Research on the biophysical properties of circulating tumor cells

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Abstract: This article reviews the biophysical properties of circulating tumor cells (CTCs) and their significance in tumor metastasis and liquid biopsy. CTCs are tumor cells that have shed from solid tumors or metastatic foci and entered the bloodstream, possessing various biological markers. They may be cleared by the immune system, enter a dormant state, or form clusters to facilitate metastasis. The physical properties of CTCs, including cell size, density, rigidity, and dielectric properties, differ from normal blood cells, providing a theoretical basis for separation and detection. The biological characteristics of CTCs include epithelial cell properties, expression of specific surface marker proteins, and the characteristics of epithelial-mesenchymal transition, which are crucial for the aggressiveness and metastatic potential of tumors. The development of CTC detection technologies, such as the Cell Search system, has been clinically applied, but the heterogeneity of tumor cells remains a major challenge in research. The development of composite separation technologies that combine various properties of CTCs is expected to improve the sensitivity, specificity, and efficiency of CTC detection, thereby playing an important role in the personalized treatment and prognostic assessment of tumors.

Keywords: Circulating Tumor Cells, Physical Properties, Biological Characteristics

1. Introduction

Circulating tumor cells (CTCs), known as liquid biopsies, are tumor cells that have shed from the primary or metastatic sites of solid tumors and entered the circulatory system. They carry biological markers from the primary or metastatic sites. In the circulation, CTCs have three possible fates: being cleared by the immune system, which is the end for most CTCs; entering a state of dormancy or stasis; or adapting to the microenvironment and being transported in the blood as single tumor cells or clusters composed of several tumor cells, lymphocytes, and adherent platelets. The formation of these clusters can provide protection, shielding them from mechanical stress and immune attacks, facilitating their adhesion to the endothelium, and being captured by potential metastatic sites to form metastatic foci, which has become a hot topic of research in recent years. The biophysical properties of CTCs are the theoretical foundation for their separation and detection, and thus this article focuses on reviewing the biophysical properties of CTCs.

2. Physical properties of CTCs

Under normal circumstances, compared to normal peripheral blood cells, CTCs have a larger nuclear/cytoplasmic ratio and a larger volume (diameter). Due to the abnormal proliferation and metabolism of tumor cells, there are changes in the composition of intracellular substances. The expression and modification of genes, the synthesis and modification of proteins, and the accumulation of some polar granular materials lead to differences in the expression of surface marker proteins, cell size, volume, morphology, and dielectric properties between tumor cells and normal cells. These differences form the theoretical basis for the separation of tumor cells.

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2.1. Cell size

Scholars at home and abroad have used microscopes, flow cytometry, and electrical measurements to determine the diameter and volume of tumor cells and peripheral blood cells. The diameter of biconcave-shaped red blood cells is 6.0 to 8.0μm; typical granulocytes, including neutrophils and eosinophils, have diameters of 12.0 to 15.0μm and 8.7 to 9.9μm, respectively; non-granulocyte lymphocytes have a wider range of diameters, with small lymphocytes at 7.0 to 10.0μm and large lymphocytes at 14.0 to 20.0μm, and monocytes have a diameter of 15.0 to 25.0μm. The diameter range of CTCs is broader, at 17.0 to 52.0μm [1-27]. Due to the high heterogeneity of tumor cells, some studies have detected smaller tumor cells, such as small-cell lung cancer cells with diameters of 7.2 to 10.0μm, smaller than lymphocytes. Therefore, some scholars have proposed that to avoid missing the detection, the Cell Search system (Veridex TM, Warren, PA) used to detect tumors should have a cell diameter greater than 4.0μm. In terms of area, the area of white blood cells is usually smaller than that of tumor cells, with the former at 140.00μm² [3] and the latter ranging from 70.00 to 796.00μm² [3-4]. Some scholars have used the difference in cell size to separate CTCs with filters; others have classified prostate cancer CTCs into three categories based on size: very small nucleated CTCs less than 8.54μm, small nucleated CTCs from 8.54 to 14.99μm, and large nucleated CTCs larger than 14.99μm. The size of CTC nuclei is related to the metastatic state of prostate cancer: very small nucleated CTCs are associated with visceral metastasis of prostate cancer, small nucleated CTCs with non-visceral metastasis, and large nucleated CTCs with no metastasis [5]. However, this classification method has certain limitations, as some studies have shown that CTC sizes vary in samples taken from different sites in the same patient, such as CTCs detected from the central venous blood of patients with metastatic breast cancer being larger than those detected from peripheral venous blood, with cell areas of 77.59μm² and 62.28μm² [4], respectively.

