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Single-cell Sequencing: Application in the Study of Disseminated Tumour Cells and Breast Cancer Treatment

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Abstract: Breast cancer is a malignant tumor originating from breast epithelial tissue. In essence, breast epithelial cells undergo gene mutation under the influence of carcinogenic factors, leading to abnormal cell proliferation and loss of organism regulation, ultimately leading to the formation of tumors with invasive and metastatic capabilities. Carcinogenic factors of breast cancer involve multiple cellular and molecular mechanisms. Among them, disseminated tumor cells (DTCs) are considered important for treating breast cancer. However, traditional bulk sequencing techniques have limitations, such as the inability to distinguish individual cell differences and dilution of information from key cell subpopulations (such as cancer stem cells and rare immune cells). Single-cell sequencing (scRNA-seq) overcomes the heterogeneity of tumors that traditional sequencing cannot capture by analysing the molecular characteristics of single cells, providing a high-resolution perspective for precise typing of breast cancer, exploration of the mechanism of the microenvironment, and personalized treatment. Through this technology, researchers can identify specific gene expression profiles of different cell subpopulations, thus providing a new basis for the molecular typing and personalized treatment of breast cancer. This article explains how single-cell sequencing is used to describe the origin of disseminated tumor cells (DTCs), analyse tumor heterogeneity, metastasis, etc., and review the current literature on the use of scRNA-seq in breast cancer treatment. In the future, cell separation and processing steps in single-cell sequencing will be further improved to ensure the accuracy of the results and broader application in clinical diagnosis and treatment.

Keywords: Disseminated tumor cells; Tumor heterogeneity analysis; Single-cell sequencing; Metastasis; scRNA-seq method

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1. Introduction

Disseminated tumor cells (DTCs) are malignant cells separated from primary tumors that spread to other parts of

the body. DTCs are the key "seeds" of breast cancer metastasis. After entering tumor cells, DTCs transfer tumor cells to the bone marrow or viscera. They first invade lymphatic vessels, causing local symptoms such as an orange peel-like skin appearance, nipple redness and swelling, or dimpling (invasion of Cooper's ligament). They subsequently spread through the lymphatic system to regional lymph nodes such as axillary, infraclavicular, and supraclavicular areas, where they form enlarged or fused lymph node masses. After entering the blood, they can spread to distant organs such as bones, liver, lungs, and brain through the circulatory system. For example, bone metastasis causes bone pain, liver metastasis causes right upper abdominal pain or distension, and brain metastasis may cause epileptic seizures or changes in consciousness. Studies have confirmed that the presence of disseminated tumor cells (DTCs) in the bone marrow is a strong independent prognostic factor for poor survival in breast cancer patients [1]. Isolating and identifying rare disseminated tumor cells (DTCs) from the circulation or premetastatic niches, such as the blood, bone marrow, and body fluids, is the premise for studying tumor metastasis mechanisms. In the study of this disease, disseminated tumor cells have become a breakthrough point for breast cancer research.

2. Technological evolution and advantages

While traditional bulk sequencing provides an average expression profile of an entire tissue sample, it often masks the signals from rare but critical cell populations, such as drug-resistant clones or dormant DTCs. In contrast, the rapid evolution of single-cell RNA sequencing (scRNA-seq) technologies, moving from plate-based methods (like Smart-seq2) to droplet-based microfluidics (like 10x Genomics), has revolutionized our ability to dissect tumor heterogeneity. This high-throughput capability allows researchers to profile thousands of cells simultaneously, creating a comprehensive "cellular atlas" of the tumor ecosystem. By analyzing the transcriptome of individual cells, we can now trace the evolutionary trajectory of cancer cells, identify the ligand-receptor interactions between tumor cells and the stromal microenvironment, and uncover the specific gene regulatory networks that drive metastasis. This level of resolution is indispensable for understanding why some breast cancer patients relapse years after apparently successful treatment, as it likely involves a small subpopulation of cells that were invisible to conventional bulk analysis methods.

3. Using single-cell sequencing to study the origin of DTCs in breast cancer

3.1. Research on the origin of DTCs

Research utilizing single-cell sequencing has challenged the traditional metastasis model. Hosseini et al. [2] demonstrated that breast cancer cells can disseminate to distant organs such as the bone marrow very early in tumor development, often before the primary tumor is clinically detectable. Their single-cell analysis revealed that these early DTCs possess a distinct molecular signature compared to late-stage metastatic cells. This finding suggests that metastasis is not necessarily a late event in tumor evolution, providing a new theoretical basis for early intervention.

