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Precision Medicine REVIEW

Single circulating tumor cell omics: Technologies and clinical applications

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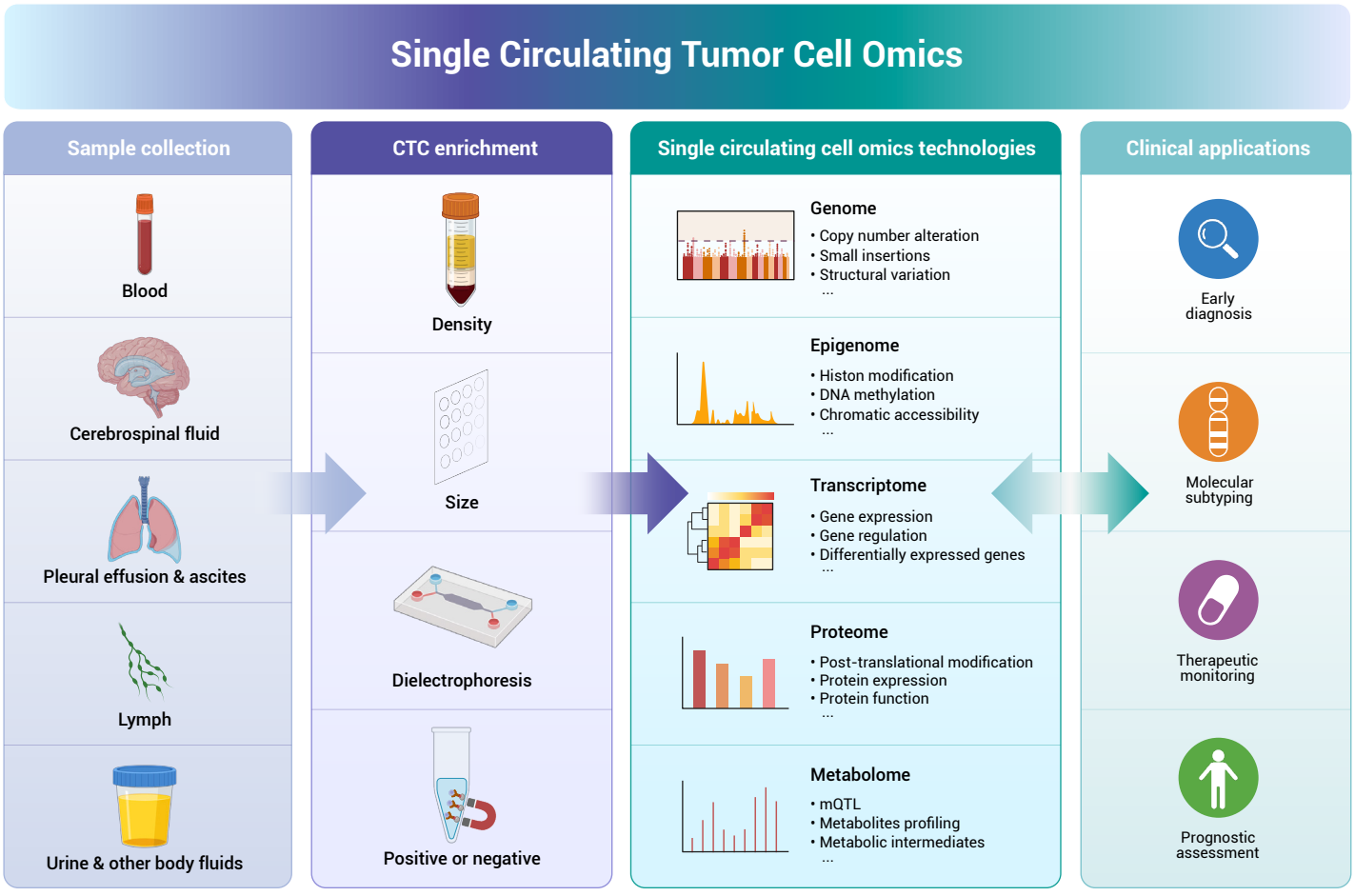
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Circulating tumor cells (CTCs) carry tumor DNA, RNA, and proteins in blood for non-invasive diagnosis.
- Single-cell omics technologies reveal CTC genomes, transcriptomes, proteomes, and metabolomes.
- CTC single-cell omics aids early detection, guides therapy, tracks drug resistance, and predicts prognosis.
- This review covers CTC enrichment, single-cell omics, and their clinical application in oncology.
- Integrating multi-omics with artificial intelligence and CTC models will advance precision cancer medicine.

REVIEW Precision Medicine

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Disseminating from primary or metastatic lesions, circulating tumor cells (CTCs) enter various body fluids—including blood, cerebrospinal fluid, and pleural effusions—acting as a liquid biopsy that provides a comprehensive molecular repository. The rapid maturation of single-cell omics has redefined CTC analysis, shifting the focus from simple enumeration to high-resolution molecular characterization. The application of these single-cell technologies has become pivotal for the next generation of precision oncology. This review first delineates the landscape of CTC enrichment, critically evaluating mainstream strategies based on biophysical and biochemical properties alongside emerging innovations. We then analyze the workflow of CTC single-cell omics analysis across five core dimensions: isolation techniques, genomics, transcriptomics, proteomics, and metabolomics. Furthermore, we highlight the clinical translational value of these approaches across four critical axes: early cancer detection, molecular subtyping for companion diagnostics, dynamic monitoring of therapeutic resistance, and prognostic risk stratification. The analysis concludes by addressing current technical bottlenecks and outlining a roadmap for integrating CTC profiling into routine clinical practice.

INTRODUCTION

Liquid biopsy has emerged as a transformative diagnostic paradigm, leveraging biofluids to interrogate pathological states. While applicable to cardiovascular and immunological conditions, its impact is most profound in oncology.[1] The molecular landscape of liquid biopsies is primarily defined by cell-free DNA (cfDNA), extracellular vesicles (EVs), and circulating tumor cells (CTCs).[2,3] Representing the "seeds" of metastasis, CTCs disseminate from primary or secondary lesions into the vasculature.[4,5] Unlike cell-free components, CTCs retain cellular integrity, offering a unique "multi-omics" snapshot—encompassing the genome, transcriptome, proteome, and metabolome—that fragment-based markers cannot provide.[2] Consequently, CTCs serve as pivotal biomarkers for early detection,[6] tracing metastatic cascades,[7] and prognostic stratification.[8] However, their clinical utility is currently hampered by their extreme rarity, often as few as one cell among billions of blood cells.[5,9] Overcoming this "needle-in-a-haystack" challenge to isolate and characterize CTCs efficiently remains a central imperative in translational cancer research.

Single-cell omics refers to a set of technologies that enable the sequencing, detection, and analysis of omics data—including the (epi)genome, transcriptome, proteome, and metabolome—at the single-cell level. This approach has become a pivotal tool in cancer research, enabling precise characterization of genetic alterations in cancer cells and facilitating a deeper understanding of the intrinsic mechanisms underlying tumor initiation and progression.[10] For example, single-cell RNA sequencing (scRNA-seq) performed on selected cells from tumors, normal tissues, and peripheral blood across different anatomical regions can generate comprehensive cellular atlases. These atlases support the inference of cell–cell interaction networks and the identification of potential molecular targets for clinical intervention.[11] The integration of single-cell sequencing with single- or multi-omics analyses applied to tissue biopsy–derived cells has emerged as a powerful strategy for investigating multiple aspects of cancer biology. These include invasion during premalignant stages, clonal evolution and intratumor heterogeneity (ITH) in primary tumors, reprogramming of the tumor microenvironment (TME), metastatic dissemination, and the development of therapeutic resistance.[12] However, tissue-based single-cell analyses capture tumor states at a single time point, lack longitudinal monitoring capacity, and depend on invasive biopsy procedures for cancer cell acquisition, which are associated with inherent clinical risks.[13] Consequently, the application of single-cell omics technologies to the detection and analysis of CTCs circumvents conventional invasive sampling approaches, enables dynamic disease monitoring, and provides critical molecular information for cancer research and clinical management.[14]

This review adopts the CTC single-cell omics analysis workflow as an organizing framework and systematically examine the major CTC enrichment strategies and their inherent limitations. Particular emphasis is placed on the feasibility of multi-target approaches for simultaneously capturing heterogeneous CTC populations. We further discuss recent technical advances in CTC single-cell omics technologies, including single-cell isolation, genomic and transcriptomic sequencing, and proteomic and metabolomic analyses. In addition, we highlight representative clinical applications of CTC single-cell omics. Finally, we provide a perspective on the current challenges and future directions of this rapidly evolving field.

CTC ENRICHMENT

The enrichment and isolation of CTCs constitute the initial and most critical step in CTC single-cell omics analysis workflows. CTCs are predominantly found in peripheral blood but occur at extremely low frequencies. Typically, only 1–1000 CTCs are present per milliliter of blood, compared with approximately 10^6–10^7 white blood cells and 10^9–10^{10} red blood cells. Gao et al. used the CytoSorter® CTC system to count EpCAM/Vimentin CTCs in 14 common cancers; even in the cancers with the highest CTC levels, the number was only tens of CTCs per 4 mL of blood.[15] This pronounced rarity poses a substantial challenge to the development and optimization of CTC separation technologies.[16,17] At present, CTC enrichment and isolation strategies fall into two categories: biochemical and biophysical methods. Biophysical methods can be further subdivided according to parameters such as density, cell size, dielectrophoretic properties, and related biophysical features. In contrast, biochemical methods are commonly divided into positive enrichment and negative enrichment strategies, depending on whether CTCs or background cells are selectively targeted. With respect to separation platforms, most existing techniques rely on magnetic bead–based systems or microfluidic devices to achieve adequate CTC enrichment and isolation.[14,16,18,19] We have summarized emerging CTC enrichment technologies successfully applied to clinical samples over the past five years, as detailed in Table 1.

Enrichment based on biophysical properties

Density-based enrichment. The scarcity of CTCs in the complex hematologic environment necessitates the removal of high-abundance bystander cells, particularly erythrocytes, as a prerequisite for adequate enrichment. This is frequently achieved through density gradient centrifugation (DGC), a label-free technique that discriminates between cell types based on differences in mass density and sedimentation coefficients (Figure 1A).[18,44] The utility of DGC relies on the specific selection of gradient media—including polyhydroxy sugars/alcohols, polysaccharides, inorganic salts, iodine compounds, and silica gels—to fractionate diverse biological components, ranging from whole cells and organelles to viruses and biomacromolecules. Prominent commercial platforms facilitating this process include Ficoll-

Table 1. CTC enrichment technologies validated on clinical samples in the past five years.

Technologies	Mechanism	Device	Spiking experiment			Number of CTCs in clinical specimens	Processing time	Target	Ability to process whole blood directly
			Capture efficiency	Purity	Viability				
MPA-Chip[20]	Size and Deformability	Microfluidic chip	92.3±3%	>90%	97%	5-22 / mL	~1 h	Non-target	No
the 3D Conductive Scaffold Microchip[21]	Immunoaffinity	Microfluidic chip	92.8%		91.3%	7-103 / mL	~ 1h	EpCAM	Yes
CMS[22]	Immunoaffinity	Microfluidic chip	80±5%			1-4 / 1.5 mL	~0.5 min	EpCAM	Yes
MN-scFv cell[23]	Immunoaffinity	Microfluidic magnetic separation	84.7%	91.9%		~20-120 / mL	~ 2 h	EpCAM	No
TDF-Cocktail-MNP[24]	Immunoaffinity	Microfluidic magnetic separation	>90%	>98%	94.25%	5-27 / 4 mL	~1.5 h	EpCAM、Vimentin、EGFR	No
AuNP-LFA Chip[25]	Immunoaffinity	LFA	90%±3.2%		80%±7%	3-8 / mL	~1 h	EpCAM	Yes
NataFace[26]	Immunoaffinity	M13 bacteriophages and Microfluidic magnetic separation	>95%	>99%	98.6%	3-24 / mL	~1 h	EpCAM	Yes
Cells-on-a-Bubble[27]	Immunoaffinity and Click chemistry	DNA glass microbubbles	98%			1-147 / mL	~0.5 h	EpCAM、EGFR、HER2	No
HSA-PIMBs[28]	Immunoaffinity	Magnetic beads	93.40–96.33%	81.21–85.89%		62-505 / 1.5 mL	~0.5 h	EpCAM	Yes
high-throughput thin filter[29]	Size	nanowell chip	76%		93%	3-15 / 5 mL	~0.5 h	Non-target	Yes
MNPs@FA[30]	Immunoaffinity	Magnetic nanoparticles	96.08%		95.47%	2-12 / 3 mL	~2 h	FR	No
CCM-CTCD system[31]	Immunoaffinity	Microfluidic magnetic separation	82-93%			12-120 / 5.4 mL	~1.5 h	Non-target	Yes
CFD-Chip[32]	Size	Microfluidic chip	>96%		>98%	0-hundreds / 5 mL	~0.2 h	Non-target	Yes
FIMMs[33]	Immunoaffinity	Magnetic nanoparticle and rotary magnetic manipulating device	79.93±3.31-92.93±3.23%		29.0±4.0-56.0±6.5%	23-56 / 1.5 mL	~1.5 h	EpCAM	No
Bait-trap chip[34]	Immunoaffinity	NCage Chip	>95%			1-21 / 2 mL	~1.5 h	EpCAM、N-cadherin	No
ieSCI-chip[35]	Inertial force and Size	Microfluidic chip	87%至98.5%	89.92±3.37%		23 / 2 mL	~1 h	Non-target	No
MNPs@hydrogel[36]	Immunoaffinity	Magnetic nanoparticles	98%		95%	1-12 / mL	~1 h	EpCAM	No
IC-MNs[37]	Immunoaffinity	Magnetic nanoparticles	>90%	98%	>90%	0-13 / mL	~1 h	EpCAM	Yes
AIMNPs[38]	Immunoaffinity	Magnetic nanoparticles	86.6–98.5%	90.5–96.5%		19-685 / 1.5 mL	~2 h	EpCAM	Yes
C6/MMSN-GPC3[39]	Immunoaffinity	Magnetic nanoparticles	96%		>80%	2-42 / 2 mL	~1 h	GPC3	No
Chimeric Ad 5/3[40]	Lentiviral infection	Fluorescence microscope	82.5-96%			0-180 / 9 mL	~24 h	hTERT	No
CTC-dChip[41]	Immunoaffinity	Microfluidic magnetic separation	>60%		>99%	2.7-122 / mL	~3 h	EpCAM、Vimentin	No
MNPs-TA[42]	Polyphenol-glycocalyx interactio	Magnetic nanoparticles	62.3–93.7%	~95%	95-97%	1-10 / mL	~1 h	glycocalyx	No
CTCelect[43]	Immunoaffinity	Microfluidic magnetic separation	51.8-86.8%			50 / mL	~2.5 h	EpCAM、Integrin α-V、CD61、CD106	Yes

Note: The processing time is an estimate of the time required to process clinical whole blood samples based on the method described in the paper; it does not include steps such as fixation and staining.

Paque™ (GE Healthcare, Chicago, IL, USA), Lymphoprep™ (STEMCELL Technologies, Vancouver, Canada), OncoQuick® gradient system (Greiner Bio-One, Kremsmunster, Austria), ACCUSPIN™ (Sigma-Aldrich, St. Louis, USA), and the CTS Rotea counter-current system (Gibco, Grand Island, USA).[45]

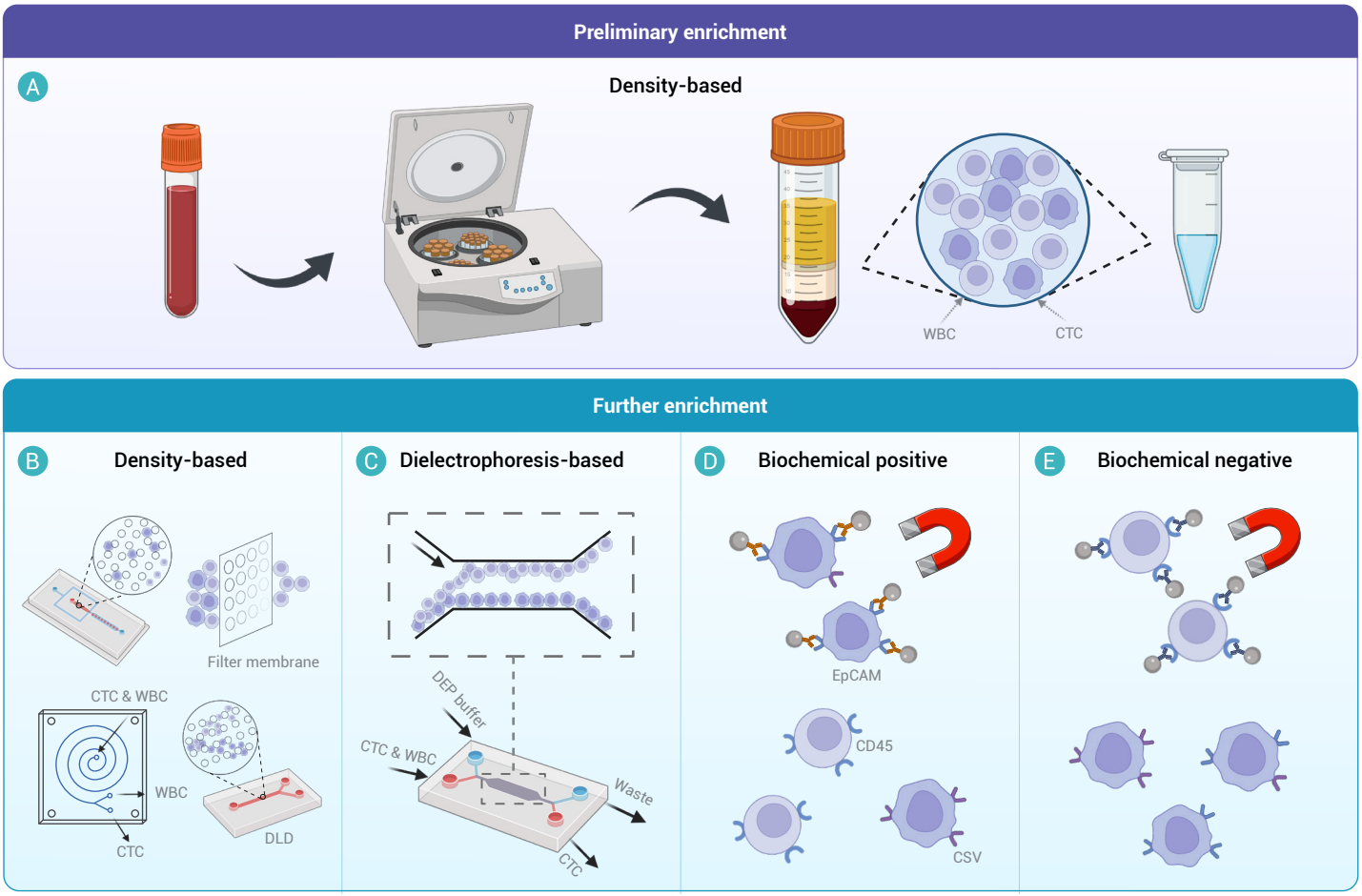

Figure 1. Common CTC enrichment methods.