2.2. Cell density

The densities of various cells in the blood are presented in Table 1[6]. Density serves as the theoretical foundation for traditional methods of CTC separation and enrichment. During Ficoll density gradient centrifugation, CTCs, plasma, and monocytes are retained together in the upper layer, while red blood cells and polymorphonuclear leukocytes sediment at the bottom. CTCs are not exclusively distributed at the interface between red blood cells and the separation medium; they can be present in both plasma and the separation medium. Therefore, during enrichment, it is essential to collect all the liquid above the red blood cell layer to prevent the loss of CTCs.

Table 1 Densities of Various Cellular Components in Blood (g/mL)

Cell Component	Density Range (g/mL)	Cell Component	Density Range (g/mL)
Neutrophils	1.080–1.085	Eosinophils	1.090–1.095
Monocytes	1.050–1.066	T Lymphocytes	1.065–1.077
B Lymphocytes	1.062–1.075	Lymphoblasts	1.065–1.077
Natural Killer Cells	1.050–1.070	Platelets	1.030–1.060
Red Blood Cells and Polymorphonuclear Neutrophils	1.090–1.110	Circulating Tumor Cells (CTCs)	<1.077

2.3. Cell rigidity

The mechanical behavior of cells is typically described by measuring the Young's modulus. The Young's modulus is a physical quantity that characterizes the properties of a material, depending solely on the material's inherent physical properties. The magnitude of the Young's modulus indicates the rigidity of the material; the greater the Young's modulus, the less likely the material is to deform.

Multiple studies have indicated that the Young's modulus of cancer cells is lower than that of healthy cells, and the Young's modulus of metastatic cancer cells is lower than that of non-metastatic cancer cells. The deformation ability of cancer cells is higher than that of white blood cells, and metastatic cancer cells

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possess stronger deformation and cell membrane repair capabilities. Research based on the Young's modulus of cells has shown that the stiffness of cancer cells is closely related to the distribution of the actin network within the cytoskeleton. A decrease in the level of actin can increase the elasticity of circulating tumor cells (CTCs), enabling them to resist the shearing forces of liquid flow and survive in peripheral blood. Atomic force microscopy studies on the viscoelastic properties of cultured cancer cell lines and normal blood cells have shown that the Young's modulus of metastatic cell lines is lower than that of normal cell lines, and the deformation ability of cancer cells is greater than that of normal blood cells [7-9]. Chen et al. [7] found that the Young's modulus of the highly metastatic prostate cancer cell line PC-3 is approximately 30 times lower than that of the non-tumorous cell line BPH-1. Measurements of the Young's modulus of CTCs in the peripheral blood of patients with castration-resistant prostate cancer and bone metastasis were similar to the results of PC-3, with a lower Young's modulus. Cross et al. [9] showed that the Young's modulus of breast cancer cells with liver metastasis is 70% lower than that of benign reactive mesothelial cells. Microrheological optical extensometry measurements of the deformation ability of individual cells revealed that the deformation ability of primary oral squamous cell carcinoma cells is 3.5 times that of healthy human keratinocytes [10]. Fluid shear flow detection of cell deformation ability found that malignant cells from the ascitic fluid of 11 patients with tumors and mesotheliomas have at least 18% higher deformation ability than non-activated monocytes and neutrophils, 5% higher than activated neutrophils, and comparable or slightly higher than the deformation ability of activated monocytes [11].

Rejniak [12] derived the trajectory of circulating tumor cells (CTCs) within blood vessels through computational mathematical modeling, demonstrating that the deformation ability of CTCs plays a crucial role in metastasis. The cytoskeleton of CTCs undergoes a series of regulatory processes to reduce their rigidity, facilitating their ability to withstand compression as they pass through the narrow spaces of the extracellular matrix, epithelial cells, and intercellular gaps (including the walls of capillaries in the circulatory system). Tumor cells insensitive to fluid shear forces experience membrane damage in their first to second generations, leading to the death of most cells and a high rate of survival loss. However, due to the activation of tumor cell membrane repair pathways by extracellular Ca ions, the cell membrane is repaired, and subsequent generations exhibit a moderate survival loss rate, producing a biphasic survival curve. Additionally, tumor gene transformation experiments have shown that oncogenes such as ras, myc, and pi3k can increase cells' resistance to fluid shear forces. This indicates that tumor cells can, during their growth, repair damaged cell membranes through external signals, dramatically resist the effects of fluid shear forces, and adapt to the blood fluid survival environment by internally regulating gene expression to self-adjust resistance to fluid shear stress.

