Pathology & Oncology Research | frontiers

TYPE Review
PUBLISHED 20 October 2025
DOI 10.3389/pore.2025.1612181

OPEN ACCESS

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RECEIVED 19 May 2025
ACCEPTED 25 September 2025
PUBLISHED 20 October 2025

CITATION
Linkner TR, Nagy ZB, Kalmár A, Farkas E, Bányai F, Szakállas N, Takács I and Molnár B (2025) Circulating tumor cells: indicators of cancer progression, plasticity and utility for therapies. *Pathol. Oncol. Res.* 31:1612181. doi: 10.3389/pore.2025.1612181

Circulating tumor cells: indicators of cancer progression, plasticity and utility for therapies

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Cancer is a deadly disease affecting millions of people worldwide. Circulating tumor cells (CTCs) represent a critical link between primary malignancies and metastasis, acting as key players in cancer dissemination, progression, and recurrence. Although rare, CTCs offer a valuable, non-invasive window into tumor biology and the evolution of disease in patients. CTCs can exist as single cells in the circulation, but some are shed and travel in larger groups, referred to as CTC clusters. These clusters possess a greater oncogenic potential compared to individual CTCs. In this review, we aim to provide insight into the dynamic biological processes underlying CTC generation, biology, and survival, with a focus on epithelial-to-mesenchymal transition (EMT) and beyond like cancer stem cells (CSCs), cellular plasticity, and senescence. A crucial aspect of CTC biology is EMT, a process that imparts cancer cells with increased motility, invasiveness, resistance to apoptosis, and the ability to intravasate and evade the immune system. Beyond EMT the cancer cells show further plasticity, allowing epithelial tumor cells to adopt mesenchymal or hybrid phenotypes, which enables adaptation to a changing microenvironment and enhances therapy resistance. Moreover, a subset of cancer cells can acquire stem cell-like properties, including self-renewal and tumor-initiating capacity. EMT, along with processes such as dedifferentiation, contributes to the generation of cancer stem cells. In recent years, studies have also highlighted the complex and paradoxical role of senescence in CTC biology. While senescence typically results in permanent cell cycle arrest, in cancer cells it may be reversible and can promote tumor cell dormancy, immune evasion, and metastatic reactivation. By exploring the connections between CTCs, EMT, CSCs, plasticity, and senescence, we aim to shed light on the unique biology of CTCs, their metastatic potential, and their contributions to tumor heterogeneity. We hope that a better understanding of these processes will help advance the development of novel biomarkers and therapeutic targets for solid tumors beyond EMT.

KEYWORDS

liquid biopsy, circulating tumor cells, cancer, EMT, senescence

Introduction

The aim of this review is to summarize recent advances regarding circulating tumor cells (CTCs), with a focus on their phenotype and plasticity. Moreover, we aim to shed light on the diagnostic properties of circulating tumor cells and cancer stem cells.

Cancer is one of the deadliest diseases affecting the human population, causing millions of deaths each year. In 2022 alone, there were 20 million newly reported cancer cases worldwide, with 9.7 million deaths. Breast carcinoma is the most common type of cancer in women (2.3 million new cases each year), while lung (1.5 million new cases each year) and prostate (1.4 million new cases each year) cancers are the most prevalent malignancies in men. Among both sexes, lung cancer is the most frequently diagnosed carcinoma with 2.5 million new cases each year [1–3].

Cancer is a disease caused by multiple mutations in a cell, leading to an altered cellular state. It is characterized by abnormal growth, spread, resource consumption, tissue disruption, and impairment of normal bodily functions. Environmental factors, viruses, bacteria, chemical agents, or radiation exposure can all contribute to cancer development [4, 5].

To fight an effective battle against cancer, understanding the disease, its progression, and developing new progression targeting therapeutic techniques is of utmost importance.

Our workgroup has previously conducted examinations of circulating tumor cells (CTCs) and CTC clusters. Using magnetic cell separation, we successfully detected cytokeratin (CK)-positive CTCs and CTC clusters in the blood of colorectal cancer patients. Additionally, our workgroup found cytokeratin positive cells in interaction with cytokeratin negative cells when investigating CTC clusters. This was the first time this was observed in colorectal carcinoma (CRC) patients. Moreover, we also observed that chemotherapy reduces the number of CTCs and clusters in the blood but does not eliminate them [6]. In another of our studies, we found that the higher the number of single CTCs in the circulation, the higher the number of epithelial cells in CTC clusters [5]. In the same study, we concluded that the number of CTC singlets, doublets, and clusters correlates with cytokeratin 20 (CK20) qPCR results from the blood of CRC patients [7]. Moreover, we have performed several liquid biopsy-based analyses on the blood of colorectal cancer patients to investigate the potential diagnostic and therapeutic implications of cell-free nucleic acids. We found that the level of cfDNA was higher in patients with non-metastatic CRC and metastatic CRC compared to individuals with remission or stable disease [8, 9].