While DGC minimizes capture bias due to its label-free nature, its performance is often hampered by the physical overlap between CTCs and leukocytes, which compromises purity. Moreover, yield reduction remains a concern, as CTCs may inadvertently migrate into the plasma fraction or form nonspecific aggregates at the vessel bottom.[45,46] To address these inefficiencies, hybrid strategies have been developed that augment DGC with immunoaffinity-based enrichment. In these workflows, microspheres functionalized with antibodies (e.g., anti-EpCAM or anti-CD146) are added to the gradient along with peripheral blood. These beads selectively bind CTCs, altering their sedimentation profile to ensure sequestration at the gradient base, thereby preventing loss to other density layers.[47,48]

Size-based enrichment. Size-based separation, which exploits differences in cell diameter, represents the most widely applied physical strategy for isolating CTCs from blood. Harouaka et al. systematically summarized the diameters and cross-sectional areas of the major cellular components in peripheral blood. They demonstrated that CTCs generally exhibit larger cell diameters than normal blood cells, supporting cell size as a reliable parameter for CTC separation.[49,50] Based on this principle, current size-dependent CTC isolation technologies can be broadly categorized into microfluidic-based separation methods and membrane-based filtration methods (Figure 1B). Among microfluidic platforms, the Parsortix® PC1 system is the first FDA-approved medical device for CTC capture and collection. It is designed to enrich CTCs from the peripheral blood of patients with metastatic breast cancer (mBC).[51] The Parsortix system (ANGLE North America, Inc., King of Prussia, PA) employs microfluidic particle separation technology, in which a disposable separation cartridge contains tapered, stepwise microchannels with gap sizes ranging from 4.5 to 10 μm. As blood flows through the cartridge, smaller blood cells traverse the constrictions, whereas larger CTCs are retained and enriched within the channel gaps.[52] In contrast, membrane-based filtration methods rely on a semipermeable membrane with a defined microporous array as the core separation element. When processed blood passes through the membrane, smaller components such as red blood cells and platelets permeate the pores, while larger cells, including CTCs, are retained on the membrane surface. Previous studies have shown that semipermeable membranes with pore sizes of approximately 8 μm can achieve high CTC recovery rates.[53] More recently, Li et al. developed a silicon nitride filter membrane and systematically evaluated its capture efficiency, white blood cell depletion rate, and CTC viability. Based on these metrics, they identified a silicon nitride membrane with a pore size of 6 μm, a thickness of 200 nm, and a porosity of 34% as optimal for high-performance CTC separation and suitable for subsequent clinical testing.[54] Meanwhile, passive systems that rely purely on channel geometry, carrier fluid, and cell properties have received attention due to their simplicity and high throughput. These include deterministic lateral displacement (DLD), pinched flow fractionation (PFF), hydrodynamic filtration, inertial migration, and viscoelastic focusing.[55] Huang et al. constructed an inertial-assisted single-cell focusing generator (I-SCF) and a water droplet deterministic lateral displacement cell sorting (D-DLD) microfluidic system (IDIC) for the precise isolation of rare CTCs from blood.[56] Wang et al. reported a method of reverse flow-enhanced inertia pinched flow fractionation (RF-iPFF) for particle separation. It separates particles by size based on the flow-induced inertial lift, and in the abruptly broadened segment, reverse flow is utilized to further enhance the separation distance between particles of different sizes.[57] In addition, Zhou et al. designed a novel multi-flow microfluidic (MFM) system for the separation of CTCs with high purity. Because lateral cell migration within microchannels is highly size-dependent, this inertial migration principle enables label-free separation of CTCs from white blood cells using MFM, without complex sample preparation.[58]

Dielectrophoresis-based enrichment. Dielectrophoresis (DEP)–based technology uses an non-uniform electric field to separate specific particles.

When an external electric field is applied, differences in dielectric properties between suspended particles and the surrounding medium lead to directional migration within the field. Depending on the dielectric contrast, particles may be attracted to regions of higher electric field intensity or repelled toward areas of lower intensity, leading to positive (pDEP) or negative (nDEP) dielectrophoresis. By modulating the strength, frequency, and spatial configuration of the applied electric field to generate a non-uniform field, specific cells can be selectively retained within or flushed out of the field, enabling the separation of target cells, viruses, and other biological entities from complex cell populations (Figure 1C). The dielectric properties of cells are highly dependent on cell type and physiological state, and dielectric parameters have been quantitatively characterized for a variety of cell types. Notably, cancer cells exhibit higher membrane capacitance compared with red blood cells (9 ± 0.8 mF/m^2) and white blood cells (ranging from 11 ± 1.1 mF/m^2 for T cells to 15.3 ± 4.3 mF/m^2 for monocytes). By tuning the electric field frequency, selective separation of specific cell populations can therefore be achieved based on these intrinsic dielectric differences.[59,60] DEPArray™ (Menarini Silicon Biosystems, S.p.A., Italy) is a representative cell-separation platform that integrates dielectrophoresis, microfluidic chip technology, and single-cell imaging. The system employs a programmable electrode array at the base of the chip to generate precisely controlled non-uniform electric fields, creating closed electrode potential wells for the capture and isolation of individual cells. Coupled with high-resolution cellular imaging, this platform enables accurate enrichment and separation of target CTCs. Furthermore, Gascoyne et al. introduced a separation factor (S) to quantify the ease of cell separation using dielectrophoresis. Cancer cells were shown to exhibit significantly higher S values than red blood cells and T cells, indicating that cancer cells can be more readily separated from these background populations by DEP, a conclusion subsequently validated experimentally.[59]

Enrichment based on biochemical properties

Positive enrichment. Biochemical-based CTC separation is the most commonly used method in practice. This approach targets molecular markers specifically expressed on CTCs to enable their enrichment and separation from other blood cells (Figures 1D-E). The CellSearch system (Menarini Silicon Biosystems, S.p.A., Italy) is the first FDA-approved platform for CTC analysis. It has been approved for CTC enumeration in breast, prostate, and colorectal cancers, and serves as the gold standard for CTC detection.[61,62] It employs immunomagnetic nanoparticles functionalized with antibodies against EpCAM to enrich epithelial-derived cells from peripheral blood. Following enrichment, captured cells are subjected to multiparametric fluorescence labeling using DAPI to stain nuclei, antibodies against CD45 to identify leukocytes, and antibodies against cytokeratins (primarily CK8, CK18, and CK19) to label epithelial cells. Cells that are EpCAM-positive, DAPI-positive, cytokeratin-positive, and CD45-negative are operationally defined as CTCs. Despite its broad application, EpCAM-based enrichment is intrinsically limited by the biological plasticity of cancer cells. EpCAM is an epithelial marker whose expression can be markedly reduced or completely lost during epithelial–mesenchymal transition (EMT), a process frequently associated with tumor invasion and metastasis.[63] In addition, EpCAM expression is absent or minimal in certain tumor types or specific disease subgroups, such as Lynch syndrome-associated tumors with EpCAM gene deletion, brain tumors and melanomas lacking EpCAM surface expression, and renal cell carcinoma characterized by low EpCAM levels.[62,64-66] Under these conditions, EpCAM-dependent enrichment strategies may fail to efficiently capture relevant CTC populations. Accordingly, molecular markers for CTC enrichment should be tailored to the biological characteristics of different cancer types. The use of alternative or multiple biomarkers offers a potential strategy to improve CTC capture efficiency and broaden the detectable CTC spectrum. Representative molecular markers used for CTC identification across common cancers are summarized in Table 2.

Table 2 categorizes common molecular markers for identifying CTCs into four groups: epithelial cell markers, mesenchymal cell markers, tumor cell markers, and site-specific cell markers. When epithelial markers fail to identify CTCs effectively, the remaining three categories can facilitate their detection. Vimentin, N-cadherin, Twist, and SNAIL are four commonly used markers of mesenchymal cells. The EMT process in tumors is frequently characterized by increased expression of mesenchymal markers, such as Vimentin, N-cadherin, Twist, and/or SNAIL, and decreased expression of epithelial markers, such as EpCAM and E-cadherin.[63,90,121,122] Relevant studies have demonstrated that microfluidic chips coated with Vimentin antibodies can capture CTCs exhibiting a mesenchymal phenotype in pancreatic cancer, with a significantly higher detection frequency than EpCAM CTCs (median of 3 cells/4 mL blood).[123] Furthermore, since CTCs are cancer cells shed from tumors into the bloodstream, tumor-specific markers and site-specific cellular markers can also distinguish CTCs from blood cells. HER2, a common proto-oncogene, is overexpressed in various cancers, including breast, thyroid, liver, prostate, and bladder cancers.[68,87,88,99,103] Tumor markers similar to HER2 can also be used to isolate CTCs. In positive enrichment of CTCs, the multi-antibody cocktail approach, which uses combined antibody capture, can substantially improve CTC capture efficiency. Chen et al. employed anti-EpCAM and anti-FRα magnetic nanospheres for the combined capture of NSCLC CTCs. In clinical samples from 41 NSCLC patients, the combined use of anti-EpCAM-MNs and anti-FRα-MNs achieved a higher detection rate (73.2%) than anti-EpCAM-MNs alone (48.8%).[74] Table 3 compares single-target and multi-target enrichment strategies across four representative technologies

Site-specific biomarkers in CTCs tend to reflect the characteristics of the primary tumor rather than those of the metastatic site. Since we cannot determine the origin of CTCs during enrichment and isolation, there are currently no specific studies comparing CTCs from primary and metastatic tumor sites. However, because CTCs detach from tumor sites and enter the circulatory system, the molecular features of primary and metastatic tumors can indirectly inform CTC characterization. Regarding specific molecular expression, Miettinen et al. detected GATA3 positivity in the vast majority of primary ductal breast cancers (163/178, 92%) and their metastatic cases (49/51, 96%).[124] Among these metastatic cases, 10 originated from the brain, and GATA3 positivity was also detected in one case of lobular breast cancer metastatic to the small intestine. However, GATA3 expression was not detected in primary meningiomas or small intestine tumors; meanwhile, Werling et al. detected high CDX2 expression in all colorectal cancer patients (including those with lung metastases), but no significant CDX2 expression was observed in primary lung cancer.[125] Regarding CTC gene expression patterns, Rossi T et al. performed GO enrichment analysis on scRNA-seq expression data from 74 CTCs in 10 patients with metastatic breast cancer, and their results were consistent with those of BC.[126] Meanwhile, Onstenk et al. analyzed the CTC gene expression profiles of 7 patients with metastatic breast cancer, finding that in only two patients did CTC characteristics correlate more strongly with lymph node metastasis than with primary tumor characteristics.[127]

Negative enrichment. In addition to the positive enrichment methods mentioned above, negative enrichment is another commonly used approach for separating CTCs based on their biological characteristics. Negative enrichment typically targets CD45 or CD66b antigens on the surface of blood cells, removing background cells, such as white blood cells, leaving CTCs in suspension. STEMCELL™ has developed the EasySep™ Direct Human CTC Enrichment Kit, which enables direct CTC enrichment from whole blood. This kit uses an antibody complex (CD2, CD14, CD16, CD19, CD45, CD61, and CD66b) combined with magnetic particles to specifically target and remove non-CTC cells. The enriched CTCs are suitable for direct downstream applications. CTCs obtained by this method theoretically encompass all subpopulations present in a sample. However, negative enrichment approaches often fail to eliminate background cells, resulting in lower CTC purity. Combining positive and negative enrichment methods increases the number of captured CTCs. Diagnostic leukapheresis (DLA) is a simplified version of leukapheresis. CTCs have densities and sizes similar to those of mononuclear cells (MNCs) in blood. DLA technology simultaneously separates white blood cells and enriches CTCs. The isolated cells are then screened to remove white blood cells or to enrich CTCs, thereby enabling CTC collection. Compared to CellSearch, DLA requires a larger initial blood volume (2.8 L vs 7.5 mL) and yields substantially more CTCs, resulting in a higher yield.[128] In a study of NSCLC patients, the DLA method yielded significantly more CTCs than CellSearch (0–216 vs 0–41).[129]

Table 2. Biomarkers for identifying circulating tumor cells in common cancers.

Cancer Types	Epithelial	Mesenchymal	Site	Tumor	References
Breast	EpCAM, E-cadherin CK5/7/8/18/19	N-cadherin, Vimentin FN1, Twist, SERPINE1/PAI1	/ GATA3, PR, ER, hMAM	HER2, MUC1, CD24/44 ALDH1	67-70
NSCLC	EpCAM CK8/18/19	N-cadherin, Vimentin Twist, MMPs, FN1	/ /	HER2, MET, TROP2, EGFR, HER3,Integrins α_v and β_1 /	71-74
SCLC	EpCAM, E-cadherin CK8/18/19	/ β-catenin, JUNB	/ SYP, CHGA	DLL3, CXCR4, PD-L1	75-79
Colorectal	EpCAM, E-Cadherin CK19/20	Vimentin Twist, SNAIL, SLUG, FOXC2, β-catenin	CEA	MUC1, EGFR, c-MET, STn c-MYC	80-84
Thyroid	EpCAM CK8/18/19	Vimentin /	TSHR /	EGFR, HER2 /	85-87
Liver	EpCAM, E-cadherin CK7/8/18/19	N-cadherin, Vimentin Twist, ZEB1/2, SNAIL, SLUG	ASGPR, GPC3 AFP	HER2 /	88-90
Gastric	EpCAM, E-cadherin CK8/18/19	MMPs, Vimentin /	/	HER2 /	91-93
Esophageal	EpCAM CK7/8/18/19/20	/ Twist	/	/	94-96
Prostate	EpCAM, E-cadherin CK5/8/18/19	Vimentin /	PSMA PSA, AR, AR-V7	EGFR, CD44/117, HER2 /	66,97-99
Pancreatic	EpCAM, E-cadherin CK7/8/18/19	Vimentin Twist	/	EGFR, GPC1, MUC1, CD166 /	100-102
Bladder	EpCAM CK5/8/13/17/18/19	/	/	HER2, STn /	66,83,103
Glioblastoma	/	CDH11, TGFBR2, Vimentin Twist, SNAI2, SERPINE1, TGFB1	A2B5 /	EGFR, c-MET Nestin, Telomerase, SOX2	104,105
Renal carcinoma	EpCAM, Cadherin 6 CK8/18/19	Vimentin Twist	/	CA9, CD44/147, CA12, EGFR, MUC1 /	61,66,106-108
Head and Neck	EpCAM Pan-CK	/	/	EGFR, c-MET, CD47, PD-L1/2 /	109-111
Cervical	EpCAM CK8/18/19	/	/	PD-L1, CD49f/133 /	112,113
Gallbladder	EpCAM CK8/18/19	Vimentin Twist	/	EGFR, HER2 /	114,115
Melanoma	/	/	MCSP MLANA, gp100, S100, Mart-1, Mitf, PAX3, GalNAc-T	ABCB5, CD133/146/271, RANK Nestin, MAGE, LNGFR, MIF,	62,116-120

Note: The biomarkers in the first row of each cell are membrane or transmembrane proteins that can be used for non-invasive capture of CTCs. The remaining biomarkers can help identify CTCs. Conventional vimentin resides within the cell, but it can also accumulate on the cell surface to form cell-surface vimentin (CSV). At present, only EpCAM and pan-CK are suitable for clinical analysis; the remaining biomarkers are used solely for research purposes.

NSCLC - Non-Small Cell Lung Cancer, **SCLC** - Small Cell Lung Cancer.

Table 3. Comparison of single-target and multi-target enrichment for four technologies.

Technologies	Single-target	Capture efficiency	Multi-target	Capture efficiency
TDF-Cocktail-MNP[24]	EpCAM	SK-BR-3 (>90%) MDA-MB-231(~33%)	EpCAM、Vimentin、EGFR	>91%
Cells-on-a-Bubble[27]	EpCAM	MDA-MB-231 (9%) A549 (21%)	EpCAM、EGFR、HER2	MDA-MB-23 (87%) A549 (93%)
Bait-trap chip[34]	EpCAM N-Cadherin	MCF-7 (EpCAM ~95%, N-Cadherin ~60%) Hela (EpCAM ~70%, N-Cadherin ~95%)	EpCAM、N-Cadherin	>97%
CTC-dChip[41]	EpCAM Vimentin	PANC-1 (EpCAM ~20%, Vimentin ~5%) LNCaP (EpCAM ~100%, Vimentin ~5%)	EpCAM、Vimentin	PANC-1 (~50%) LNCaP (~100%)

SINGLE CIRCULATING TUMOR CELL OMICS TECHNOLOGIES

Single cell isolation

The successful execution of single-cell analysis is predicated on the precise isolation of individual cells. Since standard enrichment strategies frequently fail to eliminate all background leukocytes, direct sequencing or detection of the enriched product is often infeasible, necessitating the coupling of enrichment with dedicated isolation platforms (Figure 2). Microscopic identification, followed by manual capillary aspiration, enables physical extraction of immunofluorescently labeled CTCs.[40] Although this strategy ensures high specificity, its reliance on labor-intensive micromanipulation severely limits throughput, making it unsuitable for large-scale processing. Alternatively, fluorescence-activated cell sorting (FACS) automates separation by assessing surface marker profiles marker profiles. Through the differential tagging of tumor antigens (e.g., EpCAM, CK, Vimentin) against hematopoietic exclusion markers (e.g., CD45, CD34, CD33), FACS achieves the precise isolation of CTCs based on established fluorescence gating criteria.[130-132] While FACS offers high throughput, accurate CTC discrimination often requires complex, carefully optimized fluorescence panels.[130,133] Laser capture microdissection (LCM) is an automated sample preparation technol-

ogy that precisely isolates defined target regions. Similar to FACS, LCM-based CTC isolation relies on fluorescence-based recognition; however, it can also incorporate in situ hybridization to identify CTCs based on specific genetic features, such as chromosome 8 centromere (CEP8) alterations, thereby improving isolation precision.[134] Nevertheless, LCM is limited by low throughput, as CTC identification is typically performed manually under a microscope, and the ultraviolet (UV) laser used during dissection may induce cellular damage.[133,135] To address these limitations, Kim et al. developed LIMO-seq, a CTC isolation strategy based on laser-induced forward transfer (LIFT), which enables non-contact, high-precision isolation of individual CTCs.[135] In addition, Li et al. reported an SCIGA-chip that integrates CTC single-cell isolation with on-chip cell lysis and subsequent whole-genome amplification (WGA), thereby establishing an automated workflow from whole-blood input to WGA product output and substantially simplifying the CTC single-cell sequencing process.[133]

Genomics

CTC single-cell genome sequencing generally involves two main steps: WGA of CTC genomic DNA (gDNA) followed by library preparation (Figure 2). At present, WGA methods can be broadly classified into three categories: PCR, multiple displacement amplification (MDA), and multiple annealing and looping-based amplification cycles (MALBAC). PCR-based WGA begins with the fragmentation of long gDNA molecules using physical, enzymatic, or chemical methods, followed by end repair and ligation of amplification adapters. PCR then amplifies the ligated DNA fragments. However, because PCR relies on exponential amplification, strong amplification bias can occur when the input DNA amount is low. MDA is an isothermal amplification method using phi29 DNA polymerase together with random primers. Under constant-temperature conditions, phi29 polymerase synthesizes long DNA fragments via strand displacement. MALBAC combines features of both MDA and PCR and uses a two-step amplification strategy. In this method, special MALBAC primers (5′ 27 fixed nucleotides 8 N 3′) are incorporated into newly synthesized DNA strands, forming loop structures that limit the repeated copying of the same template and enable quasi-linear amplification. The subsequent PCR step further amplifies the entire amplicon, thereby reducing amplification bias. Previous studies have shown that, compared with PCR-based WGA, MDA-based amplification of CTC genomes exhibits relatively low bias and good genome coverage, enabling genome-wide single-cell analysis.[136,137] However, due to phi29 polymerase's template-switching activity, MDA can introduce numerous small artificial inversions during amplification. These artifacts may be misidentified as true gene rearrangements in downstream bioinformatics analyses, leading to false-positive results.[138] In addition, when fixed cells are used, DNA crosslinking caused by fixatives often reduces phi29 polymerase activity, resulting in lower amplification efficiency.[139] Under these conditions, MALBAC has been reported to provide improved amplification uniformity and higher accuracy when applied to fixed cells.[29] For CTC genomic analysis, commonly used sequencing strategies include whole-genome sequencing (WGS), whole-exome sequencing (WES), and targeted sequencing. WGS involves library preparation and sequencing of all amplified DNA products, providing genome-wide information from individual cells. This method offers unbiased genome-wide coverage and is well-suited for detecting copy number alterations (CNAs) and large-scale chromosomal changes in CTCs. However, the large amount of data generated by WGS poses challenges for data processing and clinical interpretation. WES uses biotin-labeled probes or specific primers to enrich exonic regions before library preparation. By focusing on protein-coding regions, WES enables accurate detection of single-nucleotide variants (SNVs) and small insertions or deletions (INDELs) that directly affect protein function, making it valuable for identifying mutations in disease-related genes. While WGS and WES provide broad genomic information, clinical applications often require more focused analysis of specific genetic alterations. Targeted sequencing addresses this need by enriching predefined cancer-related gene panels before sequencing. This approach achieves very high sequencing depth in selected regions, improving the sensitivity for detecting low-frequency mutations and reducing background noise introduced during WGA.[140] Nevertheless, amplification bias remains unavoidable in single-cell sequencing and can introduce systematic errors, particularly in SNV analysis. To overcome this limitation, Yu et al. developed a WGA-free targeted single-cell next-generation sequencing (NGS) method for SNV detection. This method enables accurate identification of SNVs from single or small numbers of pancreatic cancer cells and was successfully applied to detect driver gene mutations in CTCs from patients with advanced pancreatic cancer. However, high sensitivity was attained only when five or more cells were analyzed, and true single-cell resolution was not attained.[141] More recently, Shen et al. developed scMet-Seq, a low-cost, high-throughput single-cell shallow WGS method based on the Tn5 transposase. This approach directly inserts PCR adapters into CTC gDNA using Tn5, thereby eliminating the need for WGA (Table 4). Using this strategy, copy number variations (CNVs) in single CTCs can be detected with high sensitivity and specificity across different cancer types and sample sources, significantly improving the efficiency and accuracy of CTC single-cell genomic analysis.[142]