2.4. Cellular dielectric properties

The cell membrane surface charge (membrane potential), membrane capacitance, membrane resistance, cytoplasmic conductivity, and dielectric constant are referred to as cellular dielectric properties. As circulating tumor cells (CTCs) enter the circulatory system, to adapt to the harsh circulatory environment and resist the shear forces of blood fluid flow, they induce changes in cell gene expression, cell membrane cytoskeleton structure, and adhesion properties under external environmental stimuli, affecting the surface charge of the cell membrane [12-13]. For example, different levels of molecular substances form a transmembrane potential inside and outside the cell membrane. Studies have shown that the biological cell membrane carries a negative charge under physiological conditions, with the membrane potential of normal living cells ranging from (-60) to (-100) mV. Due to abnormal metabolism in tumor cells, the levels of functional acid, functional base, and hydroxide constant of the cell membrane are higher than those in healthy cells, while the hydrogen ion constant is lower, causing changes in the membrane potential [13]. Metastatic breast tumor cell lines have higher membrane capacitance than lymphocytes and red blood cells, and early-stage breast tumor cell lines have higher membrane capacitance (3.0, 2.1 μ F/cm²) and cell conductivity (14.6, 8.6 mS/cm) than late-stage breast tumor cell lines [14]. Compared with white blood cells, tumor cells have high membrane capacitance and low cytoplasmic conductivity.

Cellular dielectric properties are related to the frequency of the electric current. At low frequencies, interfacial polarization, which depends on the polarity of cellular particles and the conductivity of the

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surrounding medium, predominates. Typically, below 25 kHz, the plasma membrane acts as an insulator, and living cells are considered insulators. At intermediate frequencies, both the cell's conductivity and dielectric properties are significant, and the cell's size and shape, the integrity and morphology of the cell membrane, and the cytoskeleton structure can be measured electrically. At high frequencies (10 MHz to 10 GHz), the dielectric properties of cells become important, with the nuclear-cytoplasmic ratio and the endoplasmic reticulum playing significant roles in electrical properties. Different cells have specific conductivities and dielectric properties under the influence of a certain frequency of electricity, which forms the theoretical basis for the dielectrophoretic separation of CTCs.

3. Biological characteristics of CTCs

Approximately 80% of tumors in the world are epithelial in origin, and each tumor site releases about 100 million tumor cells into the bloodstream daily, becoming CTCs. Once CTCs leave the tumor site or leave the patient along with the blood, they enter the apoptosis program [15-16]. If cell preservatives are added to the peripheral blood, CTCs can be stabilized for up to 96 hours [16]. These CTCs possess epithelial cell properties and generally contain the epithelial cell-specific surface marker protein—epithelial cell adhesion molecule (Ep CAM). This protein is not expressed on the surface of blood cells, and Ep-CAM antibodies can positively separate rare CTCs from the white blood cell population. This marker protein is the theoretical basis for the CTC separation "gold standard method" Cell Search and is also the theoretical basis for most immune separation methods. CTCs do not express the white blood cell surface marker protein CD45; using CD45 antibodies can remove white blood cells, achieving the purpose of negative selection for CTC separation. Cytoplasmic proteins of epithelial cells—cytokeratins (CKs, CK8, 18, 19)—are the confirmation markers for CTCs.

The phenotype of Ep CAM+, CKs+, and CD45- is currently a widely recognized CTC phenotype model and is also the theoretical basis for mainstream CTC separation methods. However, there is also evidence that the above phenotypes can appear in benign colorectal lesions [17], while other phenotypes can appear in the blood of patients with metastatic tumors, including CD45+ and CK+ double-positive cells. In addition to epithelial and mesothelial markers, CTC tumor cells also express tissue-specific protein markers derived from the tumor, such as prostate tumor PSA, colorectal tumor CEA, breast tumor mucin, and primary liver cell tumor sialic acid glycoprotein receptors, etc.