In this review, we aim to gather the most recent information on CTCs. Furthermore, we seek to explore their unique plasticity and highlight the significance of CK + epithelial CTC clusters in circulation. Additionally, we provide an overview of the most up-to-date techniques for CTC detection, analysis, and their relation to therapy decisions.

CTC biology and diagnostic utilization

The most lethal feature of cancer is metastasis—a process involving the invasion of distant parts of the body by cancer cells that "break away" from the primary tumor and enter the circulation. These cells are referred to as circulating tumor cells (CTCs). CTCs can travel through the bloodstream either as single cells or in clusters. CTC clusters are defined as groups of two or more CTCs with stable cell–cell junctions. Although clusters represent only a minority of CTCs found in circulation, they possess a higher metastatic potential than single CTCs. Moreover, in several cancer types, the presence of CTC clusters indicates a worse clinical outcome compared to single CTCs [10, 11]. It has been shown that in non-small cell lung cancer (NSCLC), the prevalence of CTC clusters increases with advanced cancer stages. However, no correlation was observed between the number of CTC clusters and the tumor type or stage in lung cancer indicating that cluster number may not distinguish between the most advanced disease stages. However, correlation between CTC number and prognosis was found in a meta-analysis which considered the presence of CTC but not their number or phenotype characterization [12–14].

Other than the blood stream, CTCs can also enter into the lymphatic circulation, where they can reach local lymph nodes and differentiate leading to metastases. Lymph-specific CTCs are usually non-immunogenic so they can avoid detection by the immune system, especially by cytotoxic T cells which helps them in their metastasis initiation [15–17].

Additionally, CTCs are also capable of perineural invasion (PNI), which is defined as an invasion in, around, and through the nerves. PNI is usually associated with poor clinical outcome and decreased survival in different cancer types including ductal adenocarcinoma, prostate cancer, gastric cancer, breast cancer, pancreatic ductal adenocarcinoma and colorectal carcinoma [18–20].

Since these cells are shed into the circulation, peripheral blood serves as an excellent source for the selection and analysis of CTCs. Over the past decade, multiple liquid biopsy techniques have been developed for CTC isolation and analysis. These methods can be categorized as either label-dependent or label-independent techniques. Label-dependent techniques rely on interactions between cell surface markers expressed on CTCs and specific antibodies. These antibodies can be fixed to the surface of magnetic particles or microfluidic chips to enable positive selection of CTCs from blood or negative depletion of white blood cells. These approaches typically target EpCAM, a surface protein commonly expressed on CTCs (Table 1).

TABLE 1 Main differences between single CTCs, CTC clusters and cfDNA.

Attributes	Single CTC	CTC cluster	Cell free DNA
Composition	Single cancer cells	Multiple cancer cells, often in conjugation with stromal and/or immune cells	Short DNA fragments from necrotic/apoptotic tumor cells
Survival in circulation	Low	High	Low
EMT Traits	Mainly mesenchymal	Mainly epithelial	None
Metastatic potential	Low	High	None
Prognostic value	Moderate	High. Associated with poor prognosis	High. Early cancer detection
Markers	EpCAM, Vimentin, N-cadherin	CD44, OCT4, SOX2	Mutations specific for the originating tumor (EGFR, KRAS1, BRCA1/2)

Amongst these techniques, currently the CellSearch system by Janssen Diagnostics is the only FDA approved method which utilizes EpCAM-coated ferrofluidic nanoparticles for CTC detection. Other commercially available label dependent methods are AdnaTest by Adnagen and MagSweeper by Illumina both of which are based on immunomagnetic capture of CTCs [21–23].

Label-independent detection methods, on the other hand, are based on the physical properties of CTCs, such as size. Using filters with defined pore sizes, the typically larger CTCs can be separated from smaller blood cells. Gradient centrifugation can also be employed, where lower-density cells such as erythrocytes and polymorphonuclear leukocytes settle at the bottom, while higher-density mononuclear leukocytes and CTCs remain in the upper layers. Overall, methods based on physical properties are cost-effective and preserve cell viability well. However, these techniques are often inefficient, yield low purity, and lack specificity. ISET by Rarecells diagnostics and Parylene filter by Circulogix are both based on filter based isolation and enrichment platforms available for label-free detection. Other techniques are also on the market such as RosetteSep by STEMCELL technologies and OncoQuick by Greiner BioOne which are based on density gradient separation [21–23].