Transcriptomics

Single-cell transcriptome sequencing is one of the most widely used single-cell sequencing approaches. It can be broadly divided into plate-based low-throughput methods and droplet-based high-throughput methods (Figure 2). Smart-seq2 is a widely used plate-based method and has defined a standard workflow for single-cell transcriptome analysis. This workflow includes single-cell lysis, reverse transcription to generate first-strand cDNA, second-strand cDNA synthesis and amplification, followed by cDNA fragmentation, library preparation, and sequencing. All steps can be performed in a single tube, reducing RNA loss and enabling the capture of full-length transcripts, thereby providing rich information for downstream bioinformatics analysis. Xu et al. developed a single-cell genome and transcriptome co-sequencing method termed DMF-DR-seq. In this approach, a digital microfluidic chip is used to separate the nucleus and cytoplasm of individual CTCs. Cytoplasmic RNA is sequenced using Smart-seq2, while nuclear DNA is amplified using MDA-based methods. This strategy enabled genome–transcriptome co-sequencing of single CTCs and captured abnormal transcriptional patterns driven by genomic alterations in multiple myeloma.[143] Methodological advances have led to several improved plate-based transcriptomic sequencing methods derived from Smart-seq2, including SHERRY, Smart-seq3, and FLASH-seq. In addition, CBTi-seq enables the sequencing of hundreds of cells within a single library via combinatorial barcoding. However, this method still relies on manual single-cell isolation and therefore does not support high-throughput transcriptomic sequencing of CTCs.[144-147] Droplet-based scRNA-seq methods achieve high throughput by using barcoded beads to prepare libraries from thousands to tens of thousands of cells in parallel. However, these methods usually require a minimum number of input cells. Using DLA combined with multi-marker recognition, Rieckmann et al. applied scRNA-seq (10X Genomics) to sequence 3,363 CTCs from six patients with NSCLC. This study generated the largest CTC transcriptomic dataset reported to date and revealed distinct CTC phenotypes, providing insights into potential therapeutic strategies targeting CTCs.[129] Although combining droplet-based library preparation with diagnostic leukapheresis can partially address the rarity of CTCs, it often requires large blood volumes, placing a considerable burden on patients. To reduce sample requirements, Cheng et al. developed a hydrodynamic barcoding method, Hydro-seq, that requires only 10 mL of blood. This system integrates size-based microfluidic CTC enrichment with bead-based barcoding. Individual CTCs are sequentially captured in single-cell chambers, where in situ lysis releases mRNA that hybridizes with barcoded beads. Using this approach, up to 800 cells can be sequenced per run, with scalability to 12,800 cells (Table 4).[148] Lightbody et al. proposed a CTC single-cell sequencing workflow named SWIFT-seq. This method uses CD138 magnetic beads for positive enrichment, followed by scRNA-seq (10X Genomics) and single-cell B-cell receptor sequencing (scBCR-seq, 10X Genomics). CTCs were identified based on V(D)J sequence information, and a "CTC circulation dynamics model" was proposed. This model suggests that CTC levels in peripheral blood are shaped by disease stage, proliferative potential, cytogenetic features, and circulatory capacity, offering mechanistic insight into the dissemination of multiple myeloma cells from the bone marrow into the bloodstream.[149] In addition, Brechbuhl et al. performed scRNA-seq (10X Genomics) directly on all filter-enriched peripheral blood mononuclear cells and used cell-type-specific markers to distinguish different populations. This

strategy enables simultaneous analysis of CTCs and other immune cells, providing a broader view of CTC interactions within the blood microenvironment and supporting further studies of CTC biology.[150]

Proteomics

Proteins are the primary functional molecules in living systems. Compared with DNA and RNA, protein expression at the single-cell level more closely reflects cellular states and biological activity. Although single cells contain significantly more protein than nucleic acids (approximately 50–500 pg protein versus 7 pg DNA and 10 pg RNA), the lack of effective protein amplification strategies makes single-cell proteomic analysis of CTCs technically challenging and remains a key issue (Figure 2).

Western blotting (WB) is a widely used method for qualitative and semi-quantitative protein analysis. However, conventional WB typically requires pooling cell samples to achieve sufficient protein levels for detection, thereby preventing the analysis of protein expression changes in individual CTCs. To overcome this limitation, single-cell western blotting (scWB) integrates single-cell lysis, protein separation, fixation, and immunodetection within a microfluidic chip. By relying on diffusion-based transport, scWB limits dilution of protein lysates and maintains relatively high local protein concentrations. Using this approach, up to 12 surface and intracellular signaling proteins can be quantified in individual CTCs, enabling targeted proteomic analysis at the single-cell level.[151] The Single-Cell Barcoded Chip (SCBC) is another single-cell proteomics platform that combines cell isolation with targeted protein detection. In this system, cell suspensions are introduced into a microfluidic chip containing hundreds of nanoliter-scale microchambers (approximately 2 nL each), resulting in the isolation of zero to five cells per chamber—Shi et al. pre-patterned antibody arrays within the SCBC microchambers. After cell lysis, released proteins were captured by immobilized antibodies and detected using biotin-labeled secondary antibodies and fluorescent streptavidin in a sandwich immunoassay format, allowing quantitative measurement of selected proteins.[152] BOBarray further improves single-cell isolation by increasing the number of microchambers while reducing their volume, so that most chambers contain either zero or one cell, according to Poisson statistics.[153] Given the low abundance of CTCs in clinical samples, Deng et al. introduced a poly-L-lysine (PLL) barcode pattern that captures CTCs from solution via electrostatic interactions, thereby increasing capture efficiency to approximately 55%.[154] The same group later developed a nanopore-based chip that supports CTC single-cell isolation, glucose analog imaging, intracellular signaling protein measurement, and genomic amplification. The PLL-coated surface enables higher antibody loading, thereby improving the detection sensitivity for low-abundance proteins.[155,156] Despite these advances, most of these protein detection methods rely on fluorescence-based readouts. Due to spectral overlap, their multiplexing capacity is limited, typically allowing detection of approximately a dozen proteins. This limitation restricts the depth of analysis achievable in CTC single-cell proteomics. To address this issue, alternative strategies have been explored, including DNA-barcoded antibodies combined with NGS and surface-enhanced Raman scattering (SERS)-based microfluidic immunoassays for in situ CTC analysis.[119,157] However, these methods still fall short of enabling the simultaneous detection of dozens of proteins in a single CTC. Mass cytometry (CyTOF) and imaging mass cytometry (IMC) combine flow cytometry principles with mass spectrometry. In these techniques, antibodies are labeled with metal isotopes rather than fluorescent dyes, and labeled cells are analyzed using a mass cytometer. Because metal tags do not suffer from spectral overlap, CyTOF and IMC enable the simultaneous measurement of dozens of proteins in single cells, significantly improving multiplexing capability and efficiency in CTC single-cell proteomics studies (Table 4).[158-161] Nevertheless, these approaches are still limited to targeted detection of predefined proteins and do not support unbiased proteome-wide analysis.

In contrast, mass spectrometry–based proteomics does not require prior labeling or selection of target proteins. Following protein digestion and peptide separation, amino acid sequences can be directly identified and quantified, allowing unbiased analysis of protein expression, post-translational modifications, and protein interactions.[162] Several studies have applied liquid chromatography–mass spectrometry (LC–MS) and related techniques to analyze CTC proteomes. While these methods can identify thousands of proteins, they generally rely on pooled CTC populations and do not achieve unbiased proteomic profiling at the single-cell level.[102,163-167] To advance true single-cell proteomics, Wang et al. developed an Orcs-proteomics microfluidic platform with a sickle-shaped design for rare-cell isolation and protein analysis. Using n-dodecyl-β-D-maltoside (DDM) as a mild denaturant and integrating timsTOF mass spectrometry, this system detected 973 proteins from five CTCs isolated from blood samples of patients with advanced breast cancer.[168] Tsai et al. reported a label-free single-cell proteomics method, SOP-MS, that uses surfactant-assisted integrated sample preparation in a single reaction vessel. By eliminating sample transfer steps and reducing surface adsorption losses, this method improves sensitivity. It enables the detection of 81–223 proteins in single tumor cells derived from CTC-based patient-derived xenograft (PDX) models.[169] In recent years, rapid progress in single-cell proteomics technologies, including SCoPE-MS, iProChip, plexDIA, PiSPA, One-Tip, and Chip-Tip, has enabled unbiased detection of thousands of proteins per cell, with Chip-Tip reporting more than 5,000 proteins from a single HeLa cell.[170-175] As these technologies continue to mature, their application to CTC single-cell proteomics is expected to advance liquid biopsy research substantially.

Metabolomics

Single-cell metabolomics can be broadly divided into two categories: static metabolite profiling, which mainly relies on mass spectrometry, and dynamic metabolic activity analysis, which is commonly based on fluorescence imaging and isotope tracing (Figure 2).[176] Compared with other omics dimensions, the genome of a single cell is relatively stable. Although the transcriptome and proteome can change substantially, these changes usually occur over minutes to hours. In contrast, metabolite levels can fluctuate within milliseconds to seconds. As a result, rapid quenching of enzymatic activity during sample preparation is essential to preserve accurate and unbiased metabolite profiles.[176,177] Conventional LC–MS–based metabolomics methods often lack sufficient sensitivity for single-cell analysis and are not well suited to the tiny sample volumes involved. Therefore, the development of unbiased metabolomics techniques with true single-cell resolution remains a significant challenge. Zhang et al. first used untargeted mass spectrometry–based metabolomics to compare colorectal cancer cell lines with high and low metastatic potential. Based on these results, they developed a customized single-cell quantitative mass spectrometry platform capable of measuring 11 metabolites in individual CTCs. Using this platform, they established a CTC-based classifier to predict the risk of colorectal cancer metastasis within 2 years.[178] Hiyama et al. developed live single-cell mass spectrometry (LSC-MS), in which individual CTCs are captured under microscopic observation using tapered glass microcapillaries and then directly introduced into a mass spectrometer after ionization and sonication. This method detected 216 metabolic peaks that matched entries in human metabolite databases, markedly improving detection efficiency and metabolite coverage, particularly for lipid species, in single CTCs.[179] The same group subsequently applied LSC-MS to perform untargeted metabolomic analysis of individual human CTCs and lymphocytes, revealing pronounced metabolic differences between these cell types and heterogeneity among CTCs.[180] Hu et al. applied nano-electrospray ionization atmospheric pressure chemical ionization (nanoESI-APCI) for high-sensitivity, high-coverage single-cell metabolomics analysis of primary tumor cells, CTCs, and bone metastatic cells (Table 4). This approach identified approximately 800 statistically significant metabolic features and showed that CTCs exhibit a distinct metabolic state adapted to survival in the circulatory environment.[181] When applied to clinical samples, single-cell metabolomic profiles from 120 CTCs were used to construct a classification model that separated CTCs into subgroups with different metastatic risks, enabling the prediction of metastasis within 1 year.[182] Although mass spectrometry–based metabolomics allows quantification of metabolite abundance in individual cells, it does not directly capture dynamic metabolic activity within pathways. To address this limitation, 2-NBDG, a fluorescent glucose analog that enters cells via glucose transporters and follows similar metabolic pathways, has been used to assess glucose uptake in glucose-deprived CTCs by fluorescence imaging.[155] Luan et al. developed a biofluorescence imaging–guided spatial metabolic tracking platform (BIGSMT). By

Table 4. Technical specifications for single-CTC omics analysis.

Omics	Category	Methods	Initial Quantity	Targets/cell	Platform
Genome	Targeted	WES	1	whole exons	NGS
		Target Sequencing	1	~100 genes	NGS
	Non-targeted	scMet-seq	1	whole genome	NGS
		Uni-C	1	whole genome and 3D chromatin structure	NGS
Transcriptome	Low-throughput	Smart-seq2	1	~10000 genes	NGS
		DMF-DR-seq	1	DNA: whole genome RNA: 7000-9000 genes	NGS
	High-throughput	10x Genomics	Thousands	Hundreds genes	NGS
		Hydro-seq	Hundreds	hundreds genes	NGS
Proteome	Targeted	scWB	Hundreds	~12	WB
		SCBC	Hundreds	~12	FM
		ADT	Hundreds	~15	NGS
		SERS	dozens	4	SERS
		CyTOF	millions	dozens	CyTOF
		IMC	millions	dozens	IMC
	Non-targeted	SOP-MS	1	hundreds	MS
Metabolome	Targeted	scQuantitative MS	1	12	MS
		SDM	thousands	Lactic Acid	FM
	Non-targeted	LSC-MS	1	~100	MS
		nanoESI-APCI	1	Hundreds	MS

injecting ^{13}C-labeled glucose into mice via the tail vein and combining isotope tracing with in vivo fluorescence imaging, this system enabled real-time tracking of metabolic fluxes and visualization of CTC-driven metastatic processes at specific anatomical sites, allowing spatially resolved metabolomic analysis.[183] In addition, Radfar et al. designed an arrowhead-shaped static droplet microfluidic device that integrates CTC enrichment, capture, and metabolomic screening. This platform enables precise measurement of lactate production at the single-cell level and supports high-throughput identification of metabolically active CTCs.[184]

CLINICAL APPLICATIONS OF SINGLE CIRCULATING TUMOR CELL OMICS

Although CTC detection has been introduced into clinical practice, most diagnostic applications still rely primarily on CTC enumeration to assess cancer-related indicators. This approach provides limited molecular information and does not support precise molecular diagnosis or treatment decisions.[185-187] Single-cell omics analysis of CTCs demonstrates unparalleled potential in this regard. It not only enables comprehensive molecular analysis of CTCs using NGS, mass spectrometry, and other techniques, but also facilitates sensitive, targeted detection of specific genes, proteins, and other molecules in CTCs through methods such as specific probes and fluorescence detection. This advances cancer diagnosis toward precision medicine.[14]

Early diagnosis of cancer

Early diagnosis of cancer plays a crucial role in cancer treatment. Detecting cancer or precancerous changes at an early stage enables timely intervention against tumor progression, thereby halting or slowing disease progression and reducing mortality while significantly improving survival rates.[12,188] Currently, early cancer diagnosis relies heavily on imaging studies and histopathological analysis. While these methods can identify tumor morphology and structural information, their specificity and sensitivity are limited, failing to detect minute tumor lesions (diameter ≤ 1 mm).[188,189] Single-cell omics technologies of CTCs offer a non-invasive approach to analyzing cancer cells at the molecular level, thereby advancing early cancer diagnosis. Although the FDA approved CellSearch for the use of CTC enumeration as a prognostic marker for metastatic cancer, multiple studies indicate that cancer cell dissemination occurs during early tumor development when CTCs are already released. Characterizing cancer features using these CTCs demonstrates significant potential for early cancer diagnosis.[4,6,186] DCIS has traditionally been considered a non-invasive lesion, as histological examination does not reveal obvious cancer cell dissemination. However, some studies suggest that cells within DCIS tissue may be capable of invading, entering the systemic circulation to form CTCs, and establishing local niches in distant organs, thereby facilitating metastatic spread. DNA-seq and RNA-seq analysis of these CTCs revealed mutations in multiple cancer genes and chromosomal abnormalities. Leveraging these variations could aid in understanding DCIS progression and in identifying clinically high-risk patients.[190] In lung nodule patients, Wang et al. used isolation by size of epithelial tumor cells (ISET) to detect CTCs and reported their presence in approximately 80% of nodules classified as benign. This finding challenges the traditional assumption that benign nodules do not release tumor cells into the circulation. WES further showed that CTCs derived from malignant nodules exhibited more extensive CNVs than those from benign nodules. In addition, mutations in *TP53*, *NTRK1*, and *ALK* were detected only in CTCs from malignant nodules. When combined with computed tomography (CT) imaging features, these molecular data improved the distinction between benign and malignant nodules and supported more accurate differential diagnosis.[191,192] For cancers with an unknown primary origin, where standard clinical methods often fail to identify the primary tumor, single-cell analysis of CTCs offers a promising approach. Guo et al. developed an unsupervised method, CTC-Tracer, that integrates large-scale scRNA-seq data from primary tumors with deep transfer learning. This method aligns CTC transcriptomic profiles with reference profiles from primary tumor tissues and transfers tissue-specific expression patterns to CTCs. Using this strategy, the tissue of origin of CTCs in blood samples can be inferred with high accuracy, providing essential support for

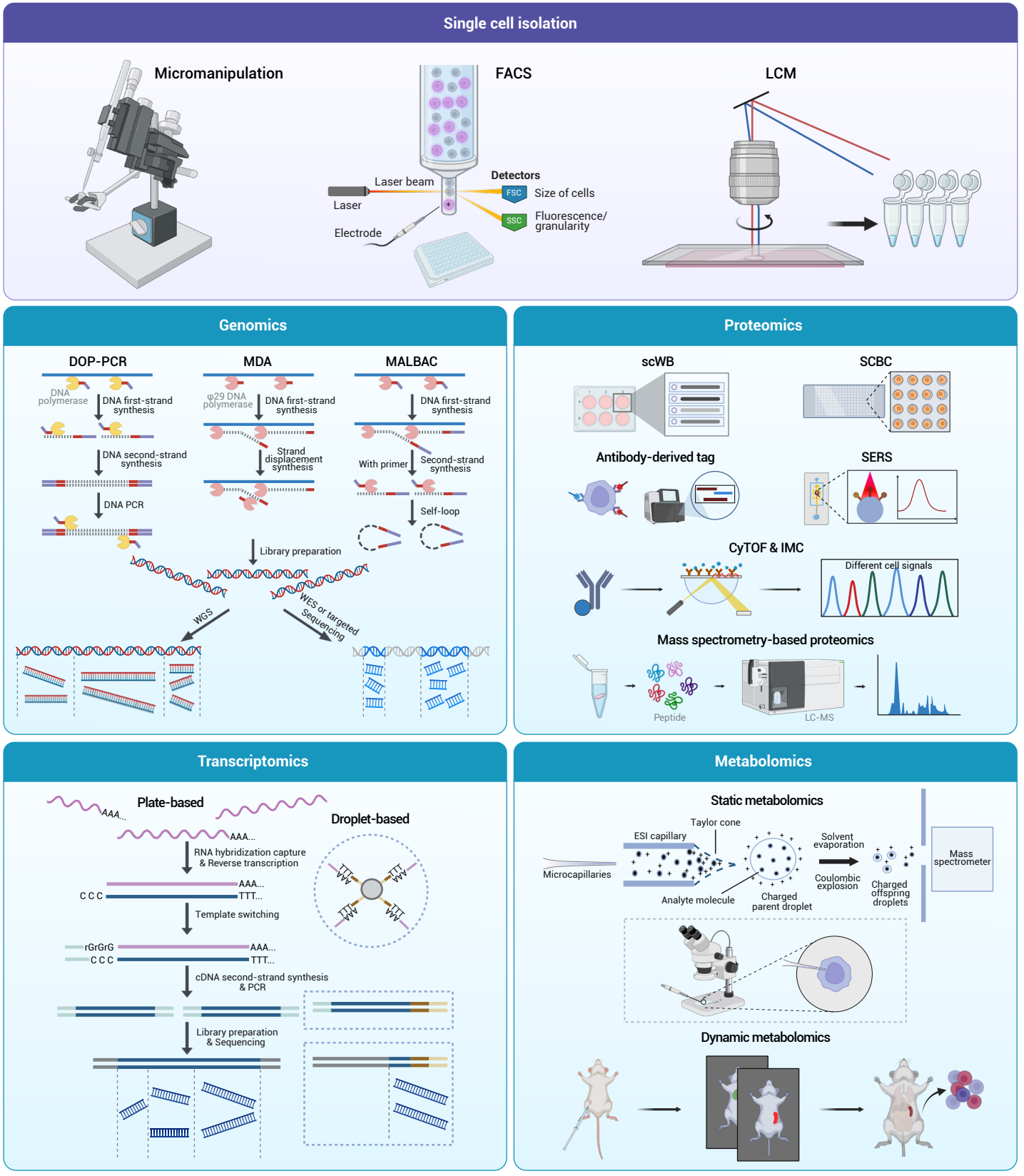

Figure 2. Single circulating tumor cell omics analysis technologies.

early diagnosis and clinical decision-making.[193]

Clinical molecular subtyping and companion diagnostics

The high heterogeneity of tumors is a key factor driving the diversity of cancer treatment approaches. Different subtypes of the same cancer often require substantially different therapeutic strategies. Currently, clinical practice still relies on traditional tissue biopsies to determine cancer subtypes. However, this method is technically challenging and invasive, making it difficult to capture tumor dynamics. In contrast, CTCs are tumor cells shed from primary or metastatic tumors into the bloodstream and carried by the circu-

latory system. These cells carry extensive genetic information, reflecting tumor progression at the molecular level. They demonstrate significant potential in clinical molecular subtyping and companion diagnostics, offering advantages over traditional tissue-based approaches.

