of research in breast cancer.

Recent research on circulating tumor intercellular proteins have demonstrated that cells in CTC clusters express more adhesion proteins, which contributes to their increased tumorigenicity. Researchers performed RNA sequencing on breast cancer cells with different migration phenotypes isolated from the same parent, characterized the adhesion behavior between cells, and calculated the respective adhesion scores. It was found that cells with weak migration phenotypes exhibited a higher adhesion score, indicating increased expression of E-cadherin. Moreover, cells with a weak migratory phenotype formed more CTC clusters *in vivo* (51), suggesting that CTC clusters express more cell-cell adhesion proteins, consistent with the understanding that the CTC clusters form as multiple CTC adhesion aggregates. Through the suspension culture of MDA-MB-231 breast cancer cells, researchers observed that these cells had a clear tendency to aggregate over time during the culture process, and the levels of fibronectin (FN) and desmosomal proteins also increased in a time-dependent manner during the aggregation process. Furthermore, the increase in the level of FN and desmosomal protein plays a key role in cell aggregation, and the expression of FN and desmosomal protein in CTC after aggregation was also significantly enhanced compared with that of single CTCs (52). The strength of cell-cell adhesion in CTC clusters may endow them with a better chance of survival in the circulation and thus higher tumorigenicity (53). Taken together, the increased expression of adhesion proteins in CTC clusters play a significant role

in their tumorigenicity and ability to survive in circulation. These findings provided valuable insights into the mechanisms underlying CTC cluster formation and into the development of more effective therapeutic strategies to prevent or target CTC clusters in breast cancer.

The acquisition of apoptosis resistance can provide CTC clusters with a stronger survival advantage than single CTCs in the same environment (54). Anoikis is a form of apoptosis that is induced by the loss of interaction between cells and the extracellular matrix, and CTCs that enter the bloodstream from primary cancer tissues must acquire anoikis resistance to survive in the absence of matrix attachment and achieve successful distant metastasis (52). A previous study on lung cancer cell line A549 revealed that it exhibited resistance to anoikis after cell aggregation (55). Similarly, another study demonstrated that breast cancer cells exhibit the same phenomenon (56). *In vitro* experiments showed that the acquisition of this anoikis resistance can promote distant metastasis of cancer (52), and researchers have detected high expression of anti-apoptotic BCL2 protein in CTC clusters, indicating the presence of this resistance (56). Collectively, these findings suggest that maintaining cell-cell adhesion contributes to the formation of cell aggregates, which in turn can endow CTC clusters with anoikis resistance, leading to a stronger survival advantage than single CTCs in the same environment. Therefore, the acquisition of anoikis resistance is a crucial factor in the formation and survival of CTC clusters, and these findings provide valuable insights into the mechanisms underlying the formation and metastasis of CTC clusters in breast cancer. They may also contribute to the development of more effective therapeutic strategies aimed at preventing or targeting CTC clusters.

Recent studies have shown that cells in CTC clusters exhibit more stemness characteristics, which may contribute to their increased metastatic potential (57). Cancer stem cells are considered the primary drivers of metastasis, and CTC aggregation has been shown to confer stem cell properties on cells (58). In a previous study regarding colon cancer, cancer stem cell cluster stemness genes, such as CD133 and Lgr5, were expressed at higher levels in cell clusters than in single cells and contributed to the formation of colon cancer cell clusters (59). Similar findings have been observed in breaster cancer. In zebrafish embryos and mouse models and *in vitro* experiments studying the metastatic ability and molecular mechanism of breast cancer CTCs and CTC clusters, it was revealed that genes associated with the cell cycle and stemness were upregulated in CTC clusters (60). Moreover, aforementioned CD44, a surface glycoprotein on cancer stem cells that promotes CTC aggregation (61), is overexpressed in CTC clusters and is involved in the maintenance of stem cell signals of various tumor cells. CD44 signals can mediate the expression of lipoprotein lipase in breast cancer stem cells promoting tumorigenesis (62). Compared to single CTCs, the aggregation of CTCs may provide cancer stem cells with the protection needed to travel to metastatic sites in order to form distant metastases more efficiently. In summary, the stemness characteristics observed in cells within CTC clusters may contribute to their increased metastatic potential, highlighting the importance of understanding the underlying mechanisms of CTC cluster formation and the role of cancer stem cells in this process.

The clustering of CTCs is a crucial factor in immune escape and contributes to their increased metastatic potential. CTCs exhibit a marked ability to evade the immune system during metastasis, and the immune escape mediated by CTC clusters is one of the reasons they are more likely to form metastases compared with single CTCs (63,64). A previous study revealed that CTC clusters recruit more immunosuppressive cells in order to protect them from being attacked by antitumor immune cells such as natural killer (NK) cells. CTC clusters are less sensitive to NK-mediated immunosuppression than single CTCs, and this is related to the fact that the aggregation of CTCs improves cell adhesion and epithelial gene expression (65). The immune escape of CTCs in the blood circulation and metastatic sites involves multiple mechanisms. For example, highly expressed PD-L1 on the surface of CTCs can mediate the immunosuppressive effect of regulatory T cells (63), and the association between neutrophils and CTCs also promotes cell cycle progression in the bloodstream and increases the metastatic potential of CTCs (66).