CTCs carry information about the originating tumor, making them highly valuable for clinical applications. CTC analysis can be used for early tumor detection, enabling treatment at an earlier, more manageable stage. Usually, the number of CTCs in early disease are low roughly ~$1/10^8$ peripheral blood mononuclear cells (PBMC), while in metastatic cancers their number is much higher at $1/10^5$–10^7 PBMCs. Moreover, the presence of CTCs in the circulation provides prognostic information, aids in predicting disease outcomes, and helps guide treatment decisions. Furthermore, molecular characterization and genome sequencing of CTCs can provide valuable insights for the development of personalized treatments [23–26].

As a few examples, Baek et al. used the fluid-assisted separation technique (FAST) to enrich CTCs from the blood of healthy donors and CRC patients. They found that CTC counts were significantly higher in CRC patients compared to healthy volunteers. Notably, all patients with stage 4 CRC were positive for CTCs [27]. Dalum et al. utilized the CellSearch system to analyze the blood of CRC patients before surgery, and reported that the presence of CTCs prior to the operation was associated with a significant decrease in recurrence-free survival [28].

Cristofanilli and colleagues, in their study of metastatic breast cancer patients, reported that the presence of more than 5 CTCs per 7.5 mL of blood was associated with shorter median progression-free survival and overall survival compared to patients with fewer than 5 CTCs [29]. According to a metaanalysis by Jin et al., the detection of CTCs in circulation is associated with poor prognosis in small cell lung cancer (SCLC) patients compared to those with non-small cell lung cancer. Moreover, they found that epithelial CTCs predict worse outcomes than mesenchymal CTCs in lung cancer patients [30].

CTC clusters

CTCs can travel as single cells in the circulation; however, CTC clusters also exist, consisting of two or more CTCs attached together. These clusters can be homotypic, involving only CTCs, or heterotypic, when blood immune cells are also attached to CTCs [31]. Immune cells, such as neutrophils, can enhance the metastatic potential and survival of these clusters (Figure 1) [32].

Moreover, cancer associated fibroblast (CAF) which is a heterogenous network of cells originating from normal fibroblasts and cells like mesenchymal stem cells or endothelial cells can be found in heterotropic CTC clusters and they can increase the metastatic potential of the CTCs [33].

Compared to single CTCs, the larger size of clusters likely enhances their ability to adhere to the endothelium and promotes extravasation. Additionally, CTC clusters have been found to show increased expression of EMT/stemness markers such as CD44, OCT4, SOX2, Nanog, and SIM3A. They also exhibit

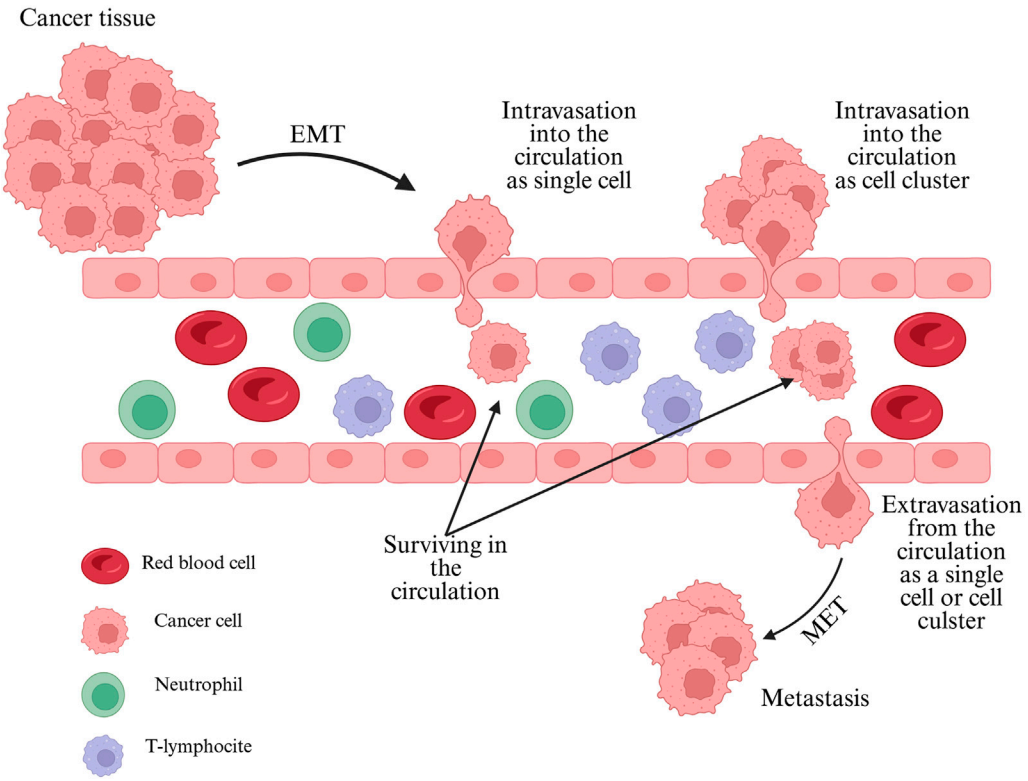

FIGURE 1
Main route of CTCs from the tumor to the secondary sites. Created in BioRender. Linkner, T. (2025) https://BioRender.com/jny6bvi.

elevated expression of cell junction proteins like plakoglobin and E-cadherin. Furthermore, the expression of markers that contribute to CTC aggregation, including KRT14, PAK2, and MUC1, is also upregulated (Table 1) [34].