Multiple studies have shown that CTCs exhibit high heterogeneity. Single-cell sequencing approaches can subdivide CTCs into distinct subpopulations from various perspectives. Phenotypically, CTCs can be classified into epithelial and EMT subtypes based on the expression of epithelial markers (e.g., CK, EpCAM, E-cadherin) and EMT markers (e.g., Vimentin, SNAIL). The EMT subtype can be further subdivided into early EMT (or partial EMT, pEMT) and advanced EMT.[150,158,161] At the functional level, epithelial-type CTCs exhibit greater activation of cellular and cyclin signaling pathways compared to EMT CTCs, while EMT CTCs demonstrate stronger anti-apoptotic capabilities. This suggests that an increased proportion of EMT CTCs may be associated with reduced therapy sensitivity.[129,194] Semaan et al. validated this concept in pancreatic cancer, demonstrating that the proportion of pEMT-CTCs correlated significantly with advanced disease, poorer progression-free and overall survival in all patients, as well as radiological treatment progression and postoperative recurrence rates. Conversely, Payne et al. observed that early-stage EMT CTCs in head and neck squamous cell carcinoma showed elevated expression of three phosphoproteins—pSTAT1, pCREB, and pERK. Combined with Rieckmann et al.'s findings in NSCLC that mesenchymal CTCs exhibit both oxidative phosphorylation and glycolytic/hypoxic phenotypes, this body of evidence suggests potential avenues for molecularly targeted therapies.[129,160] Carter et al. developed a CNA-based classifier that distinguishes chemotherapy-sensitive from chemotherapy-resistant patients, revealing significant differences in progression-free survival (PFS). This approach enables the pre-treatment assessment of individual chemosensitivity and tailored treatment adjustments, thereby achieving more precise molecular patient stratification by integrating individual characteristics.[195]

Beyond macroscopic classification of CTC expression profiles, single-cell sequencing of CTCs enables precise molecular-level detection. HER2-targeted therapy is a standard treatment for HER2+ breast cancer patients, significantly improving survival rates. However, multiple studies indicate that HER2 status may change during breast cancer progression. Monitoring CTC HER2 status via single-cell genome sequencing revealed detectable HER2+ CTCs in some HER2- patients. Adjustment of HER2-targeted therapy based on CTC HER2 status significantly prolonged progression-free survival, addressing limitations of traditional clinical staging.[134,161,196-198] Furthermore, Gasch et al. frequently detected mutations in the hotspot regions of the *PIK3CA* exons 9 and 20 in CTCs from HER2- mBC patients using two distinct WGA methods. Multiple lines of evidence confirm that single-cell sequencing of CTCs holds promise for predicting resistance to HER2-targeted therapy.[196] In metastatic castration-resistant prostate cancer (mCRPC), *AR* rearrangements represent a key mechanism of resistance to *AR*-targeted hormone therapy. However, compared to conventional *AR* amplification or mutations, their clinical detection poses greater challenges. Daniel et al. developed a strategy to identify *AR* rearrangements in CTC DNA, specifically detecting *AR* deletions and duplications via WGA. They discovered *AR* rearrangements in CTCs that were inconsistent with cfDNA, thereby more accurately capturing disease heterogeneity in CRPC patients.[138] In a study examining *BRCA2* deletions in mCRPC patients, Barnett et al. utilized scWGS to demonstrate that single-cell genome sequencing of CTCs detected *BRCA2* deletions more sensitively than bone biopsy or cfDNA sequencing. Furthermore, genomic instability observed in CTCs was significantly correlated with *BRCA2* deletions, suggesting that CTCs may serve as effective biomarkers for PARP inhibitor therapy.[199] Concurrently, Mishra et al. identified a distinct population of small, epithelial-negative prostate CTCs with neuroendocrine characteristics in mCRPC patients via RNA sequencing. Crucially, this neuroendocrine differentiation remained undetectable in metastatic tumor biopsies, demonstrating the potential of CTC single-cell sequencing to detect emerging resistance mechanisms at an early stage.[200]

Therapeutic monitoring and analysis of drug resistance mechanisms

Long-term monitoring of cancer patients is essential for clinical management, enabling adjustments to therapeutic strategies and the identification of drug resistance.[201] Clinical symptoms often appear after molecular changes have already occurred, whereas circulating tumor cells (CTCs) can reflect these changes at an earlier stage. Because CTCs can be repeatedly obtained via blood sampling, single-cell omics analysis of CTCs enables continuous assessment of a patient's molecular status. It provides real-time information on tumor evolution, thereby supporting treatment adjustments during therapy.[202-204] In a pilot study of patients with *EGFR*-mutant NSCLC, Lim et al. performed longitudinal single-cell transcriptomic analysis of CTCs from two patients receiving EGFR tyrosine kinase inhibitors. By examining EMT–related features, they captured dynamic shifts in CTCs from epithelial to mixed and mesenchymal states during treatment. They found that higher M-scores were associated with poorer clinical outcomes.[205] Blau et al. proposed the Intensive Trial of Omics in Cancer (ITOMIC) framework, which integrates DLA for continuous molecular monitoring. Using this approach in a patient with metastatic breast cancer, they characterized CTC molecular profiles, applied distributed network analysis, and predicted drug sensitivity. This strategy correctly identified the sensitivity of triple-negative breast cancer cell lines to BCL-2 inhibitors, highlighting the value of CTC-based analysis for treatment monitoring and drug screening.[206]

Several studies have reported that gene expression patterns in CTCs differ from those in matched primary tumor tissues. During mutation analysis, CTCs may reveal diverse genetic alterations earlier than tissue biopsies, offering complementary information for treatment planning.[207,208] *ALK* mutations are established oncogenic drivers in NSCLC, and although multiple selective *ALK* inhibitors are available, acquired resistance often leads to disease recurrence. Targeted sequencing of CTCs from patients resistant to crizotinib or lorlatinib identified not only known resistance-associated mutations, such as RTK–*KRAS* pathway alterations and *TP53* mutations, but also *ALK* compound mutations that were not detected in corresponding tissue biopsies. In addition, previously unreported *ALK*-independent bypass alterations involving pathways such as JAK/STAT were observed, illustrating the heterogeneity of resistance mechanisms to *ALK* inhibitors.[132] In contrast to *ALK*-targeted therapy, the mechanisms of resistance to *BRAF* inhibitors remain less well defined. In a pilot study of advanced NSCLC patients harboring $BRAF^{V600E}$ mutations, genomic sequencing of CTCs, matched tumor biopsies, and circulating cell-free DNA from patients resistant to dabrafenib with or without trametinib showed that only a single CTC retained the $BRAF^{V600E}$ mutation. Instead, CTCs showed enrichment for MAPK-related signaling, along with pathways involved in cell cycle regulation, DNA repair, and the immune response. These findings suggest that resistance in these patients was not driven by the persistence of the $BRAF^{V600E}$ mutation.[209] In metastatic melanoma, Hong et al. established short-term CTC cultures and performed WES in a patient with $BRAF^{V600E}$-mutant disease. They found that lipid biosynthesis pathways were highly activated in resistant CTC clones. Further analysis indicated that upregulation of SREBP-dependent lipid biosynthesis reduced intracellular iron levels, reactive oxygen species, and lipid peroxidation, thereby suppressing ferroptosis and contributing to resistance to *BRAF* inhibitors.[210] Endocrine therapy is widely used in estrogen receptor–positive (ER+) mBC, and mutations in *ESR1* are a well-recognized mechanism of resistance to aromatase inhibitors and gonadotropin-releasing hormone analogues. These mutations often exhibit substantial heterogeneity.[211] Paoletti et al. performed WES on 32 individual CTCs from a single patient and identified the *ESR1p.Y537S* mutation associated with endocrine resistance. Notably, they detected a unique CTC carrying a heterozygous *ESR1p.Y537S* mutation, and cells harboring this mutation showed enhanced proliferative responses to extremely low estrogen concentrations, underscoring the diversity of endocrine therapy resistance mechanisms.[207] In endocrine-refractory metastatic breast cancer, mitotic inhibitors such as docetaxel are commonly used. However, Horwitz et al. reported heterogeneous responses of cultured CTCs to docetaxel treatment. In a subset of CTCs, docetaxel induced the accumulation of CDKN1B protein, which blocked nuclear replication and drove cells into a reversible quiescent state. These cells resumed proliferation after drug withdrawal, contributing to tumor recurrence. Inhibition of AKT signaling reduced CDKN1B phosphorylation and effectively suppressed this resistance mechanism, thereby delaying disease relapse.[212]

In addition to direct analysis of patient-derived CTCs, ex vivo CTC culture and the establishment of CTC-derived xenograft (CDX) models provide important experimental platforms for studying drug resistance. Que et al.

established an NSCLC CTC cell line, CTC-TJH-01, and performed integrated transcriptomic and proteomic analyses. They found specific activation of the Src/FN1 signaling pathway in CTC clusters, which promoted cell–cell adhesion and resistance to environmental stress. Treatment with Src inhibitors disrupted CTC aggregation and invasion and reduced metastatic potential.[166,213] Stewart et al. conducted large-scale scRNA-seq (10X Genomics) of CDX models in small-cell lung cancer and reported that platinum-resistant models exhibited higher intratumoral heterogeneity scores. Drug resistance–related pathways were distributed across distinct subpopulations, and target gene expression shifted dynamically before and after treatment, providing insight into the high recurrence rate of this disease and supporting early combination therapy strategies.[214] Similarly, Suvilesh et al. established an NSCLC-derived CDX model and performed RNA-seq to assess treatment response. Their analysis revealed heterogeneous responses to carboplatin and paclitaxel, with resistant tumors showing upregulation of MYC target genes and c-MYC protein. Blocking MYC/MAX dimerization effectively reversed chemoresistance in this model.[215]

Prognosis assessment and risk of recurrence

CTCs are widely regarded as the cellular sourceof distant metastasis. Clinical studies have repeatedly reported an association between elevated CTC counts in peripheral blood and poor patient outcomes. In many cancer types, detection of five or more CTCs per 7.5 mL of blood is linked to reduced survival.[4,216-218] However, prognosis assessment based solely on CTC counts is limited. Cell number alone cannot reflect intratumoral heterogeneity or ongoing clonal evolution. Single-cell omics analysis addresses this limitation by enabling molecular analysis of individual CTCs, thereby providing additional information for evaluating recurrence risk and clinical outcome.

Genomic instability and CNAs detected in CTCs are closely associated with disease relapse and resistance to targeted therapies. Using single-cell sequencing technologies, these genomic features can be monitored over time, enabling dynamic assessment of tumor progression.[219] Chiu et al. performed whole-genome single-nucleotide polymorphism (SNP) and CNA profiling of CTCs from patients with metastatic melanoma. They observed that extensive genomic alterations in CTCs were associated with metastatic disease. Based on recurrence-related CNAs, they constructed a prognostic gene panel that included *CSMD2* and *CNTNAP5*. This panel successfully stratified melanoma patients according to recurrence risk.[220] In mCRPC, Malihi et al. analyzed 257 CTCs from 47 patients using WGS. Loss of tumor suppressor genes was more frequently detected in CTCs than in matched bulk samples. Patients whose CTCs showed concurrent loss of two or more genes, including *PTEN*, *RB1*, and *TP53*, had significantly shorter progression-free and overall survival. When genomic instability was further assessed using large-scale transition (LST) scores, longer survival was associated with lower instability and fewer tumor suppressor losses. Importantly, these associations were independent of CTC count, highlighting the prognostic value of CTC genomic features.[221] Similarly, Alves et al. conducted WES of CTCs from 29 patients with metastatic colorectal cancer. They introduced a mutation-allele tumor heterogeneity (MATH) score to quantify mutational diversity. Patients carrying non-silent mutations in *BCL9L* showed poorer overall survival, indicating that both mutational heterogeneity and specific gene alterations influence prognosis.[222] Beyond genome-wide alterations, mutations in individual cancer-related genes within CTCs also provide prognostic information. Markou et al. applied a sensitive mutation detection method and found that *PIK3CA* mutation status in CTCs could change during disease progression or relapse in breast cancer. The presence of *PIK3CA*-mutant CTCs was associated with reduced survival in metastatic patients.[223] In multiple myeloma, Lohr et al. examined 35 recurrent mutation sites and detected frequent alterations in *BRAF*, *TP53*, and *IRF4* in relapsed disease. They further showed that RNA-seq of *CCND1* and *CCND3* expression enabled molecular classification, supporting the use of CTC single-cell sequencing for disease stratification.[224] Gene expression patterns in CTCs are also linked to metastatic potential and survival. Ebright et al. combined genome-wide CRISPR activation screening in breast cancer CDX models with scRNA-seq of CTCs from metastatic breast cancer patients. They found that high expression of ribosomal protein genes correlated with shorter overall survival. Their data suggest that epithelial CTCs may promote metastasis through increased translational activity and enhanced proliferative capacity.[225] Kozuka et al. analyzed CTCs from metastatic colorectal cancer patients using stemness- and EMT-related markers together with scRNA-seq. They identified four CTC subtypes and reported that EMT-associated CTC populations were linked to shorter progression-free and overall survival. In contrast, purely epithelial CTCs showed no such association. *SPARC* and *ITGB1* were proposed as markers of EMT-related CTCs.[226] In pancreatic cancer, Rossi et al. and Nitschke et al. independently applied transcriptomic and proteomic analyses to CTCs and identified *GAS2L1* and *RARRES1* as genes associated with poor prognosis. Elevated expression of these genes was linked to reduced survival, suggesting their potential clinical relevance.[227,228]

In addition to intrinsic molecular features, interactions between CTCs and other circulating cells influence metastatic behavior. CTC-associated clusters include complexes formed with immune cells, such as neutrophils, as well as clusters composed solely of tumor cells. Szczerba et al. performed scRNA-seq and WES on isolated CTCs, CTC-neutrophil clusters, and multicellular CTC clusters from breast cancer patients and CDX models. They observed increased expression of cell cycle–related genes in CTC-neutrophil clusters. Their findings suggest that neutrophils directly support CTC proliferation and promote metastatic seeding. These clusters may therefore act as efficient metastatic units.[229] Hapach et al. investigated CTC clusters in vitro and identified two CTC subpopulations with distinct migratory capacities. In CDX models, however, the less migratory population showed greater metastatic potential. RNA sequencing revealed that this phenotype depended on E-cadherin expression, which promoted cluster formation and survival in circulation, thereby facilitating colonization of distant tissues.[230]

CHALLENGES AND PROSPECTS

Currently, clinical tumor classification largely relies on immunohistochemical testing of biopsy specimens. Although this method is well-established, such invasive procedures carry certain risks and often fail to provide timely diagnoses for early-stage tumors or tumors with unclear locations. Liquid biopsy technology, due to its non-invasive nature, enables continuous monitoring of tumor information in a patient's bodily fluids—such as peripheral blood, cerebrospinal fluid, and pleural effusion—and has demonstrated significant potential in clinical management. More than 20 years have passed since the FDA approved CellSearch for clinical testing of metastatic colorectal, breast, and prostate cancers in 2004. Liquid biopsy methods, represented by circulating tumor cell (CTC) counting, have gradually been validated in clinical practice as reliable molecular diagnostic and monitoring tools. In recent years, NGS technologies based on cfDNA/ctDNA have been approved by the FDA as companion diagnostic tools for targeted therapies for various cancers, such as Guardant360 CDx and FoundationOne Liquid CDx, which are used to monitor hundreds of genetic mutations in cancer patients. Compared to cfDNA/ctDNA, CTCs possess a complete set of genetic information, including DNA, RNA, proteins, and metabolites, which gives CTCs the potential for comprehensive characterization of tumor features. Thanks to advancements in single-cell sequencing technology, CTC-based liquid biopsy testing is currently transitioning from simple cell counting to molecular profiling. Although high-throughput sequencing and single-cell mass spectrometry can generate large-scale data sets unattainable through traditional methods, thereby accelerating clinical translation—numerous challenges must still be overcome before these approaches can be widely adopted (Figure 3).

More efficient CTC enrichment and single-cell isolation technology

Obtaining a sufficient number of high-quality CTC single cells is the most critical step in CTC single-cell omics analysis. Currently, established CTC enrichment methods still rely on strategies based primarily on EpCAM biochemical enrichment and biophysical enrichment. While the former offers higher purity, it suffers from partial CTC loss during enrichment. Later, though capable of bias-free CTC enrichment, is limited by the presence of mononuclear cells such as white blood cells, resulting in lower enrichment purity and loss of informative cells during subsequent single-cell analysis. Balancing CTC recovery rate and enrichment purity remains the top priority in the current development of CTC enrichment methods. Furthermore, current methods for obtaining single CTCs still rely on the combined use of CTC

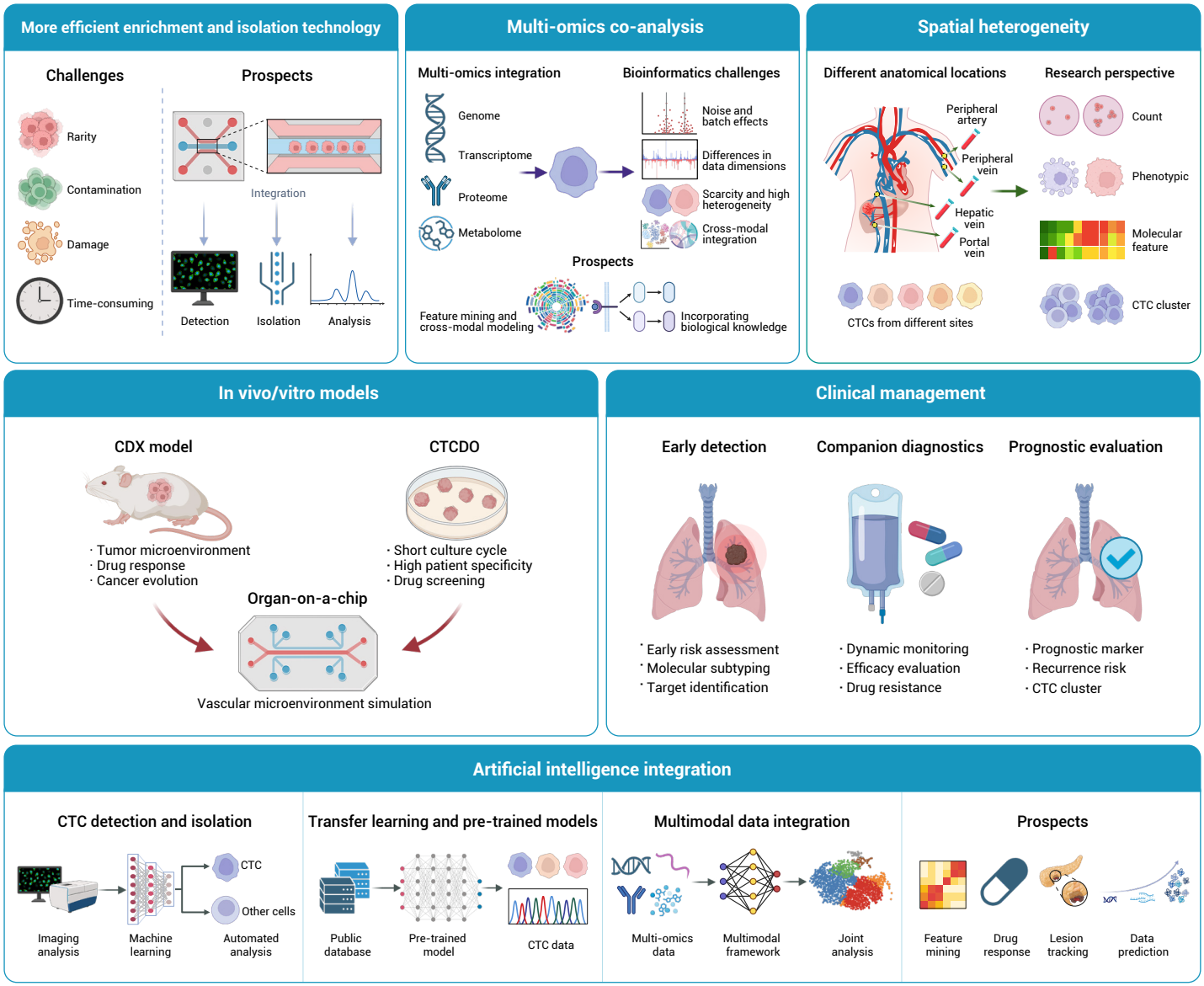

Figure 3. Challenges and prospects of single CTC omics technologies and clinical applications.

enrichment and single-cell sorting technologies. The overall workflow is relatively complex, time-consuming, and prone to causing cell damage. In recent years, with the widespread application of microfluidic technology in CTC enrichment, some technologies, such as CTCelect,[43] have integrated CTC enrichment and single-cell sorting into a single process, achieving automated single-cell separation of CTCs. Furthermore, certain single-cell omics analysis technologies, such as DMF-DR-seq,[143] iProChip,[171] and microfluidic SERS,[119] are also performed on microfluidic chips. In the future, it is anticipated that microfluidic technology will be combined with single-cell separation techniques—such as FACS and SERS—as well as other single-cell omics analysis technologies. This integration of CTC enrichment, single-cell separation, and single-cell detection and analysis is expected to make CTC acquisition more convenient and efficient.