As revealed in Table I, CTC clusters are different from single CTCs in various aspects, providing them with a greater survival advantage during metastasis. This increased survival advantage makes CTC clusters more likely to metastasize and become an important target for early diagnosis, prognostic detection indication, and clinical treatment. Overall, understanding the mechanisms underlying CTC cluster formation and immune escape is essential for the development of more effective therapeutic strategies aimed at preventing or targeting CTC clusters in breast cancer.

4. Isolation and enrichment of CTC and the clinical significance for patients with metastatic breast cancer

Various methods have been developed for the isolation of CTCs based on their physical or chemical characteristics that differ from those of other cells. Examples of these methods include microfluidic separation technology based on the mechanism of magnetophoresis (67,68), and size-based microfilters that capture CTCs according to the their size and hardness compared to other cells (69). The choice of separation method depends on various factors, including the type of cancer and the equipment available. For breast cancer, the CellSearch system, is the most widely used clinical method for the monitoring of metastatic breast cancer. This system has been approved by the FDA and automates the processing of CTC counts, thus playing a significant role in monitoring metastatic cancer, predicting progression-free survival and overall survival. The CellSearch system works by counting CTCs that are positive for EpCAM and keratin-positive, and only requires 7.5 ml of peripheral blood for each test. The separation process invovles two parts, which are completed by different instruments. The first step is AutoPrep, which automatically captures CTCs, followed by immunostaining of the captured cells, and then the semi-automated fluorescence microscopy CellTrack Analyzer distinguishes white blood cells from CTCs by immunofluorescence staining of captured cells with anti-keratin and anti-CD45 antibodies. Experiments have revealed that the CellSearch system has a sensitivity of >90% for the detection of EpCAM and keratin-positive cancer cells (70). However, the high purchase price of the equipment

Table I. Comparison of single CTCs and CTC clusters.

Type of CTC	Description	DNA of stemness and proliferation-associated transcription factors	Cell-cell adhesion proteins	Anoikis resistance	Stemness characteristics	Immune escape
CTC clusters	The size of CTC clusters can range from two tumor cells to >100 cells; it exists in clusters	• OCT4, NANOG, SOX2, SIN3A as well as the DNA of other stemness and proliferation-associated transcription factors are specifically hypomethylated	Expression of intercellular adhesion proteins such as E-cadherin, fibronectin and desmosomal protein are significantly increased	Anti-apoptotic protein BCL2 exhibits high expression	• Stem genes such as CD133 and Lgr5 are highly expressed in cell clusters	• CTC clusters are insensitive to NK cell-mediated immunity due to cell adhesion and upregulation of epithelial gene expression
					• The cell cycle and stemness are upregulated in CTC clusters	• The high expression of PD-L1 mediates the immunosuppressive effect of Treg
		• Cancer suppressor gene ADAM23 expresses epigenetic silencing by promoting hypermethylation			• The surface glycoprotein on cancer stem cells CD44 is expressed at a high level in CTC clusters	
Single CTCs	Cancer cells that have shed off from developed tumor tissues and entered the vasculature or lymphatics, and are circulating in peripheral blood vessels	Compared to CTC clusters, the DNA of stemness and proliferation-associated transcription factors is methylated in a single CTC, resulting in epigenetic silencing	Expression of single CTC surface adhesion protein is low, and there is a tendency to increase with cell agglomeration fibronectin and desmosomal proteins during suspension culture	A single CTC lacking anoikis resistance has a higher number of apoptosis cells in the circulatory system, which affects the transfer efficiency	A single CTC lacks protection against cancer stem cells in the circulatory system and is more difficult to form distant metastases	It is difficult for a single CTC to escape the attack of the immune system and the survival rate is low

CTC, circulating tumor cell.

and the high cost of a single test, as well as the fact that the detection of CTCs is a long-term dynamic process, has put great economic pressure on patients. Therefore, numerous medical institutions and patients do not choose to isolate CTCs using this method.