It has been documented that the presence of circulating CTC clusters is associated with worse prognosis in various types of cancer. Additionally, CTC clusters may be protected from shear forces, anoikis, and immune surveillance while in circulation. Moreover, the metastatic potential of CTC clusters is significantly higher than that of single CTCs [35]. The main differences between CTCs, CTC clusters, and cell-free DNA are shown in Table 1.

CTC heterogeneity

CTC heterogeneity can be divided into morphological and phenotypic heterogeneity of epithelial and mesenchymal cells in addition to tissue tumor heterogeneity which describes the genetic and somatic diversity within the primary tumor or between primary tumor and metastasises. Morphological heterogeneity refers to the different sizes and shapes that CTCs can take. This categorization also includes CTC clusters. In contrast, phenotypic heterogeneity refers to differences in gene expression patterns and cell surface markers [10, 36, 37].

The ability of CTCs to change their phenotype in response to environmental changes is referred to as CTC plasticity. One of the main expressions of CTC plasticity is a process called EMT [38]. This is the primary mechanism by which CTCs are formed. During EMT, epithelial tumor cells lose their adhesive ability and epithelial characteristics and acquire a mesenchymal phenotype, which results in mobile, highly metastatic CTCs. If they survive long enough in the circulation in the end they reach a distant organ, where CTCs undergo a reverse process known as mesenchymal-to-epithelial transition (MET) [23]. The ability of CTCs to transition back and forth between these cell states is referred to as EMT plasticity [39].

Epithelial to mesenchymal transition

EMT is a complex process involving many molecular and cellular changes, such as the downregulation of epithelial markers (e.g., cytokeratins, E-cadherin, and claudins) and the upregulation of mesenchymal proteins (e.g., vimentin, N-cadherin, and fibronectin), which increase the mobility and invasiveness of the cell. The changes observed during EMT are

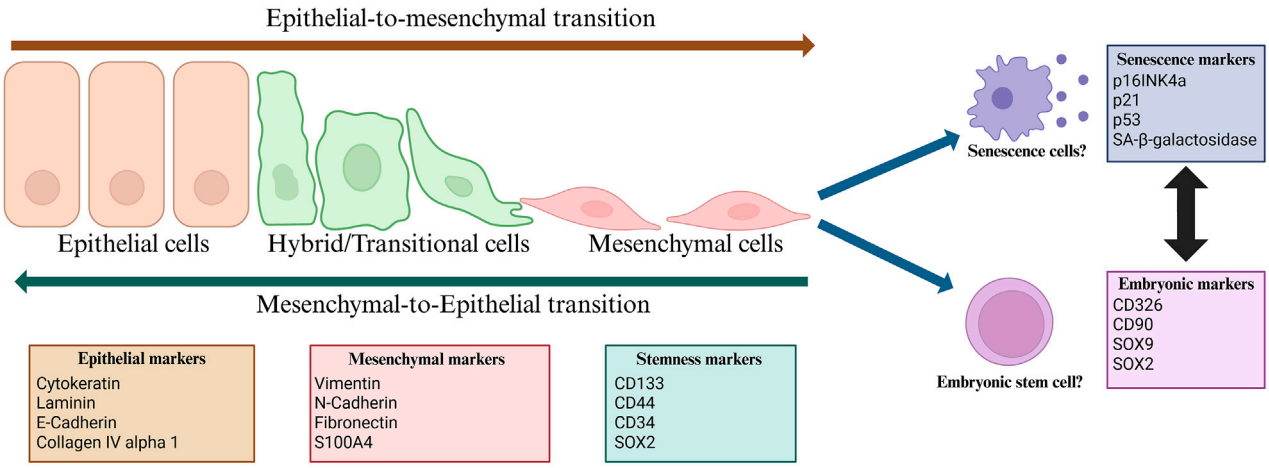

FIGURE 2
Basic overview of the process of EMT with basic markers and the possible stages after the mesenchymal state. Created in BioRender. Linkner, T. (2025) https://BioRender.com/tp6wqbm.

regulated by transcription factors known as EMT-inducing transcription factors (EMT-TFs), such as Snail-1, Snail-2 (Slug), ZEB1, and Twist (Figure 2) [40]. It is widely accepted that the process of EMT generates multiple hybrid phenotypes along the epithelial-mesenchymal axis, contributing to tumor heterogeneity. Both epithelial and mesenchymal states are believed to harbor limited metastatic potential; however, certain hybrid phenotypes can possess a higher degree of EMT plasticity, enabling them to survive and adapt to different microenvironments encountered during metastatic spread [40].