Single CTC multi-omics co-analysis

Although numerous single-cell omics analysis technologies for CTC genomics, transcriptomics, proteomics, and metabolomics have emerged, only DMF-DR-seq (genomics-transcriptomics) and Uni-C (genomics-3D genomics) address CTC multi-omics analysis, which severely limits the ability to obtain comprehensive molecular information from individual CTCs.[231] Although traditional single-cell multi-omics analysis technologies such as CITE-seq and REAP-seq can simultaneously analyze the transcriptome and cell surface proteins, they rely on high-throughput scRNA platforms, and the rarity of CTCs limits their further application in this context.[232,233] Certain technologies, such as nanoSPLITS and scSTAP, utilize nanodroplet fractionation to divide the contents of a single cell into multiple groups for separate multi-omics detection and analysis.[234,235] Although these techniques are suitable for in-depth multi-omics analysis of single cells, the high heterogeneity of CTCs means that the loss of a substantial fraction of cellular material during sample partitioning may prevent accurate characterization of CTCs' biological features. Technologies such as OCAM, scPMA, and scMAPs utilize the biochemical characteristics of different intracellular components and their responses to various treatment methods to enable sequential multi-omics detection within a single cell.[236,237] This strategy effectively overcomes the sample scarcity and heterogeneity of CTCs and is expected to be further applied in CTC multi-omics analysis in the future, jointly revealing the molecular landscape within CTCs.

Apart from CTC multi-omics detection technologies, CTC multi-omics bioinformatics processing also poses a major challenge in multi-omics co-analysis due to differences in data dimensions across omics, noise introduced by detection methods, and variations in analytical approaches. Currently, bioinformatics tools used for single-cell multi-omics co-analysis primarily focus on (1) dimensionality reduction, (2) batch correction, (3) clustering, (4) classification, (5) feature selection, (6) imputation, and (7) spatial

registration.[238,239] However, most of these tools are designed for high-throughput single-cell multi-omics detection technologies and are not suitable for analyzing low-throughput, deep single-cell multi-omics data. Although some researchers have utilized WNN algorithms for integrated analysis of different modalities or employed machine learning models to process and analyze multi-omics data, these approaches overlook cross-modal biological characteristics (such as the time lag between the transcriptome and proteome). Furthermore, due to the scarcity of CTC samples and their high internal heterogeneity, it is difficult to train a universal analytical framework.[235] To address these issues, we believe that future CTC multi-omics bioinformatics analysis should shift from being data-driven to knowledge-driven. Rather than merely performing dimensionality reduction and analysis on data from different omics modalities, it should incorporate existing biological knowledge—such as gene function, pathway classification, and gene interactions—to achieve a precise, comprehensive interpretation of CTC biological information.

Spatial heterogeneity of CTC

CTCs are tumor cells that have detached from primary or metastatic tumor tissue and entered the circulatory system; like tumor tissue, CTCs also exhibit spatiotemporal heterogeneity. The temporal heterogeneity of CTCs has been reported in numerous studies, covering aspects ranging from cell count to phenotype, from early to late stages of tumor development, and at different time points before and after tumor treatment. However, there is a scarcity of research on the spatial heterogeneity of CTCs, primarily because the vast majority of existing studies capture CTCs through blood sampling from peripheral venous blood, thereby overlooking the inherent spatial heterogeneity of CTCs. Given the complexity of the blood microenvironment, CTCs are exposed to shear stress, immune cell binding, and the formation of cellular aggregates as they circulate through the bloodstream. This spatial heterogeneity may result in CTCs in peripheral venous blood failing to represent the most representative characteristics of the tumor tissue. Relevant studies have reported that CTC counts in the portal vein are significant predictors of liver metastasis within 6 months post-surgery for gallbladder or pancreatic cancer.[240] In PC patients, the percentage of M-CTCs in portal venous blood is also a significant prognostic biomarker.[241] Furthermore, CTC and circulating tumor microemboli burden in hepatic veins and peripheral circulation predicted postoperative lung metastasis and intrahepatic recurrence, respectively.[242] Based on these findings, tumor-proximal liquid biopsy is proposed to improve the diagnostic and prognostic performance of circulating tumor cells.[243] However, most current studies on the spatial heterogeneity of CTCs rely primarily on CTC counts, making it difficult to capture their biological characteristics. By leveraging single-cell omics analysis techniques, we can further model the molecular features of CTC spatial heterogeneity and investigate deeper biomedical questions. For example, Sun et al. compared the transcriptomic profiles of CTCs captured from different vascular sites in HCC patients and revealed that CCL5 acts as a key mediator promoting CTC immune evasion by recruiting and regulating regulatory T cells (Tregs) via the TGF-β1-p38-MAX signaling pathway.[244] Currently, research on CTC spatial heterogeneity is largely confined to small-scale studies, and no large-scale clinical studies have yet confirmed the advantages of tumor-proximal liquid biopsy over peripheral venous blood biopsy. In the future, single-cell omics detection and analysis technologies should be utilized to further investigate the optimal anatomical sites for CTC sampling across different cancer types, disease stages, and research objectives. Furthermore, in recent years, an increasing number of studies have focused on the role of CTC clusters in tumor metastasis and prognostic evaluation. In addition to the spatial heterogeneity of CTCs, the spatiotemporal heterogeneity of CTC clusters should also be further investigated in the future.

Combined with in vivo/vitro models

Traditional patient-derived xenograft (PDX) models require the collection of tumor tissue via surgery or biopsy, which is then implanted into immunodeficient mice through heterotopic or orthotopic. Although these models can preserve tumor characteristics to the greatest extent in vitro, the need for tumor biopsy severely limits their further application. In recent years, CTC-derived xenograft (CDX) models—which use circulating tumor cells (CTCs) instead of traditional biopsy tissue for transplantation into immunodeficient mice—have proven to be reliable models for disease modeling and drug screening.[245] Furthermore, when combined with single-cell omics analysis of CTCs, these models enable functional simulation at the molecular level. On the one hand, CDX models can simulate the complexity of tumors in vivo and in vitro, and their response to standard chemotherapy is similar to that of the donor patient receiving the same treatment. This characteristic, combined with single-cell analysis, facilitates more accurate modeling of tumor responses to specific therapies, thereby identifying drug targets and pathways with greater therapeutic potential. On the other hand, constructing CDX models using CTCs collected at different spatiotemporal points for single-cell omics analysis can better recreate the tumor evolution process, further deepening our understanding of tumor metastasis mechanisms.[246]

Although CDX models offer unique advantages in studying tumor genetic profiles and evolutionary mechanisms, they often fail to fully replicate the parent tumor. This limitation restricts the use of many anticancer drugs, including those with poor solubility or stability, suboptimal pharmacokinetics, insufficient tumor specificity, and severe side effects. Additionally, the development cycle is lengthy, success rates are low, and such models are not suitable for precision medicine in clinical settings. CTCs have been shown to retain the characteristics of cancer stem cells (CSCs) and can undergo phenotypic transitions similar to those of CSCs. By leveraging this feature, CTC-derived organoid models (CTCDO) can be established. Compared to traditional tumor tissue-derived organoids, CTCDO models retain the unique biological characteristics of both the primary tumor and CTCs, enabling a more accurate in vitro reflection of the properties of CTCs in the bloodstream and thereby further elucidating the mechanisms of early metastasis.[247,248] Furthermore, compared to CDX, CTCDO has a shorter culture cycle (~2 weeks) and stronger patient specificity. Combined with multi-omics analysis, it enables targeted high-throughput drug screening for patients in vitro, supporting precision diagnosis and treatment of tumors. However, whether it is CDX or CTCDO, culture success rates are often low due to the challenges of CTC acquisition—including low yield, scarcity, and low purity. Furthermore, there is currently no standardized protocol for constructing CTC in vitro models, particularly for CTCDO. These models often overlook the fluid dynamics of blood and the complexity of the microenvironment during cultivation, failing to fully simulate tumor characteristics. In the future, with the emergence of more efficient CTC enrichment strategies, researchers will need to develop a targeted, effective standard protocol tailored to different cancer types and application scenarios. Combined with single-cell multi-omics analysis of CTCs, this will advance precision diagnosis and treatment of tumors. During CTCDO culture, researchers should also consider incorporating blood cells such as neutrophils. Combining organ-on-a-chip technology to simulate the physical vascular environment with single-cell omics analysis would enable more accurate modeling of CTC survival and metastasis, thereby facilitating the translation of this approach from the laboratory to clinical practice.

Combined with artificial intelligence

Artificial intelligence (AI) has advanced rapidly in the biomedical field in recent years, and combining AI with existing single-cell analysis techniques holds promise for ushering in a new paradigm in CTC single-cell analysis. Traditional CTC identification relies on immunofluorescence staining analysis or visual identification of cellular features; the former often fails to fully identify CTCs due to their low abundance and weak fluorescence intensity, while the latter frequently results in misidentification because CTCs resemble white blood cells in morphology. AI algorithms, particularly those that incorporate multiple markers for identification, offer new solutions to these challenges. Traditional machine learning models, such as random forests and XGBoost, and deep learning models, such as convolutional neural networks and Transformers, can extract more detailed features—such as fluorescence intensity, cell radius, and nuclear size—significantly improving CTC detection efficiency and accuracy.[249-251] When combined with CTC enrichment and sorting technologies, this approach holds promise for achieving efficient CTC detection and analysis without relying on any biochemical methods.[252] Furthermore, CTC samples are scarce and highly heterogeneous. Compared to traditional single-cell omics analysis of tumor tissues, the available data

volume is limited, which severely restricts further biological analysis of CTCs. By leveraging the similarities between CTCs and tumor cells and employing transfer learning methods, the shortcomings of insufficient CTC sample data can be effectively addressed. By leveraging pre-trained models such as Geneformer, scGPT, and CTC-Tracer in conjunction with specific CTC single-cell detection and analysis data, researchers can not only address the dropout effects and high noise associated with single-cell omics using existing knowledge but also perform cell type annotation, multi-batch integration, multi-omic integration, perturbation response prediction, and lesion tracking even with limited data.[193,253,254] However, single-cell omics data are often characterized by high dimensionality, heterogeneity, sparsity, and nonlinear variations. Traditional statistical methods often struggle to uncover the underlying biological mechanisms. Although algorithms like WNN can facilitate joint analysis of different omics data, their capacity for complex modeling is limited. By leveraging deep learning models—particularly multimodal learning frameworks based on autoencoders, variational inference, generative adversarial networks (GANs), and Transformer architectures—it is possible to more effectively capture the complex nonlinear relationships between different omics, thereby achieving higher-precision molecular analysis of CTCs.[255-259] Furthermore, integrating existing multi-omics datasets with deep learning enables the cross-referencing of omics data from different sources, thereby addressing the limitations of insufficient clinical CTC data. In the future, leveraging large-scale public single-cell databases alongside specific CTC single-cell omics data, AI technology is expected to enable the prediction of other omics data from a single omics dataset, thereby enhancing the accuracy of CTC single-cell analysis. Furthermore, by utilizing specific perturbation models, it is expected to simulate in vitro the multi-omics responses of CTCs under drug pressure or immune attack, thereby advancing precision medicine in oncology.[260]

Clinical management

As CTC single-cell omics technologies mature and are integrated with complementary experimental approaches, it will become an essential part of clinical patient management. Because CTCs are easy to obtain and carry comprehensive information, they can play a unique role at every stage of tumor diagnosis and treatment. Looking ahead, we anticipate CTC single-cell omics analysis to become a routine clinical monitoring tool and a key component of precision oncology, providing comprehensive molecular profiling. In early cancer diagnosis and treatment: i. Combining CTC counts with conventional imaging can help distinguish benign from malignant tumors in early-stage patients, accurately assess tumor risk, and compensate for the limited sensitivity of imaging. ii. Complete characterization of genetic and molecular expression in early-stage patients' CTCs—using genomic, transcriptomic, proteomic, and metabolomic data—allows preliminary diagnosis based on broader CTC categories, such as classic EMT-related subtypes or PD-L1/2-related subtypes relevant to immunotherapy. iii. Focusing on specific gene expression: current cancer molecular typing relies on immunohistochemistry (IHC), which has drawbacks like difficulty in sample collection and long turnaround times. In early diagnosis and treatment, using CTC gene expression information can precisely identify molecules linked to specific cancer subtypes—such as *ER*, *HER2*, and *ALK*—compensating for the limitations of traditional clinical classification. iv. Finding targeted therapy targets: targeted therapy is a key cancer treatment. Selecting targeted drugs based on specific gene mutations can substantially improve treatment outcomes. CTC genomic sequencing can effectively identify actionable genetic targets, provide mutation profiles, and help select suitable drugs. In the future, combining this with high-throughput drug screening in CDX models may enable accurate prediction of treatment outcomes. During clinical treatment: i. Continuously monitor changes in patient CTCs during therapy to track shifts in tumor molecular profiles, evaluate treatment effectiveness, assess therapeutic approaches, and adjust treatment plans accordingly for effective companion diagnostics. ii. Combine known drug-resistance mutation data with targeted, ongoing monitoring of specific CTC molecules to predict patient resistance early. Integrate this with clinical information to optimize therapeutic strategies. In prognosis evaluation: i. Screen for specific prognostic markers (e.g., *PTEN*, *TP53*) to assess treatment response early. Use bioinformatics tools to estimate recurrence risk. ii. Apply highly sensitive detection of metastasis-related biomarkers and develop tailored molecular detection panels for different cancer types and metastatic sites to improve metastasis risk quantification. iii. Characterize features specific to CTC clusters. Integrate cluster classification, metastatic potential, and specific gene expression with other indicators to improve prognostic monitoring and assessment.

REFERENCES

1. Xie W., Suryaprakash S., Wu C., et al. (2023). Trends in the use of liquid biopsy in oncology. *Nat. Rev. Drug Discov.* **22**:612–613. DOI:10.1038/d41573-023-00111-y
2. Zhu Z., Hu E., Shen H., et al. (2023). The functional and clinical roles of liquid biopsy in patient-derived models. *J. Hematol. Oncol.* **16**:36. DOI:10.1186/s13045-023-01433-5
3. Yu W., Hurley J., Roberts D., et al. (2021). Exosome-based liquid biopsies in cancer: opportunities and challenges. *Ann. Oncol.* **32**:466–477. DOI:10.1016/j.annonc.2021.01.074
4. Lin D., Shen L., Luo M., et al. (2021). Circulating tumor cells: biology and clinical significance. *Signal Transduct. Target. Ther.* **6**:404. DOI:10.1038/s41392-021-00817-8
5. Ring A., Nguyen-Strauli B. D., Wicki A., et al. (2023). Biology, vulnerabilities and clinical applications of circulating tumour cells. *Nat. Rev. Cancer* **23**:95–111. DOI:10.1038/s41568-022-00536-4
6. Lawrence R., Watters M., Davies C. R., et al. (2023). Circulating tumour cells for early detection of clinically relevant cancer. *Nat. Rev. Clin. Oncol.* **20**:487–500. DOI:10.1038/s41571-023-00781-y
7. Diamantopoulou Z., Castro-Giner F., Schwab F. D., et al. (2022). The metastatic spread of breast cancer accelerates during sleep. *Nature* **607**:156–162. DOI:10.1038/s41586-022-04875-y
8. Sharifi M. N., Sperger J. M., Taylor A. K., et al. (2025). High-purity CTC RNA sequencing identifies prostate cancer lineage phenotypes prognostic for clinical outcomes. *Cancer Discov.* DOI:10.1158/2159-8290.CD-24-1509 %J Cancer Discovery.
9. Rawal S., Yang Y. P., Cote R., et al. (2017). Identification and Quantitation of Circulating Tumor Cells. *Annu. Rev. Anal. Chem. (Palo Alto Calif.)* **10**:321–343. DOI:10.1146/annurev-anchem-061516-045405
10. Li Y., Xu F., Qiao J., et al. (2023). Into the microscale: Low-input sequencing technologies and applications in medicine. *Innov. Med.* **1**:100041. DOI:10.59717/j.xinn-med.2023.100041.
11. Zhang L., Li Z., Skrzypczynska K. M., et al. (2020). Single-Cell Analyses Inform Mechanisms of Myeloid-Targeted Therapies in Colon Cancer. *Cell* **181**:442-459 e429. DOI:10.1016/j.cell.2020.03.048.
12. Lim B., Lin Y. and Navin N. (2020). Advancing Cancer Research and Medicine with Single-Cell Genomics. *Cancer Cell* **37**:456–470. DOI:10.1016/j.ccell.2020.03.008
13. Alix-Panabieres C. and Pantel K. (2025). Advances in liquid biopsy: From exploration to practical application. *Cancer Cell* **43**:161–165. DOI:10.1016/j.ccell.2024.11.009
14. Nikanjam M., Kato S. and Kurzrock R. (2022). Liquid biopsy: current technology and clinical applications. *J. Hematol. Oncol.* **15**:131. DOI:10.1186/s13045-022-01351-y
15. Gao Y., Fan W.-H., Song Z., et al. (2021). Comparison of circulating tumor cell (CTC) detection rates with epithelial cell adhesion molecule (EpCAM) and cell surface vimentin (CSV) antibodies in different solid tumors: a retrospective study. *PeerJ* **9**. DOI:10.7717/peerj.10777.
16. Descamps L., Le Roy D. and Deman A. L. (2022). Microfluidic-Based Technologies for CTC Isolation: A Review of 10 Years of Intense Efforts towards Liquid Biopsy. *Int. J. Mol. Sci.* **23**. DOI:10.3390/ijms23041981.
17. Ding P., Wang Z., Wu Z., et al. (2020). Aptamer-based nanostructured interfaces for the detection and release of circulating tumor cells. *J. Mater. Chem. B* **8**:3408–3422. DOI:10.1039/c9tb02457c
18. Gabriel M. T., Calleja L. R., Chalopin A., et al. (2016). Circulating Tumor Cells: A Review of Non-EpCAM-Based Approaches for Cell Enrichment and Isolation. *Clin. Chem.* **62**:571–581. DOI:10.1373/clinchem.2015.249706
19. Ahn J. C., Teng P. C., Chen P. J., et al. (2021). Detection of Circulating Tumor Cells and Their Implications as a Biomarker for Diagnosis, Prognostication, and Therapeutic Monitoring in Hepatocellular Carcinoma. *Hepatology* **73**:422–436. DOI:10.1002/hep.31165
20. Rahmanian M., Sartipzadeh Hematabad O., Askari E., et al. (2023). A micropillar array-based microfluidic chip for label-free separation of circulating tumor cells: The best micropillar geometry. *J. Adv. Res.* **47**:105–121. DOI:10.1016/j.jare.2022.08.005
21. Cheng S.-B., Chen M.-M., Wang Y.-K., et al. (2021). A Three-Dimensional Conductive Scaffold Microchip for Effective Capture and Recovery of Circulating Tumor Cells with High Purity. *Anal. Chem.* **93**:7102–7109. DOI:10.1021/acs.analchem.1c00785
22. Arora S., D'Souza A., Aland G., et al. (2022). Antibody mediated cotton-archetypal substrate for enumeration of circulating tumor cells and chemotherapy outcome in 3D tumors. *Lab Chip* **22**:1519–1530. DOI:10.1039/d2lc00004k
23. Chen J., Lin Z., Wan X., et al. (2025). Magnetic Genetically Engineered Cells Constructed via Microfluidic Squeezing for Highly Efficient Capture of Circulating Tumor Cells. *Small* **21**. DOI:10.1002/smll.202503795.
24. Zhang C., Zhang X., Zhang J., et al. (2026). Multivalent capture of circulating tumor cells using tetrahedral DNA framework-targeted nanomagnetic beads integrated