The detection of CTCs has become an increasingly important tool in clinical practice for *in vitro* non-invasive early diagnosis, individualized treatment, and prognostic prediction. However, the clinical application of CTCs faces numerous challenges, including the cost and efficiency of isolating CTCs and the difficulty in standardizing the identification of CTCs, which limits their clinical utility (71). As previosuly reported (72) numerous clinical trials and studies have demonstrated that the presence of CTCs is significantly associated with poor prognosis in patients with various cancers. The clinical monitoring of the number and status of CTCs is therefore of great significance in formulating treatment strategies and predicting the prognosis of patients with metastatic breast

cancer. In-depth studies of CTCs and their clinical applications will help guide the development of new diagnostic and therapeutic strategies, inhibit cancer metastasis, and ultimately benefit patients. Despite the current limitations, the close association between CTCs and clinical practice provides a promising avenue for improving the diagnosis and treatment of breast cancer.

Current treatment strategies for breast cancer mainly target the primary tumor tissue and have limited effect on the initial cells in the initial stage of metastasis (73), which increase the likelihood of metastasis. Thus, developing treatment strategies that target CTCs has become a new direction for breast cancer treatment, and there are increasing clinical applications. Inhibiting the metastatic ability of CTCs by targeting them with drugs can be an effective therapeutic approach for patients with breast cancer with a high risk of metastasis. For patients with early-stage breast cancer, cluster-targeted therapy is currently being developed to identify CTC clusters in patient groups. It helps identify patients with high risk of metastasis and initiates anti-cluster treatment early, which can reduce the ability of cancer cells to metastasize and prevent the occurrence of metastasis (74). For patients with HER2-positive metastatic breast cancer, targeted HER2 therapy can significantly decrease the total number of CTCs in patients (75). Furthermore, the status of CTCs can predict the benefit of radiotherapy on overall survival in patients with early breast cancer and detectable CTCs (76). CTC count can be a reliable biomarker method (77) to guide treatment selection between single-agent endocrine therapy and chemotherapy in patients with hormone receptor-positive, ERBB2-negative metastatic breast cancer (78). The combined monitoring of circulating tumor DNA and CTCs after surgery can also help with the prediction of the risk of disease recurrence (79). Overall, the development of treatment strategies that target CTCs offers a promising approach to breast cancer treatment, and the combined monitoring of CTCs and other biomarkers has the potential to improve prognosis and treatment selection for patients with breast cancer.

The presence of CTCs is strongly associated with poor prognosis of patients with breast cancer (80). CTCs serve as a non-negligible prognostic biomarker in metastatic breast cancer (81). CTC counts based on the CellSearch detection platform (70) have been revealed to be an independent predictor of survival in patients with metastatic breast cancer (82). In HER2-negative metastatic breast cancer, epithelial-mesenchymal transition marker-based CTC detection can identify disease progression and treatment resistance in advance, and combined criteria considering both CTC count and the proportion of CTCs expressing mesenchymal markers are significantly associated with prognosis (83). A longitudinal assessment of CTCs and CTC clusters in patients with metastatic breast cancer showed an increase in their prognostic value over time (84). The status of CTCs changes dynamically during treatment of metastatic breast cancer, which affects prognostic judgements. Therefore, the timing of CTC detection must be well grasped when using CTCs to assess prognosis. A previous study revealed that the CTC counts and status in the later stages of treatment were more reliable for predicting the prognosis status of patients (85). In addition to metastatic breast cancer, the detection rate of CTC is 39% in non-metastatic inflammatory breast cancer, which has strong independent prognostic value (86).

5. Summary and prospects

CTCs are shed from primary tumors or metastases and enter the bloodstream, playing a crucial role in the understanding of breast cancer and other cancer metastasis mechanisms. Investigating CTCs is of great significance due to their ability to initiate tumor metastasis in distant organs. Understanding the molecular mechanisms through which CTCs promote tumor metastasis is an important area of research. CTCs have been identified to form clusters, which exhibit a higher metastatic potential than single CTCs. This enhanced potential can be attributed to differences in the expression of intercellular adhesion proteins, levels of DNA methylation, anti-apoptotic properties, and immune escape mechanisms. Consequently, targeting CTC clusters has emerged as a novel approach for intervening in breast cancer metastasis.

Targeting CTC clusters offers a promising strategy for reducing the risk of breast cancer metastasis, whether by disrupting their collective shedding or dissociating them in the circulation. The distinctive characteristics exhibited by CTC clusters, including altered intercellular adhesion protein expression, DNA methylation patterns, anti-apoptotic properties, and immune escape mechanisms, confer a survival advantage and ultimately contribute to their heightened metastatic potential.

Currently, the clinical application of CTCs primarily revolves around predicting treatment efficacy and prognosis based on CTC enumeration and characterization. Combined assessment of CTCs with other biomarkers enables effective monitoring of disease progression and prognosis prediction. However, the high cost associated with CTC isolation and monitoring limits their widespread clinical implementation. Future advancements in CTC detection technology should focus on achieving higher enrichment efficiency and standardization to meet the clinical demands. Continued progress in the clinical application of CTCs holds the potential to significantly benefit patients with breast cancer.

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Competing interests

The authors declare that they have no competing interests.

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