The process leading to metastasis is complex, involving several biological steps. First, metastatic cells must undergo EMT, detach from the primary tumor, invade the bloodstream, survive in circulation, disseminate into distant organs, extravasate, undergo MET, colonize, and form micrometastasis. Only a fraction of CTCs are capable of undergoing metastatic transformation; these cells are referred to in the literature as circulating cancer stem cells [41].

Balcik-Ercin et al. found in their colorectal carcinoma-derived CTC cell line, that the expression of SIX1, an EMT marker important for the mesenchymal profile, was downregulated. This suggests that tumor cells can utilize alternative pathways to activate genes that promote their plasticity and invasiveness. Furthermore, they found that the MET transcription factor GRHL2 was overexpressed in their CTC lines. GRHL2 may stabilize the epithelial-mesenchymal hybrid phenotype and support cell migration [38].

Seo et al. investigated the phenotypic heterogeneity of CTCs in SCLC using assays to characterize rare cells. In an EpCAM-targeted assay, utilizing a variety of biomarkers, they observed a wide range of CK and EpCAM expression in the CTC population. Their single-cell sequencing results reinforce the presence of tumor cell plasticity by indicating that a phenotypically heterogeneous population of cells can be genomically stable. Recent evidence suggests that cancer cells exhibit a hybrid mesenchymal and epithelial character, and this plasticity is associated with their metastatic ability and poor patient prognosis [42].

It has been described that the activation of the EMT program does not always result in a fully mesenchymal phenotype. It is likely that a partial EMT status is achieved in both non-transformed and cancer cells, the resulting hybrid cells carry both epithelial and mesenchymal markers. Moreover, these hybrid cells are more likely to acquire stemness [33]. Indeed, it has been shown that with EMT induction, breast cancer cells can acquire cancer stem cell markers, such as CD44 [43, 44].

The fact that stemness markers can be expressed by cells undergoing EMT opens the possibility that differentiated mesenchymal cells can also acquire stemness characteristics, leading to the formation of new mesenchymal stem cells.

Cancer stem cells

There are multiple therapies that can be implemented to treat cancer, such as radiotherapy, surgery, and chemotherapy. However, cancer cells can develop resistance to chemotherapy, which is a major factor in therapy failure and poor patient survival [45, 46].

Due to the stress generated by the changing environment and therapy, genetic mutations occur in cancer cells, leading to cancer heterogeneity and, in turn, therapy resistance. Heterogeneity among patients due to environmental, somatic, and germline

factors is called intertumoral, while uneven distributions of genetically diverse subpopulations of cancer cells in the same tumor are referred as intratumoral heterogeneity. Moreover, the differences between a primary tumor and the metastasis in a patient are also called intertumoral heterogeneity [47–49].

One of the factors contributing to intratumoral heterogeneity is the presence of cancer stem cells (CSCs), a subset of cancer cells possessing stem cell characteristics such as self-renewal and the ability to differentiate [50, 51]. CSCs were first identified in acute myeloid leukemia (AML) after transplanting isolated CD34+/CD38− cancer cells into non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. Since then, CSCs have been described in a variety of hematological and solid tumors, such as pancreatic, breast, and colon malignancies [52]. The origin of CSCs is highly debated, with multiple hypotheses suggesting that they arise from either adult stem cells, mutated adult progenitor cells, or cancer cells that gain stem-like properties through dedifferentiation. CSCs can be separated from normal stem cells via the expression of specific cell surface markers such as CD133, CD24, CD44, epithelial cell adhesion molecule (EpCAM), and CD200. Moreover, intracellular proteins have also been used as markers of CSCs, such as aldehyde dehydrogenase 1 (ALDH1) (Figure 2) [53–55].

There's a connection between the previously mentioned EMT and cancer stemness. The expression of EMT inducing transcription factors such as ZEB1, SNAIL1 and 2 by cancer cells initiates the expression of stem cell markers SOX2, BMI1 and OCT4. It is described that mesenchymal and stemness traits, characterise cancer stem cells within the tumor mass. This indicates that CSCs have specific abilities similar to embryonic stem cells [56].

Cancer senescence

Chemo- and radiotherapy induce DNA damage in differentiated cancer cells, which in turn leads to therapy-induced senescence (TIS). Senescence is a cell state characterized by prolonged cell-cycle arrest, enhanced secretory capacity, macromolecular damage, and altered metabolism. The main defining characteristic of senescence is stable growth arrest, which ensures that damaged or transformed cells do not preserve and perpetuate their genomes. During this process, specific molecular markers are activated, such as p16INK4a/Rb and p53/p21CIP1. Senescence also has physiological roles; the process is triggered in response to damage and allows the suppression of potentially dysfunctional, transformed, or aged cells. However, the aberrant accumulation of senescent cells during aging has potential detrimental effects, such as contributing to renal dysfunction and type II diabetes (Figure 2) [57].