microfluidic device. *Colloids Surf. B. Biointerfaces* **257**. DOI:10.1016/j.colsurfb.2025.115142.
25. Pedrosa V. A., Chen K., George T. J., et al. (2023). Gold Nanoparticle-Based Microfluidic Chips for Capture and Detection of Circulating Tumor Cells. *Biosensors* **13**. DOI:10.3390/bios13070706.
26. Cao Y., Xu Y., Luo T., et al. (2025). Nanotentacle-Armed Flexible Affinity Interface Enables Streamlined Capture, Labeling, and Release of Circulating Tumor Cells. *ACS Nano* **19**:30115–30124. DOI:10.1021/acsnano.5c06198
27. Xiang Y., Zhang H., Lu H., et al. (2023). Bioorthogonal Microbubbles with Antifouling Nanofilm for Instant and Suspended Enrichment of Circulating Tumor Cells. *ACS Nano* **17**:9633–9646. DOI:10.1021/acsnano.3c03194
28. Zhou X., Zhang Y., Kang K., et al. (2022). Controllable Environment Protein Corona-Disguised Immunomagnetic Beads for High-Performance Circulating Tumor Cell Enrichment. *Anal. Chem.* **94**:4650–4657. DOI:10.1021/acs.analchem.1c04587
29. Zhang Y., Han C., Li K., et al. (2025). Single circulating tumor cell sequencing based on improved high-porosity membranes and nanoporous microchambers. *Biosens. Bioelectron.* **276**:117263. DOI:10.1016/j.bios.2025.117263
30. Pan Y., Wang Z., Ma J., et al. (2022). Folic Acid-Modified Fluorescent-Magnetic Nanoparticles for Efficient Isolation and Identification of Circulating Tumor Cells in Ovarian Cancer. *Biosensors* **12**. DOI:10.3390/bios12030184.
31. Woo H. J., Kim S.-H., Kang H. J., et al. (2022). Continuous centrifugal microfluidics (CCM) isolates heterogeneous circulating tumor cells via full automation. *Theranostics* **12**:3676–3689. DOI:10.7150/thno.72511
32. Liu Z., Huang Y., Liang W., et al. (2021). Cascaded filter deterministic lateral displacement microchips for isolation and molecular analysis of circulating tumor cells and fusion cells. *Lab Chip* **21**:2881–2891. DOI:10.1039/d1lc00360g
33. Yu Y., Zhang Y., Chen Y., et al. (2023). Floating Immunomagnetic Microspheres for Highly Efficient Circulating Tumor Cell Isolation under Facile Magnetic Manipulation. *ACS Sensors* **8**:1858–1866. DOI:10.1021/acssensors.3c00420
34. Jiang W., Han L., Li G., et al. (2023). Bait-trap chip for accurate and ultrasensitive capture of living circulating tumor cells. *Acta Biomater.* **162**:226–239. DOI:10.1016/j.actbio.2023.03.019
35. Abdulla A., Zhang T., Li S., et al. (2022). Integrated microfluidic single-cell immunoblotting chip enables high-throughput isolation, enrichment and direct protein analysis of circulating tumor cells. *Microsyst. Nanoeng.* **8**. DOI:10.1038/s41378-021-00342-2.
36. Wang Z., Wu Z., Sun N., et al. (2021). Antifouling hydrogel-coated magnetic nanoparticles for selective isolation and recovery of circulating tumor cells. *J. Mater. Chem. B* **9**:677–682. DOI:10.1039/d0tb02380a
37. Jiang X., Zhang X., Guo C., et al. (2022). Protein corona-coated immunomagnetic nanoparticles with enhanced isolation of circulating tumor cells. *Nanoscale* **14**:8474–8483. DOI:10.1039/d2nr01568d
38. Zhou X., Zhang Y., Kang K., et al. (2022). Artificial cell membrane camouflaged immunomagnetic nanoparticles for enhanced circulating tumor cell isolation. *J. Mater. Chem. B* **10**:3119–3125. DOI:10.1039/d1tb02676c
39. Chu Q., Mu W., Lan C., et al. (2021). High-Specific Isolation and Instant Observation of Circulating Tumour Cell from HCC Patients via Glypican-3 Immunomagnetic Fluorescent Nanodevice. *Int. J. Nanomed. Volume* **16**:4161–4173. DOI:10.2147/ijn.S307691
40. Song J. W., Suh J., Lee S. W., et al. (2022). Isolation and Genomic Analysis of Single Circulating Tumor Cell Using Human Telomerase Reverse Transcriptase and Desmoglein-2. *Small Methods* **6**:e2100938. DOI:10.1002/smtd.202100938
41. Cha J., Cho H., Chung J.-S., et al. (2023). Effective Circulating Tumor Cell Isolation Using Epithelial and Mesenchymal Markers in Prostate and Pancreatic Cancer Patients. *Cancers (Basel)* **15**. DOI:10.3390/cancers15102825.
42. Ding P., Wang Z., Wu Z., et al. (2021). Tannic Acid (TA)-Functionalized Magnetic Nanoparticles for EpCAM-Independent Circulating Tumor Cell (CTC) Isolation from Patients with Different Cancers. *ACS Appl. Mater. Interfaces* **13**:3694–3700. DOI:10.1021/acsami.0c20916
43. Stiefel J., Freese C., Sriram A., et al. (2022). Characterization of a novel microfluidic platform for the isolation of rare single cells to enable CTC analysis from head and neck squamous cell carcinoma patients. *Eng. Life Sci.* **22**:391–406. DOI:10.1002/elsc.202100133
44. Sun Y. and Sethu P. (2018). Low-stress Microfluidic Density-gradient Centrifugation for Blood Cell Sorting. *Biomed. Microdevices* **20**. DOI:10.1007/s10544-018-0323-3.
45. Eslami S. Z., Cortes-Hernandez L. E., Thomas F., et al. (2022). Functional analysis of circulating tumour cells: the KEY to understand the biology of the metastatic cascade. *Br. J. Cancer* **127**:800–810. DOI:10.1038/s41416-022-01819-1
46. Danova M., Torchio M. and Mazzini G. (2011). Isolation of rare circulating tumor cells in cancer patients: technical aspects and clinical implications. *Expert Rev. Mol. Diagn.* **11**:473–485. DOI:10.1586/erm.11.33
47. Park J. M., Kim M. S., Moon H. S., et al. (2014). Fully automated circulating tumor cell isolation platform with large-volume capacity based on lab-on-a-disc. *Anal. Chem.* **86**:3735–3742. DOI:10.1021/ac403456t
48. Huang Q., Wang F. B., Yuan C. H., et al. (2018). Gelatin Nanoparticle-Coated Silicon Beads for Density-Selective Capture and Release of Heterogeneous Circulating Tumor Cells with High Purity. *Theranostics* **8**:1624–1635. DOI:10.7150/thno.23531
49. Harouaka R. A., Nisic M. and Zheng S. Y. (2013). Circulating tumor cell enrichment based on physical properties. *Journal of Laboratory Automation* **18**:455–468. DOI:10.1177/2211068213494391
50. Ferreira M. M., Ramani V. C. and Jeffrey S. S. (2016). Circulating tumor cell technologies. *Mol. Oncol.* **10**:374–394. DOI:10.1016/j.molonc.2016.01.007
51. Ciccioli M., Kim K., Khazan N., et al. (2024). Identification of circulating tumor cells captured by the FDA-cleared Parsortix® PC1 system from the peripheral blood of metastatic breast cancer patients using immunofluorescence and cytopathological evaluations. *J. Exp. Clin. Cancer Res.* **43**. DOI:10.1186/s13046-024-03149-x.
52. Miller M. C., Robinson P. S., Wagner C., et al. (2018). The Parsortix™ Cell Separation System—A versatile liquid biopsy platform. *Cytom. A* **93**:1234–1239. DOI:10.1002/cyto.a.23571
53. Zabaglo L., Ormerod M. G., Parton M., et al. (2003). Cell filtration - laser scanning cytometry for the characterisation of circulating breast cancer cells. *Cytom. A* **55A**:102–108. DOI:10.1002/cyto.a.10071.
54. Shirai K., Guan G., Meihui T., et al. (2022). Hybrid double-spiral microfluidic chip for RBC-lysis-free enrichment of rare cells from whole blood. *Lab Chip* **22**:4418–4429. DOI:10.1039/d2lc00713d
55. Zhou J., Tu C., Liang Y., et al. (2018). Isolation of cells from whole blood using shear-induced diffusion. *Sci. Rep.* **8**. DOI:10.1038/s41598-018-27779-2.
56. Zhao Y.-N., Zhang X., Bai J.-J., et al. (2024). Inertial and Deterministic Lateral Displacement Integrated Microfluidic Chips for Epithelial–Mesenchymal Transition Analysis. *Anal. Chem.* **96**:18187–18194. DOI:10.1021/acs.analchem.4c04366
57. Wang S., Xu Q., Zhang Z., et al. (2023). Reverse flow enhanced inertia pinched flow fractionation. *Lab Chip* **23**:4324–4333. DOI:10.1039/d3lc00473b
58. Zhou J., Kulasinghe A., Bogseth A., et al. (2019). Isolation of circulating tumor cells in non-small-cell-lung-cancer patients using a multi-flow microfluidic channel. *Microsyst. Nanoeng.* **5**. DOI:10.1038/s41378-019-0045-6.
59. Gascoyne P. R., Wang X. B., Huang Y., et al. (1997). Dielectrophoretic Separation of Cancer Cells from Blood. *IEEE Transactions on Industry Applications* **33**:670–678. DOI:10.1109/28.585856
60. Becker F. F., Wang X. B., Huang Y., et al. (1995). Separation of human breast cancer cells from blood by differential dielectric affinity. *Proc. Natl. Acad. Sci. U. S. A.* **92**:860–864. DOI:10.1073/pnas.92.3.860
61. Allard W. J., Matera J., Miller M. C., et al. (2004). Tumor Cells Circulate in the Peripheral Blood of All Major Carcinomas but not in Healthy Subjects or Patients With Nonmalignant Diseases. *Clin. Cancer Res.* **10**:6897–6904. DOI:10.1158/1078-0432.CCR-04-0378 %J Clinical Cancer Research. DOI:10.1158/1078-0432.CCR-04-0378%JClinicalCancerResearch
62. Marsavela G., Aya-Bonilla C. A., Warkiani M. E., et al. (2018). Melanoma circulating tumor cells: Benefits and challenges required for clinical application. *Cancer Lett.* **424**:1–8. DOI:10.1016/j.canlet.2018.03.013
63. Liu X., Li J., Cadilha B. L., et al. (2019). Epithelial-type systemic breast carcinoma cells with a restricted mesenchymal transition are a major source of metastasis. *Sci. Adv.* **5**:eaav4275. DOI. DOI:10.1126/sciadv.aav4275
64. Hodan R., Gupta S., Weiss J. M., et al. (2024). Genetic/Familial High-Risk Assessment: Colorectal, Endometrial, and Gastric, Version 3.2024, NCCN Clinical Practice Guidelines In Oncology. *J. Natl. Compr. Canc. Netw.* **22**:695-711. DOI:10.6004/jnccn.2024.0061.
65. Eibl R. H. and Schneemann M. (2021). Liquid Biopsy and Primary Brain Tumors. *Cancers (Basel)* **13**. DOI:10.3390/cancers13215429.
66. Gorin M. A., Verdone J. E., van der Toom E., et al. (2017). Circulating tumour cells as biomarkers of prostate, bladder, and kidney cancer. *Nat. Rev. Urol.* **14**:90–97. DOI:10.1038/nrurol.2016.224
67. Loibl S., Poortmans P., Morrow M., et al. (2021). Breast cancer. *Lancet* **397**:1750–1769. DOI:10.1016/S0140-6736(20)32381-3
68. Yu M., Bardia A., Wittner B. S., et al. (2013). Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition. *Science* **339**:580–584. DOI. DOI:10.1126/science.1228522
69. Markou A. and Lianidou E. S. (2011). Circulating Tumor Cells in Breast Cancer: Detection Systems, Molecular Characterization, and Future Challenges. *Clin. Chem.* **57**:1242–1255. DOI:10.1373/clinchem.2011.165068.
70. Zhou J., Zhu X., Wu S., et al. (2020). Epithelial-mesenchymal transition status of circulating tumor cells in breast cancer and its clinical relevance. *Cancer Biol. Med.* **17**:169–180. DOI:10.20892/j.issn.2095-3941.2019.0118
71. Odintsov I. and Sholl L. M. (2024). Prognostic and predictive biomarkers in non-small cell lung carcinoma. *Pathology* **56**:192–204. DOI:10.1016/j.pathol.2023.11.006
72. Andrikou K., Rossi T., Verlicchi A., et al. (2023). Circulating Tumour Cells: Detection and Application in Advanced Non-Small Cell Lung Cancer. *Int. J. Mol. Sci.* **24**. DOI:10.3390/ijms242216085.
73. Scharpenseel H., Hanssen A., Loges S., et al. (2019). EGFR and HER3 expression in circulating tumor cells and tumor tissue from non-small cell lung cancer patients. *Sci. Rep.* **9**:7406. DOI:10.1038/s41598-019-43678-6
74. Chen L., Peng M., Li N., et al. (2018). Combined use of EpCAM and FRα enables the high-efficiency capture of circulating tumor cells in non-small cell lung cancer. *Sci. Rep.* **8**. DOI:10.1038/s41598-018-19391-1.
75. Welsch E., Holzer B., Schuster E., et al. (2024). Prognostic significance of circulating tumor cells and tumor related transcripts in small cell lung cancer: A step further to clinical implementation. *Int. J. Cancer* **154**:2189–2199. DOI:10.1002/ijc.34886

76. Hamilton G., Hochmair M., Rath B., et al. (2016). Small cell lung cancer: Circulating tumor cells of extended stage patients express a mesenchymal-epithelial transition phenotype. *Cell Adh. Migr.* **10**:360–367. DOI:10.1080/19336918.2016.1155019
77. Papakonstantinou D., Roumeliotou A., Pantazaka E., et al. (2024). Integrative analysis of circulating tumor cells (CTCs) and exosomes from small-cell lung cancer (SCLC) patients: a comprehensive approach. *Mol. Oncol.* DOI:10.1002/1878-0261.13765.
78. Hou J.-M., Krebs M. G., Lancashire L., et al. (2012). Clinical Significance and Molecular Characteristics of Circulating Tumor Cells and Circulating Tumor Microemboli in Patients With Small-Cell Lung Cancer. *Signal Transduct. Target. Ther.* **30**:525–532. DOI:10.1200/jco.2010.33.3716
79. Ricordel C., Chaillot L., Vlachavas E. I., et al. (2023). Genomic characteristics and clinical significance of CD56+ circulating tumor cells in small cell lung cancer. *Sci. Rep.* **13**:3626. DOI:10.1038/s41598-023-30536-9
80. Shou X., Li Y., Hu W., et al. (2019). Six-gene Assay as a new biomarker in the blood of patients with colorectal cancer: establishment and clinical validation. *Mol. Oncol.* **13**:781–791. DOI:10.1002/1878-0261.12427
81. Satelli A., Mitra A., Brownlee Z., et al. (2015). Epithelial-mesenchymal transitioned circulating tumor cells capture for detecting tumor progression. *Clin. Cancer Res.* **21**:899–906. DOI:10.1158/1078-0432.CCR-14-0894
82. Hinz S., Hendricks A., Wittig A., et al. (2017). Detection of circulating tumor cells with CK20 RT-PCR is an independent negative prognostic marker in colon cancer patients - a prospective study. *BMC Cancer* **17**:53. DOI:10.1186/s12885-016-3035-1
83. Neves M., Azevedo R., Lima L., et al. (2019). Exploring sialyl-Tn expression in microfluidic-isolated circulating tumour cells: A novel biomarker and an analytical tool for precision oncology applications. *N. Biotechnol.* **49**:77–87. DOI:10.1016/j.nbt.2018.09.004
84. Wei C., Yang C., Wang S., et al. (2019). Crosstalk between cancer cells and tumor associated macrophages is required for mesenchymal circulating tumor cell-mediated colorectal cancer metastasis. *Molecular Cancer* **18**. DOI:10.1186/s12943-019-0976-4.
85. Liang M. X., Fei Y. J., Yang K., et al. (2022). Potential values of circulating tumor cell for detection of recurrence in patients of thyroid cancer: a diagnostic meta-analysis. *BMC Cancer* **22**:954. DOI:10.1186/s12885-022-09976-5
86. Yu H. W., Park E., Lee J. K., et al. (2024). Analyzing circulating tumor cells and epithelial-mesenchymal transition status of papillary thyroid carcinoma patients following thyroidectomy: a prospective cohort study. *Int. J. Surg.* **110**:3357–3364. DOI:10.1097/JS9.0000000000001284
87. Dai Y. J., Qiu Y. B., Jiang R., et al. (2017). Concomitant high expression of ERalpha36, EGFR and HER2 is associated with aggressive behaviors of papillary thyroid carcinomas. *Sci. Rep.* **7**:12279. DOI:10.1038/s41598-017-12478-1
88. Xu W., Cao L., Chen L., et al. (2011). Isolation of circulating tumor cells in patients with hepatocellular carcinoma using a novel cell separation strategy. *Clin. Cancer Res.* **17**:3783–3793. DOI:10.1158/1078-0432.CCR-10-0498
89. Li Y. M., Xu S. C., Li J., et al. (2013). Epithelial-mesenchymal transition markers expressed in circulating tumor cells in hepatocellular carcinoma patients with different stages of disease. *Cell Death Dis.* **4**:e831. DOI:10.1038/cddis.2013.347
90. Gerber T. S., Ridder D. A., Goeppert B., et al. (2024). N - cadherin: A diagnostic marker to help discriminate primary liver carcinomas from extrahepatic carcinomas. *Int. J. Cancer* **154**:1857–1868. DOI:10.1002/ijc.34836
91. Li Y., Zhang X., Liu D., et al. (2018). Evolutionary Expression of HER2 Conferred by Chromosome Aneuploidy on Circulating Gastric Cancer Cells Contributes to Developing Targeted and Chemotherapeutic Resistance. *Clin. Cancer Res.* **24**:5261–5271. DOI:10.1158/1078-0432.CCR-18-1205 %J Clinical Cancer Research. DOI:10.1158/1078-0432.CCR-18-1205%JClinicalCancerResearch
92. Zheng X., Fan L., Zhou P., et al. (2017). Detection of Circulating Tumor Cells and Circulating Tumor Microemboli in Gastric Cancer. *Transl. Oncol.* **10**:431–441. DOI:10.1016/j.tranon.2017.02.007
93. Mimori K., Fukagawa T., Kosaka Y., et al. (2008). A large-scale study of MT1-MMP as a marker for isolated tumor cells in peripheral blood and bone marrow in gastric cancer cases. *Ann. Surg. Oncol.* **15**:2934–2942. DOI:10.1245/s10434-008-9916-z
94. Gopalan V. and Lam A. K. (2018). Circulatory Tumor Cells in Esophageal Adenocarcinoma. In Esophageal Adenocarcinoma: Methods and Protocols, A.K. Lam, ed. (Springer New York), pp. 177-186. 10.1007/978-1-4939-7734-5_16.
95. Joo D. C., Kim G. H., I H., et al. (2024). Clinical Implications of Circulating Tumor Cells in Patients with Esophageal Squamous Cell Carcinoma: Cancer-Draining Blood Versus Peripheral Blood. *Cancers (Basel)* **16**. DOI:10.3390/cancers16162921.
96. Xi L., Nicastri D. G., El-Hefnawy T., et al. (2007). Optimal markers for real-time quantitative reverse transcription PCR detection of circulating tumor cells from melanoma, breast, colon, esophageal, head and neck, and lung cancers. *Clin. Chem.* **53**:1206–1215. DOI:10.1373/clinchem.2006.081828
97. Cho H., Byun S.-S., Son N.-H., et al. (2024). Impact of Circulating Tumor Cell–Expressed Prostate-Specific Membrane Antigen and Prostate-Specific Antigen Transcripts in Different Stages of Prostate Cancer. *Clin. Cancer Res.* **30**:1788–1800. DOI:10.1158/1078-0432.CCR-23-3083 %J Clinical Cancer Research. DOI:10.1158/1078-0432.CCR-23-3083%JClinicalCancerResearch
98. Kilercik M., Ozgur E., Sahin S., et al. (2024). Detection of circulating tumor cells in non-metastatic prostate cancer through integration of a microfluidic CTC enrichment system and multiparametric flow cytometry. *PLoS One* **19**:e0312296. DOI:10.1371/journal.pone.0312296
99. Day K. C., Lorenzatti Hiles G., Kozminsky M., et al. (2017). HER2 and EGFR Overexpression Support Metastatic Progression of Prostate Cancer to Bone. *Cancer Res.* **77**:74–85. DOI:10.1158/0008-5472.CAN-16-1656
100. Nagrath S., Jack R. M., Sahai V., et al. (2016). Opportunities and Challenges for Pancreatic Circulating Tumor Cells. *Gastroenterology* **151**:412–426. DOI:10.1053/j.gastro.2016.05.052
101. Zhao X. H., Wang Z. R., Chen C. L., et al. (2019). Molecular detection of epithelial-mesenchymal transition markers in circulating tumor cells from pancreatic cancer patients: Potential role in clinical practice. *World J. Gastroenterol.* **25**:138–150. DOI:10.3748/wjg.v25.i1.138
102. Signorelli R., Giret T. M., Umland O., et al. (2022). ALCAM: A Novel Surface Marker on EpCAM(low) Circulating Tumor Cells. *Biomedicines* **10**. DOI:10.3390/biomedicines10081983.
103. Rink M., Chun F. K., Dahlem R., et al. (2012). Prognostic Role and HER2 Expression of Circulating Tumor Cells in Peripheral Blood of Patients Prior to Radical Cystectomy: A Prospective Study. *Eur. Urol.* **61**:810–817. DOI:10.1016/j.eururo.2012.01.017
104. MacArthur K. M., Kao G. D., Chandrasekaran S., et al. (2014). Detection of Brain Tumor Cells in the Peripheral Blood by a Telomerase Promoter-Based Assay. *Cancer Res.* **74**:2152–2159. DOI:10.1158/0008-5472.Can-13-0813
105. Sullivan J. P., Nahed B. V., Madden M. W., et al. (2014). Brain Tumor Cells in Circulation Are Enriched for Mesenchymal Gene Expression. *Cancer Discov.* **4**:1299–1309. DOI:10.1158/2159-8290.Cd-14-0471
106. GRADILONE A., IACOVELLI R., CORTESI E., et al. (2011). Circulating Tumor Cells and "Suspicious Objects" Evaluated Through CellSearch® in Metastatic Renal Cell Carcinoma. *Clin. Cancer Res.* **31**:4219–4221.
107. Bade R. M., Schehr J. L., Emamekhoo H., et al. (2021). Development and initial clinical testing of a multiplexed circulating tumor cell assay in patients with clear cell renal cell carcinoma. *Mol. Oncol.* **15**:2330–2344. DOI:10.1002/1878-0261.12931
108. Palmela Leitao T., Miranda M., Polido J., et al. (2021). Circulating tumor cell detection methods in renal cell carcinoma: A systematic review. *Crit. Rev. Oncol. Hematol.* **161**:103331. DOI:10.1016/j.critrevonc.2021.103331
109. Tinhofer I., Konschak R., Stromberger C., et al. (2014). Detection of circulating tumor cells for prediction of recurrence after adjuvant chemoradiation in locally advanced squamous cell carcinoma of the head and neck. *Ann. Oncol.* **25**:2042–2047. DOI:10.1093/annonc/mdu271
110. Chikamatsu K., Tada H., Takahashi H., et al. (2019). Expression of immune-regulatory molecules in circulating tumor cells derived from patients with head and neck squamous cell carcinoma. *Oral Oncol.* **89**:34–39. DOI:10.1016/j.oraloncology.2018.12.002
111. Strati A., Koutsodontis G., Papaxoinis G., et al. (2017). Prognostic significance of PD-L1 expression on circulating tumor cells in patients with head and neck squamous cell carcinoma. *Ann. Oncol.* **28**:1923–1933. DOI:10.1093/annonc/mdx206
112. Peeters D. J., Brouwer A., Van den Eynden G. G., et al. (2015). Circulating tumour cells and lung microvascular tumour cell retention in patients with metastatic breast and cervical cancer. *Cancer Lett.* **356**:872–879. DOI:10.1016/j.canlet.2014.10.039
113. Gies S., Melchior P., Molnar I., et al. (2025). PD-L1(+) CD49f(+) CD133(+) Circulating tumor cells predict outcome of patients with vulvar or cervical cancer after radio- and chemoradiotherapy. *J. Transl. Med.* **23**:321. DOI:10.1186/s12967-025-06277-w
114. Yan C., Xiao Y., Zhang W., et al. (2023). Circulating Tumor Cells are an Independent Risk Factor for Poor Prognosis in Patients with Gallbladder Adenocarcinoma. *Ann. Surg. Oncol.* **30**:7966–7975. DOI:10.1245/s10434-023-14231-7
115. Mishra S., Kumari S. and Husain N. (2024). Liquid biopsy in gallbladder carcinoma: Current evidence and future prospective. *J. Liq. Biopsy* **6**. DOI:10.1016/j.jlb.2024.100280.
116. Khoja L., Lorigan P., Zhou C., et al. (2013). Biomarker Utility of Circulating Tumor Cells in Metastatic Cutaneous Melanoma. *J. Invest. Dermatol.* **133**:1582–1590. DOI:10.1038/jid.2012.468
117. Fusi A., Reichelt U., Busse A., et al. (2011). Expression of the Stem Cell Markers Nestin and CD133 on Circulating Melanoma Cells. *J. Invest. Dermatol.* **131**:487–494. DOI:10.1038/jid.2010.285
118. Khoja L., Lorigan P., Dive C., et al. (2015). Circulating tumour cells as tumour biomarkers in melanoma: detection methods and clinical relevance. *Ann. Oncol.* **26**:33–39. DOI:10.1093/annonc/mdu207
119. Reza K. K., Dey S., Wuethrich A., et al. (2021). In Situ Single Cell Proteomics Reveals Circulating Tumor Cell Heterogeneity during Treatment. *ACS Nano* **15**:11231–11243. DOI:10.1021/acsnano.0c10008
120. Koyanagi K., O'Day S. J., Boasberg P., et al. (2010). Serial Monitoring of Circulating Tumor Cells Predicts Outcome of Induction Biochemotherapy plus Maintenance Biotherapy for Metastatic Melanoma. *Clin. Cancer Res.* **16**:2402–2408. DOI:10.1158/1078-0432.Ccr-10-0037
121. Serrano-Gomez S. J., Maziveyi M. and Alahari S. K. (2016). Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. *Mol. Cancer* **15**. DOI:10.1186/s12943-016-0502-x.
122. Kuburich N. A., den Hollander P., Pietz J. T., et al. (2022). Vimentin and cytokeratin:

Good alone, bad together. *Semin. Cancer Biol.* **86**:816–826. DOI:10.1016/j.semcancer.2021.12.006
123. Wei T., Zhang X., Zhang Q., et al. (2019). Vimentin-positive circulating tumor cells as a biomarker for diagnosis and treatment monitoring in patients with pancreatic cancer. *Cancer Lett.* **452**:237–243. DOI:10.1016/j.canlet.2019.03.009
124. Miettinen M., McCue P. A., Sarlomo-Rikala M., et al. (2014). GATA3: A Multispecific But Potentially Useful Marker in Surgical Pathology: A Systematic Analysis of 2500 Epithelial and Nonepithelial Tumors. *Am. J. Surg. Pathol.* **38**:13–22. DOI:10.1097/PAS.0b013e3182a0218f
125. Werling R. W., Yaziji H., Bacchi C. E., et al. (2003). CDX2, a Highly Sensitive and Specific Marker of Adenocarcinomas of Intestinal Origin: An Immunohistochemical Survey of 476 Primary and Metastatic Carcinomas. *Am. J. Surg. Pathol.* **27**:303–310.
126. Rossi T., Bandini S., Zanoni M., et al. (2025). Transcriptomic profiling of circulating tumor cells from metastatic breast cancer patients reveals new hints in their biological features and phenotypic heterogeneity. *Exp. Hematol. Oncol.* **14**:67. DOI:10.1186/s40164-025-00659-y
127. Onstenk W., Sieuwerts A. M., Weekhout M., et al. (2015). Gene expression profiles of circulating tumor cells versus primary tumors in metastatic breast cancer. *Cancer Lett.* **362**:36–44. DOI:10.1016/j.canlet.2015.03.020
128. Stoecklein N. H., Fluegen G., Guglielmi R., et al. (2023). Ultra-sensitive CTC-based liquid biopsy for pancreatic cancer enabled by large blood volume analysis. *Mol. Cancer* **22**. DOI:10.1186/s12943-023-01880-1.
129. Rieckmann L.-M., Spohn M., Ruff L., et al. (2024). Diagnostic leukapheresis reveals distinct phenotypes of NSCLC circulating tumor cells. *Mol. Cancer* **23**. DOI:10.1186/s12943-024-01984-2.
130. Chauhan A., Pal A., Sachdeva M., et al. (2024). A FACS-based novel isolation technique identifies heterogeneous CTCs in oral squamous cell carcinoma. *Front. Oncol.* **14**:1269211. DOI:10.3389/fonc.2024.1269211
131. Lambros M. B., Seed G., Sumanasuriya S., et al. (2018). Single-Cell Analyses of Prostate Cancer Liquid Biopsies Acquired by Apheresis. *Clin. Cancer Res.* **24**:5635–5644. DOI:10.1158/1078-0432.CCR-18-0862
132. Pailler E., Faugeroux V., Oulhen M., et al. (2019). Acquired Resistance Mutations to ALK Inhibitors Identified by Single Circulating Tumor Cell Sequencing in ALK-Rearranged Non-Small-Cell Lung Cancer. *Clin. Cancer Res.* **25**:6671–6682. DOI:10.1158/1078-0432.CCR-19-1176
133. Li R., Jia F., Zhang W., et al. (2019). Device for whole genome sequencing single circulating tumor cells from whole blood. *Lab Chip* **19**:3168–3178. DOI:10.1039/c9lc00473d
134. Li Y., Jiang X., Zhong M., et al. (2022). Whole Genome Sequencing of Single-Circulating Tumor Cell Ameliorates Unraveling Breast Cancer Heterogeneity. *Breast Cancer (Dove Med Press)* **14**:505–513. DOI:10.2147/BCTT.S388653
135. Kim O., Lee D., Chungwon Lee A., et al. (2019). Whole Genome Sequencing of Single Circulating Tumor Cells Isolated by Applying a Pulsed Laser to Cell-Capturing Microstructures. *Small* **15**:e1902607. DOI:10.1002/smll.201902607
136. Kojima M., Harada T., Fukazawa T., et al. (2021). Single-cell DNA and RNA sequencing of circulating tumor cells. *Sci. Rep.* **11**:22864. DOI:10.1038/s41598-021-02165-7
137. Liu H. E., Triboulet M., Zia A., et al. (2017). Workflow optimization of whole genome amplification and targeted panel sequencing for CTC mutation detection. *NPJ Genom. Med.* **2**:34. DOI:10.1038/s41525-017-0034-3
138. Daniel M., Knutson T. P., Sperger J. M., et al. (2021). AR gene rearrangement analysis in liquid biopsies reveals heterogeneity in lethal prostate cancer. *Endocr. Relat. Cancer* **28**:645–655. DOI:10.1530/erc-21-0157
139. Deger T., Mendelaar P. A. J., Kraan J., et al. (2022). A pipeline for copy number profiling of single circulating tumour cells to assess intrapatient tumour heterogeneity. *Mol. Oncol.* **16**:2981–3000. DOI:10.1002/1878-0261.13174
140. Yadav D., Patil-Takbhate B., Khandagale A., et al. (2023). Next-Generation sequencing transforming clinical practice and precision medicine. *Clin. Chim. Acta* **551**. DOI:10.1016/j.cca.2023.117568.
141. Yu J., Gemenetzis G., Kinny-Koster B., et al. (2020). Pancreatic circulating tumor cell detection by targeted single-cell next-generation sequencing. *Cancer Lett.* **493**:245–253. DOI:10.1016/j.canlet.2020.08.043
142. Shen X., Dai J., Guo L., et al. (2024). Single-cell low-pass whole genome sequencing accurately detects circulating tumor cells for liquid biopsy-based multi-cancer diagnosis. *NPJ Precis. Oncol.* **8**:30. DOI:10.1038/s41698-024-00520-1
143. Xu X., Lin L., Yang J., et al. (2022). Simultaneous single-cell genome and transcriptome sequencing in nanoliter droplet with digital microfluidics identifying essential driving genes. *Nano Today* **46**. DOI:10.1016/j.nantod.2022.101596.
144. Di L., Fu Y., Sun Y., et al. (2020). RNA sequencing by direct tagmentation of RNA/DNA hybrids. *Proc. Natl. Acad. Sci. U. S. A.* **117**:2886–2893. DOI:10.1073/pnas.1919800117
145. Hagemann-Jensen M., Ziegenhain C., Chen P., et al. (2020). Single-cell RNA counting at allele and isoform resolution using Smart-seq3. *Nat. Biotechnol.* **38**:708–714. DOI:10.1038/s41587-020-0497-0
146. Hahaut V., Pavlinic D., Carbone W., et al. (2022). Fast and highly sensitive full-length single-cell RNA sequencing using FLASH-seq. *Nat. Biotechnol.* **40**:1447–1451. DOI:10.1038/s41587-022-01312-3
147. He L., Dang K., Sun Q., et al. (2026). High-Resolution Multiplexed Sequencing of Single-Cell Full-length Transcriptome Via Combinational Barcoded Tn5 Transposon Insertion. *Adv. Sci.* **13**:e16013. DOI:10.1002/advs.202516013
148. Cheng Y. H., Chen Y. C., Lin E., et al. (2019). Hydro-Seq enables contamination-free high-throughput single-cell RNA-sequencing for circulating tumor cells. *Nat. Commun.* **10**:2163. DOI:10.1038/s41467-019-10122-2
149. Lightbody E. D., Sklavenitis-Pistofidis R., Wu T., et al. (2025). SWIFT-seq enables comprehensive single-cell transcriptomic profiling of circulating tumor cells in multiple myeloma and its precursors. *Nat. Cancer* **6**:1595–1611. DOI:10.1038/s43018-025-01006-0
150. Brechbuhl H. M., Vinod-Paul K., Gillen A. E., et al. (2020). Analysis of circulating breast cancer cell heterogeneity and interactions with peripheral blood mononuclear cells. *Mol. Carcinog.* **59**:1129–1139. DOI:10.1002/mc.23242
151. Sinkala E., Sollier-Christen E., Renier C., et al. (2017). Profiling protein expression in circulating tumour cells using microfluidic western blotting. *Nat. Commun.* **8**:14622. DOI:10.1038/ncomms14622
152. Shi Q., Qin L., Wei W., et al. (2012). Single-cell proteomic chip for profiling intracellular signaling pathways in single tumor cells. *Proc. Natl. Acad. Sci. U. S. A.* **109**:419–424. DOI:10.1073/pnas.1110865109
153. Yang L., Wang Z., Deng Y., et al. (2016). Single-Cell, Multiplexed Protein Detection of Rare Tumor Cells Based on a Beads-on-Barcode Antibody Microarray. *Anal. Chem.* **88**:11077–11083. DOI:10.1021/acs.analchem.6b03086
154. Deng Y., Zhang Y., Sun S., et al. (2014). An integrated microfluidic chip system for single-cell secretion profiling of rare circulating tumor cells. *Sci. Rep.* **4**:7499. DOI:10.1038/srep07499
155. Zhang Y., Tang Y., Sun S., et al. (2015). Single-Cell Codetection of Metabolic Activity, Intracellular Functional Proteins, and Genetic Mutations from Rare Circulating Tumor Cells. *Anal. Chem.* **87**:9761–9768. DOI:10.1021/acs.analchem.5b01901
156. Song Y., Tian T., Shi Y., et al. (2017). Enrichment and single-cell analysis of circulating tumor cells. *Chem. Sci.* **8**:1736–1751. DOI:10.1039/c6sc04671a
157. Wang C., Yang L., Wang Z., et al. (2019). Highly multiplexed profiling of cell surface proteins on single circulating tumor cells based on antibody and cellular barcoding. *Anal. Bioanal. Chem.* **411**:5373–5382. DOI:10.1007/s00216-019-01666-9
158. Chai S., Ruiz-Velasco C., Naghdloo A., et al. (2022). Identification of epithelial and mesenchymal circulating tumor cells in clonal lineage of an aggressive prostate cancer case. *NPJ Precis. Oncol.* **6**:41. DOI:10.1038/s41698-022-00289-1
159. Karabacak N. M., Zheng Y., Dubash T. D., et al. (2022). Differential Kinase Activity Across Prostate Tumor Compartments Defines Sensitivity to Target Inhibition. *Cancer Res.* **82**:1084–1097. DOI:10.1158/0008-5472.CAN-21-2609
160. Payne K., Brooks J., Batis N., et al. (2023). Feasibility of mass cytometry proteomic characterisation of circulating tumour cells in head and neck squamous cell carcinoma for deep phenotyping. *Br. J. Cancer* **129**:1590–1598. DOI:10.1038/s41416-023-02428-2
161. Welter L., Zheng S., Setayesh S. M., et al. (2023). Cell State and Cell Type: Deconvoluting Circulating Tumor Cell Populations in Liquid Biopsies by Multi-Omics. *Cancers (Basel)* **15**. DOI:10.3390/cancers15153949.
162. Guo T., Steen J. A. and Mann M. (2025). Mass-spectrometry-based proteomics: from single cells to clinical applications. *Nature* **638**:901–911. DOI:10.1038/s41586-025-08584-0
163. Bartkowiak K., Mohammadi P. M., Nissen P., et al. (2025). Discovery of a sushi domain-containing protein 2-positive phenotype in circulating tumor cells of metastatic breast cancer patients. *Sci. Rep.* **15**:3913. DOI:10.1038/s41598-025-87122-4
164. Horrmann A., Arafa A., Kamalanathan K., et al. (2025). A liquid biopsy-based proteomic assay to identify circulating tumor cell (CTC) –derived druggable targets in cancer patients. *J. Clin. Oncol.* **43**:258–258. DOI:10.1200/JCO.2025.43.5_suppl.258
165. Huang M. S., Fu L. H., Yan H. C., et al. (2022). Proteomics and liquid biopsy characterization of human EMT-related metastasis in colorectal cancer. *Front. Oncol.* **12**:790096. DOI:10.3389/fonc.2022.790096
166. Que Z., Xi Z., Qi D., et al. (2025). Src/FN1 pathway activation drives tumor cell cluster formation and metastasis in lung cancer: A promising therapeutic target. *Sci. Adv.* **11**:eadv7377. DOI. DOI:10.1126/sciadv.adv7377
167. Vuille J. A., Tanriover C., Antmen E., et al. (2025). The E3 ligase HECTD4 regulates COX-2-dependent tumor progression and metastasis. *Proc. Natl. Acad. Sci. U. S. A.* **122**:e2425621122. DOI:10.1073/pnas.2425621122
168. Wang L., Abdulla A., Wang A., et al. (2022). Sickle-like Inertial Microfluidic System for Online Rare Cell Separation and Tandem Label-Free Quantitative Proteomics (Orcs-Proteomics). *Anal. Chem.* **94**:6026–6035. DOI:10.1021/acs.analchem.2c00679
169. Tsai C. F., Zhang P., Scholten D., et al. (2021). Surfactant-assisted one-pot sample preparation for label-free single-cell proteomics. *Commun. Biol.* **4**:265. DOI:10.1038/s42003-021-01797-9
170. Budnik B., Levy E., Harmange G., et al. (2018). SCoPE-MS: mass spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation. *Genome Biol.* **19**:161. DOI:10.1186/s13059-018-1547-5
171. Gebreyesus S. T., Siyal A. A., Kitata R. B., et al. (2022). Streamlined single-cell proteomics by an integrated microfluidic chip and data-independent acquisition mass spectrometry. *Nat. Commun.* **13**:37. DOI:10.1038/s41467-021-27778-4