Furthermore, epithelial-origin tumor cells possess the reversible characteristics of epithelial-mesenchymal transition [18]. This characteristic is the source of CTC generation and also ensures the survival of CTCs in the blood and their invasion outside the blood vessels. The transformation of epithelial cells into mesenchymal cells causes some CTCs to express weakened epithelial cell markers, downregulate epithelial cell markers (CK and E-Cadherin), and upregulate mesenchymal cell markers (vimentin, N-Cadherin, fibronectin, plastin3, activated matrix metalloproteinases, MMPs), which is conducive to tumor cells detaching from intercellular adhesion, giving them variability and invasiveness, producing strong DNA repair capabilities, and making them more resistant to chemotherapy drugs [19]. Therefore, CTCs that do not express or have low expression of epithelial markers often indicate that they have strong invasiveness and self-repair ability, reducing the prognosis of patients.

In the peripheral blood of advanced tumors, there is the appearance of CTC clusters. Early studies believed that CTC clusters can avoid CTC anoikis associated with the loss of basement membrane attachment, protecting tumor cells from resisting the harsh environment in blood circulation and fluid shear forces. The clinical significance lies in the assessment of tumor patient prognosis, especially in clarifying the number, size, and composition of cells in CTC clusters [20-21]. The cell adhesion protein discoidin can serve as a marker for single or clustered CTCs. The reduced expression of discoidin increases the motility of tumor cells, promotes the epithelial-mesenchymal transition of tumor cells, and tumor metastasis. High expression of discoidin increases the number of clustered CTCs, increases the metastatic potential of breast tumors, and shortens the survival rate of patients [22].

Through the comparison of biological characteristics of CTCs and primary solid tumors, it has been found that some treatment target gene mutations differ between CTCs and solid tumors, such as EGFR mutations in lung tumor CTCs, HER2 mutations in breast tumors, and TM-PRSS2-ERG gene translocations in prostate tumors, etc. These changes make CTCs more likely to transfer to other tissues

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and resist chemotherapy drugs. Therefore, studying the mutation of drug target genes in CTCs in peripheral blood is helpful for the selection of individualized treatment for tumor patients.

4. Significance of CTC detection

Although CTCs are rare cells in peripheral blood, they can be dynamically monitored in real-time in the peripheral blood, which is significantly superior to tissue specimens and is known as "liquid biopsy." A large number of literature reports have shown that CTC detection is related to tumor metastasis, patient prognosis, and efficacy evaluation, but it has no statistical significance in relation to tumor staging and lymph node involvement [23]. The application of CTC biological characteristics is beneficial for individualized treatment of tumors. For example, classifying CTCs using epithelial-mesenchymal transition markers helps to identify more invasive CTC subpopulations, providing effective evidence for the clinical selection of appropriate treatment plans; selecting treatment strategies based on CTC genetic phenotypes, marker gene variants, and point mutations [24-25]. In addition, enriching CTCs can utilize techniques such as flow cytometry, RT-PCR technology, FISH, immunocytochemistry, and even single-cell sequencing to study the genetic information of CTCs, explore mechanisms of tumor occurrence, metastasis, drug resistance, predict patient prognosis, outcomes, and responses to drugs, etc.

5. Conclusion

Numerous studies have shown that compared to normal blood cells in the peripheral blood, CTCs generally have physical properties such as larger cell volume, lower density, lower rigidity, and higher membrane capacitance. They also possess epithelial cell surface marker proteins and certain tissue-specific marker proteins. These attributes form the theoretical basis for the separation and enrichment of CTCs in the peripheral blood, leading to the establishment of detection platforms represented by Cell Search, which has been approved by the U.S. Food and Drug Administration for clinical use. However, the heterogeneity of tumor cells is the biggest challenge in CTC research and is also a key point in understanding tumor diseases. Detection platforms established based on a single attribute of tumor cells have certain rates of false negatives and false positives. Therefore, researchers are combining different attributes of tumor cells to establish composite separation technologies to improve the sensitivity, specificity, and efficiency of CTC separation and enrichment, such as Bio Fluidica (Baton Rouge, LA) and On-Q-ity Inc. With the understanding and application of the biophysical characteristics of tumor cells, the development of CTC separation technology will be promoted, making CTC detection an indispensable monitoring method in the prevention and treatment of tumors.

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