The senescent state of cancer cells can be beneficial as these cells induce inflammation and attract immune cells, which clear the senescent cancer cells. One of the main characteristics of senescent cells is the senescence-associated secretory phenotype (SASP). They secrete interleukins and other ligands that can negatively affect cancer initiation and progression.

However, the previously mentioned TIS also induces cancer remodeling and promotes CSC generation. Moreover, senescent tumor cells can cause changes in the tumor microenvironment, further promoting cancer development. SASP can also provide a positive environment for tumor progression. It was shown that SASP components can promote cancer cell growth, invasion, metastasis, and tumor vascularization [54, 58, 59].

Cancer cells can escape the senescent state through the acquisition of genetic and epigenetic features, which make them plastic. Additionally, via the paracrine action of SASP, cells in close proximity to tumor cells can be imparted with tumorigenic capacities. Senescence escape and cellular reprogramming via SASP are essential components of epithelial tumor progression. Tumor cells achieve the previously mentioned plasticity through the initiation of EMT [60].

Polyploid senescence cells

Polyploid cells are large, multicellular entities formed by cell fusion and/or endoreduplication [61]. In the case of cancer, polyploid giant cancer cells arise due to genotoxic stress caused by chemo and/or radiotherapy. They mostly exhibit features of senescence, and they also give rise to aneuploid or diploid daughter cells, which can undergo mitosis. This might be responsible for the heterogeneous nature of cancer cells. Additionally, they can secrete an array of cytokines, chemokines, and growth factors which influences the tumor microenvironment and contributes to poor prognosis like therapy resistance [62, 63]. Polyploid tumor cells are able to differentiate into different types of cells, including adipose tissue or bone, which indicates that these cells possess cancer stem cell properties [64]. Like senescence, polyploidy can develop in response to therapy. The connection between senescence, polyploidy, and therapy has been observed in multiple cancer types. Cancer cells, when exposed to DNA-damaging agents, develop polyploidy upon entering senescence. Senescent polyploid cells are involved in the generation of proliferating progeny cells. This likely occurs through depolyploidization, during which mononucleated daughter cells are created from the multinucleated tumor, either by budding or asymmetric cell division. Depolyploidization can be a way for cancer cell to escape senescence [65].

Circulating tumor cells and circulating DNA an overlap is to find

Liquid biopsy-based monitoring of cancer is a promising, non-invasive method which usually involves blood or urine

TABLE 2 Examples of biomarkers which can be detected with ctDNA analysis and their clinical utility and prognostic relevance with the most common mutations in associated cancer.

Gene	Associated cancer	Mutations in associated cancer	Prognostic/therapeutic relevance in associated cancer	Clinical utility in associated cancer	Source
TP53	Ovarian, head and neck, breast	R175H, R248Q	Poor prognosis	Prognosis and therapy prediction	[77–82]
EGFR	Lung, colon	L858R, T790M, C797S	Therapy prediction monitoring	Therapy selection, disease monitoring	[83–89]
KRAS	Pancreatic, lung, colon	G12D, G12V, G12C	Response to inhibitors	Disease and treatment monitoring	[90–95]
BRAF V600E	Melanoma, colon	V600E, V600K	Response to inhibitors	Treatment monitoring, survival prediction	[96–99]
PIK3CA	Breast, colon, endometrial	H1047R, E545K	Poor prognosis	Survival prediction	[100–106]
SEPT9	Colon	Methylation in the promoter region	Poor prognosis	Early diagnosis, survival prediction	[75, 107–109]
BRCA1/2	Ovarian, breast	Frameshift, splicing mutations	Response to inhibitors	Survival prediction	[110–112]
HER2	Breast	S310F, L755S	Poor prognosis	Treatment and relapse monitoring	[113–116]
CTNNB1	Liver	S45F, D32Y	Prognostic indicator	Treatment and tumor dynamics monitoring	[117–120]

collection, followed by the analysis of extracellular vesicles, circulating tumor cells (CTCs), or circulating tumor DNA (ctDNA) [66].

ctDNA is a form of nucleic acid released mainly from apoptotic or necrotic tumor cells into the circulation. In the peripheral blood, ctDNA circulates in the form of nucleosomes, which can be isolated, and their genetic and epigenetic properties can be analyzed to provide information about the originating tumor (Table 1) [67].