172. Derks J., Leduc A., Wallmann G., et al. (2023). Increasing the throughput of sensitive proteomics by plexDIA. *Nat. Biotechnol.* **41**:50–59. DOI:10.1038/s41587-022-01389-w
173. Wang Y., Guan Z. Y., Shi S. W., et al. (2024). Pick-up single-cell proteomic analysis for quantifying up to 3000 proteins in a Mammalian cell. *Nat. Commun.* **15**:1279. DOI:10.1038/s41467-024-45659-4
174. Ye Z., Sabatier P., Martin-Gonzalez J., et al. (2024). One-Tip enables comprehensive proteome coverage in minimal cells and single zygotes. *Nat. Commun.* **15**:2474. DOI:10.1038/s41467-024-46777-9
175. Ye Z., Sabatier P., van der Hoeven L., et al. (2025). Enhanced sensitivity and scalability with a Chip-Tip workflow enables deep single-cell proteomics. *Nat. Methods* **22**:499–509. DOI:10.1038/s41592-024-02558-2
176. Liang L., Sun F., Wang H., et al. (2021). Metabolomics, metabolic flux analysis and cancer pharmacology. *Pharmacol. Ther.* **224**:107827. DOI:10.1016/j.pharmthera.2021.107827
177. Sun F., Li H., Sun D., et al. (2025). Single-cell omics: experimental workflow, data analyses and applications. *Sci. China Life Sci.* **68**:5–102. DOI:10.1007/s11427-023-2561-0
178. Zhang W., Xu F., Yao J., et al. (2023). Single-cell metabolic fingerprints discover a cluster of circulating tumor cells with distinct metastatic potential. *Nat. Commun.* **14**:2485. DOI:10.1038/s41467-023-38009-3
179. Hiyama E., Ali A., Amer S., et al. (2015). Direct Lipido-Metabolomics of Single Floating Cells for Analysis of Circulating Tumor Cells by Live Single-cell Mass Spectrometry. *Anal. Sci.* **31**:1215–1217. DOI:10.2116/analsci.31.1215
180. Abouleila Y., Onidani K., Ali A., et al. (2019). Live single cell mass spectrometry reveals cancer-specific metabolic profiles of circulating tumor cells. *Cancer Sci.* **110**:697–706. DOI:10.1111/cas.13915
181. Hu X., Xu T., Xia S., et al. (2025). Metabolic Adaptation Study of Tumor Cells during Lung Cancer Bone Metastasis in Mice Based on Single-Cell Metabolome Analysis. *J. Am. Soc. Mass Spectrom.* **36**:1959–1969. DOI:10.1021/jasms.5c00177
182. Xu Y., Hu X., Yuan Y., et al. (2025). Prediction of Lung Cancer Metastasis Risk Based on Single-Cell Metabolic Profiling of Circulating Tumor Cells. *Adv. Sci.* **12**:e08878. DOI:10.1002/advs.202508878
183. Luan H., Chen S., Lian J., et al. (2024). Biofluorescence imaging-guided spatial metabolic tracing: In vivo tracking of metabolic activity in circulating tumor cell-mediated multi-organ metastases. *Talanta* **280**:126696. DOI:10.1016/j.talanta.2024.126696
184. Radfar P., Ding L., de la Fuente L. R., et al. (2023). Rapid metabolomic screening of cancer cells via high-throughput static droplet microfluidics. *Biosens. Bioelectron.* **223**:114966. DOI:10.1016/j.bios.2022.114966
185. Dai C. S., Mishra A., Edd J., et al. (2025). Circulating tumor cells: Blood-based detection, molecular biology, and clinical applications. *Cancer Cell* **43**:1399–1422. DOI:10.1016/j.ccell.2025.07.008
186. Raza A., Khan A. Q., Inchakalody V. P., et al. (2022). Dynamic liquid biopsy components as predictive and prognostic biomarkers in colorectal cancer. *J. Exp. Clin. Cancer Res.* **41**. DOI:10.1186/s13046-022-02318-0.
187. Lianidou E. S., Mavroudis D. and Georgoulias V. (2013). Clinical challenges in the molecular characterization of circulating tumour cells in breast cancer. *Br. J. Cancer* **108**:2426–2432. DOI:10.1038/bjc.2013.265
188. Crosby D., Bhatia S., Brindle K. M., et al. (2022). Early detection of cancer. *Science* **375**. DOI:10.1126/science.aay9040.
189. Batool S. M., Yekula A., Khanna P., et al. (2023). The Liquid Biopsy Consortium: Challenges and opportunities for early cancer detection and monitoring. *Cell Rep. Med.* **4**. DOI:10.1016/j.xcrm.2023.101198.
190. Rupp B., Nagpal N., Thanasiu B., et al. (2025). Multiplex characterization of circulating tumor cells from ductal carcinoma in situ patients suggests early tumor dissemination. *Cancer Lett.* **623**:217703. DOI:10.1016/j.canlet.2025.217703
191. Xu C., Xu X., Shao W., et al. (2023). CTCs Detection and Whole-exome Sequencing Might Be Used to Differentiate Benign and Malignant Pulmonary Nodules. *Zhongguo Fei Ai Za Zhi* **26**:449–460. DOI:10.3779/j.issn.1009-3419.2023.106.12
192. Wang S., Xu C., Xu X., et al. (2025). The Role of Circulating Tumor Cell as a Promising Biomarker in the Evaluation of Pulmonary Nodules: A Prospective Study. *Cancer Res. Treat.* DOI:10.4143/crt.2024.841.
193. Guo X., Lin F., Yi C., et al. (2022). Deep transfer learning enables lesion tracing of circulating tumor cells. *Nat. Commun.* **13**:7687. DOI:10.1038/s41467-022-35296-0
194. Kojima M., Harada T., Fukazawa T., et al. (2023). Single-cell next-generation sequencing of circulating tumor cells in patients with neuroblastoma. *Cancer Sci.* **114**:1616–1624. DOI:10.1111/cas.15707
195. Carter L., Rothwell D. G., Mesquita B., et al. (2016). Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. *Nat. Med.* **23**:114–119. DOI:10.1038/nm.4239
196. Gasch C., Oldopp T., Mauermann O., et al. (2016). Frequent detection of PIK3CA mutations in single circulating tumor cells of patients suffering from HER2 - negative metastatic breast cancer. *Mol. Oncol.* **10**:1330–1343. DOI:10.1016/j.molonc.2016.07.005
197. Rimawi M. F., Schiff R. and Osborne C. K. (2015). Targeting HER2 for the Treatment of Breast Cancer. *Annu. Rev. Med.* **66**:111–128. DOI:10.1146/annurev-med-042513-015127
198. Van Poznak C., Somerfield M. R., Bast R. C., et al. (2015). Use of Biomarkers to Guide Decisions on Systemic Therapy for Women With Metastatic Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J. Clin. Oncol.* **33**:2695–2704. DOI:10.1200/jco.2015.61.1459
199. Barnett E. S., Schultz N., Stopsack K. H., et al. (2023). Analysis of BRCA2 Copy Number Loss and Genomic Instability in Circulating Tumor Cells from Patients with Metastatic Castration-resistant Prostate Cancer. *Eur. Urol.* **83**:112–120. DOI:10.1016/j.eururo.2022.08.010
200. Mishra A., Huang S. B., Dubash T., et al. (2025). Tumor cell-based liquid biopsy using high-throughput microfluidic enrichment of entire leukapheresis product. *Nat. Commun.* **16**:32. DOI:10.1038/s41467-024-55140-x
201. Lu X., Cheng L., Yang C., et al. (2024). Crosstalk between bladder cancer and the tumor microenvironment: Molecular mechanisms and targeted therapy. *Innov. Med.* **2**. DOI:10.59717/j.xinn-med.2024.100094.
202. King J. D., Casavant B. P. and Lang J. M. (2014). Rapid translation of circulating tumor cell biomarkers into clinical practice: technology development, clinical needs and regulatory requirements. *Lab Chip* **14**:24–31. DOI:10.1039/c3lc50741f
203. Maltoni R., Gallerani G., Fici P., et al. (2016). CTCs in early breast cancer: A path worth taking. *Cancer Lett.* **376**:205–210. DOI:10.1016/j.canlet.2016.03.051
204. Moon D. H., Lindsay D. P., Hong S., et al. (2018). Clinical indications for, and the future of, circulating tumor cells. *Adv. Drug Del. Rev.* **125**:143–150. DOI:10.1016/j.addr.2018.04.002
205. Lim M., Park J., Lowe A. C., et al. (2020). A lab-on-a-disc platform enables serial monitoring of individual CTCs associated with tumor progression during EGFR-targeted therapy for patients with NSCLC. *Theranostics* **10**:5181–5194. DOI:10.7150/thno.44693
206. Blau C. A., Ramirez A. B., Blau S., et al. (2016). A Distributed Network for Intensive Longitudinal Monitoring in Metastatic Triple-Negative Breast Cancer. *J. Natl. Compr. Canc. Netw.* **14**:8–17. DOI:10.6004/jnccn.2016.0003
207. Paoletti C., Cani A. K., Larios J. M., et al. (2018). Comprehensive Mutation and Copy Number Profiling in Archived Circulating Breast Cancer Tumor Cells Documents Heterogeneous Resistance Mechanisms. *Cancer Res.* **78**:1110–1122. DOI:10.1158/0008-5472.Can-17-2686
208. Franken A., Honisch E., Reinhardt F., et al. (2020). Detection of ESR1 Mutations in Single Circulating Tumor Cells on Estrogen Deprivation Therapy but Not in Primary Tumors from Metastatic Luminal Breast Cancer Patients. *J. Mol. Diagn.* **22**:111–121. DOI:10.1016/j.jmoldx.2019.09.004
209. Mezquita L., Oulhen M., Aberlenc A., et al. (2024). Resistance to BRAF inhibition explored through single circulating tumour cell molecular profiling in BRAF-mutant non-small-cell lung cancer. *Br. J. Cancer* **130**:682–693. DOI:10.1038/s41416-023-02535-0
210. Hong X., Roh W., Sullivan R. J., et al. (2021). The Lipogenic Regulator SREBP2 Induces Transferrin in Circulating Melanoma Cells and Suppresses Ferroptosis. *Cancer Discov.* **11**:678–695. DOI:10.1158/2159-8290.Cd-19-1500
211. Cani A. K., Dolce E. M., Darga E. P., et al. (2022). Serial monitoring of genomic alterations in circulating tumor cells of ER-positive/HER2-negative advanced breast cancer: feasibility of precision oncology biomarker detection. *Mol. Oncol.* **16**:1969–1985. DOI:10.1002/1878-0261.13150
212. Horwitz E., Dubash T. D., Szabolcs A., et al. (2025). CDKN1B (p27/kip1) enhances drug-tolerant persister CTCs by restricting polyploidy following mitotic inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* **122**:e2507203122. DOI:10.1073/pnas.2507203122
213. Que Z., Luo B., Zhou Z., et al. (2019). Establishment and characterization of a patient-derived circulating lung tumor cell line in vitro and in vivo. *Cancer Cell Int.* **19**. DOI:10.1186/s12935-019-0735-z.
214. Stewart C. A., Gay C. M., Xi Y., et al. (2020). Single-cell analyses reveal increased intratumoral heterogeneity after the onset of therapy resistance in small-cell lung cancer. *Nat. Cancer* **1**:423–436. DOI:10.1038/s43018-019-0020-z
215. Suvilesh K. N., Nussbaum Y. I., Radhakrishnan V., et al. (2022). Tumorigenic circulating tumor cells from xenograft mouse models of non-metastatic NSCLC patients reveal distinct single cell heterogeneity and drug responses. *Mol. Cancer* **21**. DOI:10.1186/s12943-022-01553-5.
216. Huebner H., Fasching P. A., Gumbrecht W., et al. (2018). Filtration based assessment of CTCs and CellSearch® based assessment are both powerful predictors of prognosis for metastatic breast cancer patients. *BMC Cancer* **18**. DOI:10.1186/s12885-018-4115-1.
217. Pereira-Veiga T., Schneegans S., Pantel K., et al. (2022). Circulating tumor cell-blood cell crosstalk: Biology and clinical relevance. *Cell Rep.* **40**. DOI:10.1016/j.celrep.2022.111298.
218. Deng Z., Wu S., Wang Y., et al. (2022). Circulating tumor cell isolation for cancer diagnosis and prognosis. *eBioMedicine* **83**. DOI:10.1016/j.ebiom.2022.104237.
219. Ahmed A., Greene S. B., Dago A. E., et al. (2016). Chromosomal Instability Estimation Based on Next Generation Sequencing and Single Cell Genome Wide Copy Number Variation Analysis. *PLoS One* **11**. DOI:10.1371/journal.pone.0165089.
220. Hoon D. S. B., Morton D. L., Irie R. F., et al. (2014). Genome-Wide Characterization of Circulating Tumor Cells Identifies Novel Prognostic Genomic Alterations in Systemic Melanoma Metastasis. *Clin. Chem.* **60**:873–885. DOI:10.1373/clinchem.2013.213611
221. Malihi P. D., Graf R. P., Rodriguez A., et al. (2020). Single-Cell Circulating Tumor Cell

Analysis Reveals Genomic Instability as a Distinctive Feature of Aggressive Prostate Cancer. *Clin. Cancer Res.* **26**:4143–4153. DOI:10.1158/1078-0432.CCR-19-4100
222. Alves J. M., Estévez-Gómez N., Piñeiro R., et al. (2025). Genomic diversity and BCL9L mutational status in circulating tumor cells predict overall survival in metastatic colorectal cancer. *Cell. Oncol.* **48**:1809–1820. DOI:10.1007/s13402-025-01109-x
223. Markou A., Farkona S., Schiza C., et al. (2014). PIK3CA Mutational Status in Circulating Tumor Cells Can Change During Disease Recurrence or Progression in Patients with Breast Cancer. *Clin. Cancer Res.* **20**:5823–5834. DOI:10.1158/1078-0432.Ccr-14-0149
224. Lohr J. G., Kim S., Gould J., et al. (2016). Genetic interrogation of circulating multiple myeloma cells at single-cell resolution. *Sci. Transl. Med.* **8**:363ra147. DOI:10.1126/scitranslmed.aac7037
225. Ebright R. Y., Lee S., Wittner B. S., et al. (2020). Deregulation of ribosomal protein expression and translation promotes breast cancer metastasis. *Science* **367**:1468–1473. DOI:10.1126/science.aay0939
226. Kozuka M., Battaglin F., Jayachandran P., et al. (2021). Clinical Significance of Circulating Tumor Cell Induced Epithelial-Mesenchymal Transition in Patients with Metastatic Colorectal Cancer by Single-Cell RNA-Sequencing. *Cancers (Basel)* **13**. DOI:10.3390/cancers13194862.
227. Rossi T., Gallerani G., Angeli D., et al. (2020). Single-Cell NGS-Based Analysis of Copy Number Alterations Reveals New Insights in Circulating Tumor Cells Persistence in Early-Stage Breast Cancer. *Cancers (Basel)* **12**. DOI:10.3390/cancers12092490.
228. Nitschke C., Markmann B., Tolle M., et al. (2022). Characterization of RARRES1 Expression on Circulating Tumor Cells as Unfavorable Prognostic Marker in Resected Pancreatic Ductal Adenocarcinoma Patients. *Cancers (Basel)* **14**. DOI:10.3390/cancers14184405.
229. Szczerba B. M., Castro-Giner F., Vetter M., et al. (2019). Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature* **566**:553–557. DOI:10.1038/s41586-019-0915-y
230. Hapach L. A., Carey S. P., Schwager S. C., et al. (2021). Phenotypic Heterogeneity and Metastasis of Breast Cancer Cells. *Cancer Res.* **81**:3649–3663. DOI:10.1158/0008-5472.Can-20-1799
231. Gao X., Li X., Xu W., et al. (2025). Identification of multiple genomic alterations and prediction of neoantigens from circulating tumor cells at the single-cell level. *Nat. Commun.* **16**. DOI:10.1038/s41467-025-62215-w.
232. Peterson V. M., Zhang K. X., Kumar N., et al. (2017). Multiplexed quantification of proteins and transcripts in single cells. *Nat. Biotechnol.* **35**:936–939. DOI:10.1038/nbt.3973
233. Stoeckius M., Hafemeister C., Stephenson W., et al. (2017). Simultaneous epitope and transcriptome measurement in single cells. *Nat. Methods* **14**:865–868. DOI:10.1038/nmeth.4380
234. Fulcher J. M., Markillie L. M., Mitchell H. D., et al. (2024). Parallel measurement of transcriptomes and proteomes from same single cells using nanodroplet splitting. *Nat. Commun.* **15**. DOI:10.1038/s41467-024-54099-z.
235. Jiang Y.-R., Zhu L., Cao L.-R., et al. (2023). Simultaneous deep transcriptome and proteome profiling in a single mouse oocyte. *Cell Rep.* **42**. DOI:10.1016/j.celrep.2023.113455.
236. Wu J., Xu Q.-Q., Jiang Y.-R., et al. (2024). One-Shot Single-Cell Proteome and Metabolome Analysis Strategy for the Same Single Cell. *Anal. Chem.* **96**:5499–5508. DOI:10.1021/acs.analchem.3c05659
237. He Y., Yuan H., Liang Y., et al. (2023). On-capillary alkylation micro-reactor: a facile strategy for proteo-metabolome profiling in the same single cells. *Chem. Sci.* **14**:13495–13502. DOI:10.1039/d3sc05047e
238. Hu Y., Wan S., Luo Y., et al. (2024). Benchmarking algorithms for single-cell multi-omics prediction and integration. *Nat. Methods* **21**:2182–2194. DOI:10.1038/s41592-024-02429-w
239. Liu C., Ding S., Kim H. J., et al. (2025). Multitask benchmarking of single-cell multimodal omics integration methods. *Nat. Methods* **22**:2449–2460. DOI:10.1038/s41592-025-02856-3
240. Tien Y. W., Kuo H.-C., Ho B.-I., et al. (2016). A High Circulating Tumor Cell Count in Portal Vein Predicts Liver Metastasis From Periampullary or Pancreatic Cancer. *Medicine* **95**. DOI:10.1097/md.0000000000003407.
241. Dong X., Ma Y., Zhao X., et al. (2020). Spatial heterogeneity in epithelial to mesenchymal transition properties of circulating tumor cells associated with distant recurrence in pancreatic cancer patients. *Ann. Transl. Med.* **8**:676–676. DOI:10.21037/atm-20-782
242. Sun Y.-F., Guo W., Xu Y., et al. (2018). Circulating Tumor Cells from Different Vascular Sites Exhibit Spatial Heterogeneity in Epithelial and Mesenchymal Composition and Distinct Clinical Significance in Hepatocellular Carcinoma. *Clin. Cancer Res.* **24**:547–559. DOI:10.1158/1078-0432.CCR-17-1063 %J Clinical Cancer Research. DOI:10.1158/1078-0432.CCR-17-1063%JClinicalCancerResearch
243. Buscail E., Chiche L., Laurent C., et al. (2019). Tumor - proximal liquid biopsy to improve diagnostic and prognostic performances of circulating tumor cells. *Mol. Oncol.* **13**:1811–1826. DOI:10.1002/1878-0261.12534
244. Sun Y. F., Wu L., Liu S. P., et al. (2021). Dissecting spatial heterogeneity and the immune-evasion mechanism of CTCs by single-cell RNA-seq in hepatocellular carcinoma. *Nat. Commun.* **12**:4091. DOI:10.1038/s41467-021-24386-0
245. Kahounová Z., Pícková M., Drápela S., et al. (2023). Circulating tumor cell-derived preclinical models: current status and future perspectives. *Cell Death Dis.* **14**. DOI:10.1038/s41419-023-06059-6.
246. Lallo A., Schenk M. W., Frese K. K., et al. (2017). Circulating tumor cells and CDX models as a tool for preclinical drug development. *Transl. Lung Cancer Res.* **6**:397–408. DOI:10.21037/tlcr.2017.08.01
247. Qu S., Xu R., Yi G., et al. (2024). Patient-derived organoids in human cancer: a platform for fundamental research and precision medicine. *Mol. Biomed.* **5**. DOI:10.1186/s43556-023-00165-9.
248. Li T., Deng B., Li S., et al. (2025). Circulating Tumor Cell - Derived Organoids: Current Progress, Applications, and Future. *MedComm – Future Medicine* **4**. DOI:10.1002/mef2.70030.
249. Wang P.-X., Zhong Y.-C., Duan B., et al. (2025). Exploring morphological heterogeneity of circulating tumor cells: machine learning-based approach for cell identification and prognostic implications. *Sci. Bull.* **70**:2070–2074. DOI:10.1016/j.scib.2025.04.048
250. Zhu S., Zhu Z., Ni C., et al. (2024). Liquid Biopsy Instrument for Ultra-Fast and Label-Free Detection of Circulating Tumor Cells. *Research* **7**. DOI:10.34133/research.0431.
251. Zhang W., Lu Y., Su C., et al. (2023). Confinement-guided ultrasensitive optical assay with artificial intelligence for disease diagnostics. *Innov. Med.* **1**. DOI:10.59717/j.xinn-med.2023.100023.
252. Mallery K., Bristow N. R., Heller N., et al. (2026). Circulating tumor cell detection in cancer patients using in-flow deep learning holography. *NPJ Biosens.* **3**. DOI:10.1038/s44328-026-00084-z.
253. Theodoris C. V., Xiao L., Chopra A., et al. (2023). Transfer learning enables predictions in network biology. *Nature* **618**:616–624. DOI:10.1038/s41586-023-06139-9
254. Cui H., Wang C., Maan H., et al. (2024). scGPT: toward building a foundation model for single-cell multi-omics using generative AI. *Nat. Methods* **21**:1470–1480. DOI:10.1038/s41592-024-02201-0
255. Lin X., Tian T., Wei Z., et al. (2022). Clustering of single-cell multi-omics data with a multimodal deep learning method. *Nat. Commun.* **13**. DOI:10.1038/s41467-022-35031-9.
256. Baek S., Song K. and Lee I. (2025). Single-cell foundation models: bringing artificial intelligence into cell biology. *Exp. Mol. Med.* **57**:2169–2181. DOI:10.1038/s12276-025-01547-5
257. Wang G., Zhao J., Lin Y., et al. (2025). scMODAL: a general deep learning framework for comprehensive single-cell multi-omics data alignment with feature links. *Nat. Commun.* **16**. DOI:10.1038/s41467-025-60333-z.
258. Lopez R., Regier J., Cole M. B., et al. (2018). Deep generative modeling for single-cell transcriptomics. *Nat. Methods* **15**:1053–1058. DOI:10.1038/s41592-018-0229-2
259. Ergen C., Pour Amiri V. V., Kim M., et al. (2025). Scvi-hub: an actionable repository for model-driven single-cell analysis. *Nat. Methods* **22**:1836–1845. DOI:10.1038/s41592-025-02799-9
260. Cao C., Zhao W., Guo J., et al. (2025). Leveraging artificial intelligence and machine learning for unraveling pathogenesis and advancing precision medicine in autoimmune diseases. *Innov. Med.* **3**. DOI:10.59717/j.xinn-med.2025.100154.

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AUTHOR CONTRIBUTIONS

All authors contributed significantly to the research. W.Z. was responsible for the conceptualization, study design, and drafting of the initial manuscript. L.H. and C.M. reviewed and corrected the entire manuscript. W.W. and H.W. contributed to improving the article's images. K.Y., J.S., and P.Q. participated in discussions and assisted in manuscript revision. B.H. was responsible for proofreading the manuscript to ensure that it contained no medical errors. Y.H. and X.Z. were responsible for the overall content revision and final proofreading. All authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

No new data were created or analyzed in this study.