A few examples are listed below for the utilization and shortcomings of CTCs and ctDNA in the diagnosis of different epithelial cancers. Both CTCs and ctDNA can be used in the early detection of colorectal cancer and can be used in prognosis and treatment response monitoring, as well [68]. However, the level of CTCs is usually low in CRC patients, especially in the early phase of the disease. On the other hand, ctDNA can be detected more easily and provide real-time molecular information to monitor treatment response and relapse [69].

In early stage breast cancer, CTCs are present in low numbers and difficult to analyze, while ctDNA is more readily detectable and useful for monitoring tumor response, drug resistance, and mutations. In metastatic breast cancer, ctDNA efficiently tracks treatment response and tumor heterogeneity, whereas elevated CTC levels serve as prognostic markers [70].

CTCs are more common in small cell lung cancer (SCLC) than in non-small cell lung cancer (NSCLC) [71]. Despite this fact, in NSCLC, CTCs provide more informative mutation detection than ctDNA because of more sensitive genotyping [72]. However, as mentioned before CTC counts are highest in SCLC due to rapid tumor growth and early spread, making them better prognostic markers than ctDNA in this subtype [73]. In NSCLC, both CTCs and ctDNA can serve as diagnostic, prognostic, and therapeutic monitoring tools [74].

Our workgroup previously carried out experiments where with high sensitivity we detected the septin 9 gene (*SEPT9*) from circulation which is an excellent marker of CRC [75]. Moreover in a separate study we also detected that compared to healthy tissue, SEPT9 is hypermethylated in adenoma and CRC cells. Our results indicated that changes in the SEPT9 methylation reflects the cellular progression towards malignancy in the colon mucosa [76]. A list of biomarkers which can be detected with ctDNA analysis are shown on Table 2 with relevant mutations and associated cancers.

In a study, Kong et al. found mutations in CTCs and ctDNA that matched those in the primary tumor. They also discovered that the top mutated genes in CTCs and ctDNA had prognostic value when applied to existing cohorts of cancer [121].

Koyanagi et al. also found in their research that, in stage IV melanoma patients, the number of CTCs correlated with the methylation of ctDNA molecules [122]. Additionally, in the peripheral blood of breast cancer patients, the level of ctDNA correlated with the presence of CTCs. This potentially suggests that CTCs are a major source of ctDNA, or that high numbers of CTCs and ctDNA are both features of a more aggressive tumor [123]. This correlation between ctDNA and CTCs was also

observed in another study. Furthermore, methylated ctDNA and CTCs correlated with aggressive tumor biology and advanced disease [124].

Therapy of minimal residual disease (MRD), cancer relapse based on circulating tumor cells

MRD is defined as a small number of cancer cells that remain in the body after treatment and can cause disease relapse [66].

For the tumor to detoriate, many pathophysiological cascades are required, such as the loss of cellular adhesion, increased cancer motility, invasiveness, entry into and survival in the circulation, and extravasation into the surrounding tissue. Circulating tumor cells (CTCs) represent an important phase in these processes [125, 126].

Liquid biopsy-based methods are non-invasive and provide an accurate method for monitoring the stage of the tumor. Before surgery, CTCs are much more informative about the tumor and correlates with disease stage compared to ctDNA [69, 126, 127]. However, ctDNA is much better at monitoring therapy and relapse as it is an accurate real-time biomarker of solid tumors and also a method to analyze MRD [69, 128]. Additionally ctDNA detection in the circulation of postoperative patients has a 100% possibility of predicting tumor relapse [129]. Furthermore, in a study, Radovich and colleagues found that the presence of ctDNA and CTCs after neoadjuvant chemotherapy correlates with cancer relapse in triple-negative breast cancer. A part of the observed patient group were positive for one marker, such that the sensitivity for recurrence detection went from 79% with ctDNA alone and 62% with CTC alone to 90% when combined [130].

Moreover, CTC detection and analysis also provide information about MRD and late-stage recurrence. In colorectal cancer (CRC) patients, CTC positivity before surgery significantly reduces overall survival (OS) and progression-free survival (PFS) compared to CTC-negative patients. Additionally, CTCs can be used as independent prognostic indicators of PFS and OS in advanced CRC. Furthermore, there are differences between the subtypes of CTCs. Mesenchymal-type CTCs are predominantly found in patients with metastatic CRC [130].

In the last few years, immune checkpoint therapies gained huge attention in the treatment of cancer. These methods are based on the inhibition of immune cell inactivating signals generated by cancer cells through cell surface molecules like PD-1 or CTLA-4 [131]. Most of the CTCs are eliminated by the immune system, however a subset of cells can evade the immune surveillance through various ways. One of the escape mechanisms are based on plasticity. For example, through epithelial-to-mesenchymal transition, cancer cells can increase PD-L1 expression on their surface, induce regulatory T cells, or inhibit dendritic cell functions, all of which helps them evade the immune system. In the light of this information, targeting tumor cell plasticity can sensitize cancer cells to immune-mediated cell death [132, 133].

Adaptive cancer therapy based on cancer cell plasticity

Tumor cell plasticity is a non-mutational process that contributes to drug resistance. Plasticity includes the reactivation of developmental programs such as epithelial-to-mesenchymal transition (EMT), acquisition of cancer stem cell properties, and trans-differentiation [134]. Plasticity provides the tumor with the ability to shift between different states, from low tumorigenic potential to an undifferentiated cancer stem cell-like state [135]. Alterations in the cancer state are caused by changes in the tumor microenvironment, genetic or epigenetic changes, or selective pressure from treatment. There is also evidence suggesting that cancer cells have intrinsic plasticity, which helps the tumor adapt to the changing microenvironment. This flexibility in the cell state may contribute to therapy resistance [136]. It was described that CTCs with stem or mesenchymal characteristics are more aggressive and less susceptible to chemotherapy in case of breast cancer [137]. EMT which is associated with the increased invasiveness of tumor cells are also involved in the generation of resistance mechanisms. Inhibition of EMT was shown to reduce chemotherapy resistance [138]. Moreover, it was described that inhibition of EMT transcription factors can reduce cancer stem cells [139]. In case of senescence, it was observed that transcription factors which promote EMT can reduce senescence in cancer cells. However the mechanisms behind this process are not yet fully understood [140, 141].

Additionally, numerous factors are involved in the cancer plasticity-mediated therapy resistance, such as transcription factors like SOX2 or ZEB1 [142, 143]. Epigenetic modifications, such as DNA methylation, are also significant factors in therapy resistance [144]. Indeed, Caamano et al found that methylation in CTCs were associated with changes in gene expression which contributes to CTC therapy resistance [145].

Strategies to combat plasticity-induced therapy resistance can be categorized as: prevention of the emergence of plasticity, selective elimination of emerging therapy-resistant plastic cells, and reversion of the phenotypic switch [136].

Cancer therapy has the potential to initiate the creation of a therapy-resistant cancer cell population. Cancer is highly heterogeneous, while therapy is often administered in a linear, strict manner [146]. Meanwhile, adaptive therapy employs a treatment strategy based on tumor evolution. After treatment, the tumor is different compared to its pre-treatment state, which means that the following treatment should be applied differently. Adaptive therapy

needs to adjust treatment strategies in light of the changing tumor [147].

Conclusion

CTCs are a pivotal and critical point in the progression and understanding of cancer especially metastasis. Their presence in the circulation of the patient either as single cells or clusters highlights their important role in cancer dissemination. Due to their unique ability to mirror the genetic characteristics of the originating tumor CTCs provide valuable, minimally invasive means of accessing real-time information about the biology of the tumors. However, due to changes like EMT, CTCs can diverge phenotypically from the original tumor. As EMT usually activated in tumor cell sub-populations during dissemination CTCs carry the phenotypic information of the originating cell population. Despite, it has been demonstrated that CTCs carry prognostic and diagnostic utility in detecting MRD, guiding therapeutic decisions and monitoring relapse especially when utilized alongside cfDNA.

Looking forward, advances in CTC isolation and characterization techniques may provide a way for more precise and personalized therapy. With the integration of multi-omics approaches like single-cell sequencing and artificial intelligence researchers could further enhance the ability to profiling these rare cells and also offer deeper insights into the evolution of the tumor and its resistance to therapy.

Finally, leveraging CTCs in clinical practice holds promise for early detection and better monitoring and also for targeted treatment development which could transform and improve cancer care and ultimately patient outcomes. When used in conjunction with ctDNA CTCs can provide a more comprehensive view of tumor dynamics as ctDNA offers insight into genetic alterations while CTCs allow phenotypic and functional analyses. However, there are still challenges remain before CTC-based approaches can be fully utilized in routine clinical use. These include the extremely low abundance of CTCs in early-stage disease compared to ctDNA, limitations in current isolation and detection technologies, and the lack of standardized protocols across platforms. Furthermore, heterogeneity among CTCs, including variable expression of surface markers due to processes like EMT, can lead to false negatives and complicate interpretation. Addressing these technical and biological hurdles through continued innovation and validation in large clinical studies will be important for fully realizing the potential of CTCs in precision oncology.

Author contributions

Data collection was done by TL, ZN, AK, EF, FB, and NS. Text was composed by TL, ZN, and AK. Proofreading was done by BM and IT. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This project financed from the Hungarian Scientific Research Fund (NKFI-143002), from the NRDI Fund FK0201NEPE/TKP-NKTA-47 and from the Fund National Cardiovascular Laboratory RRF-2.3.1-21-2022-00003. This project has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development, and Innovation Fund also, financed under the KDP-2023/C2270480.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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