4. Background on DTCs promoting metastasis in tumours

Metastasis is the leading cause of death in patients with breast cancer (BC). Therefore, studying metastasis is crucial for identifying a cure for breast cancer. Disseminated tumor cells (DTCs) detach from the primary site and en-

ter the bloodstream to become circulating tumor cells (CTCs), which are the precursors of metastasis. Single-cell sequencing of bone marrow-derived epithelial-like cells, conducted concurrently with analysis of intratumoral genetic heterogeneity from bulk DNA, is an effective method for identifying and studying DTCs, providing insights into the metastatic process.

4.1. Single-cell analysis of circulating tumor cells

Lawson et al. conducted comprehensive single-cell analysis of circulating tumor cells (CTCs) [3]. They identified significant heterogeneity within CTC populations, finding that cells expressing stem-cell-like markers and those undergoing epithelial-to-mesenchymal transition (EMT) are key drivers of metastasis. This highlights the power of scRNA-seq in identifying rare, metastasis-competent subpopulations that traditional bulk sequencing might miss.

4.2. Dynamic Epithelial-Mesenchymal Transition (EMT) phenotypes

The process of metastasis is often driven by Epithelial-Mesenchymal Transition (EMT), but single-cell analysis has revealed that this is not a binary switch but a dynamic spectrum. Yu et al. utilized RNA sequencing on circulating tumor cells isolated from breast cancer patients and identified a dual expression of epithelial and mesenchymal markers within the same cells [4]. This "hybrid" E/M phenotype allows tumor cells to retain the cell-cell adhesion properties necessary for survival in clusters while simultaneously acquiring the migratory and invasive capabilities of mesenchymal cells. Their study showed that the proportion of these hybrid cells fluctuates during treatment and correlates with disease progression. This finding challenges the classical view that cells must undergo a complete mesenchymal transition to metastasize, suggesting instead that cellular plasticity and the ability to cycle between states are the true drivers of metastatic spread.

4.3. The role of CTC clusters in metastasis

Beyond single circulating tumor cells, recent single-cell studies have highlighted the critical role of CTC clusters—groups of tumor cells traveling together in the bloodstream. Aceto et al. demonstrated that although CTC clusters are rarer than single CTCs in the blood of breast cancer patients, they possess a 23- to 50-fold increased metastatic potential [5]. Single-cell sequencing of these clusters revealed that the cell-cell junctions within the cluster (mediated by proteins like plakoglobin) protect the tumor cells from anoikis (cell death induced by detachment) and immune attack in the circulation. Furthermore, the analysis showed that cells within these clusters exhibit a distinct hypomethylation pattern compared to single CTCs, particularly in binding sites for stemness-associated transcription factors. This suggests that the clustering of cells is not merely a physical aggregation but a biological state that promotes stemness and survival, making these clusters the primary drivers of polyclonal metastasis. Understanding the molecular biology of these clusters via single-cell resolution offers new avenues for therapy, such as targeting cell adhesion molecules to dissociate clusters into less metastatic single cells.

5. Single-cell sequencing was used for heterogeneity analysis of disseminated tumor cells (DTCs)

5.1. Reasons for using single-cell sequencing for tumor heterogeneity analysis

Single-cell sequencing of cells isolated from bone marrow aspirates of localized breast cancer patients, in combination with intratumoral genetic heterogeneity analysis of bulk tumor DNA, is a powerful method for identifying

true DTCs and their biology and for subsequent studies on tumor progression. Specifically, tumor heterogeneity means that within a single tumor, not all tumor cells and tumor tissues possess a single set of tumor characteristics but rather have their own relatively specific and different features and clinical manifestations. This characteristic is unique to tumors and leads to differences in treatment and prognosis. In simple terms, tumor heterogeneity refers to intratumoral heterogeneity within a cancer. scRNA-seq can assess this at single-cell resolution, thereby effectively alleviating some issues that traditional chemotherapy cannot address. scRNA-seq provides complete gene expression patterns for individual cells that are masked in bulk analysis.

5.2. Unravelling subclonal heterogeneity in TNBC

Single-cell RNA sequencing has been particularly instrumental in understanding triple-negative breast cancer (TNBC). Karaayvaz et al. analyzed TNBC tumors at single-cell resolution and revealed distinct subclonal populations [6]. They identified specific metabolic pathways and gene signatures that drive therapy resistance, which are often masked in bulk sequencing data. These findings suggest that molecular profiling at the single-cell level is more detailed and accurate, potentially guiding clinicians to select more effective treatment options for distinct tumor subclones.

5.3. Characterizing the immune microenvironment in TNBC

Tumor heterogeneity is not limited to cancer cells; the immune microenvironment surrounding the tumor also displays significant diversity that influences prognosis. Savas et al. utilized single-cell RNA sequencing to profile T cells isolated from human breast cancer tissues [7]. They identified a specific subset of CD8+ T cells known as tissue-resident memory T cells (TRM), which are characterized by the expression of immune checkpoint molecules and cytotoxic markers. The abundance of these TRM cells was found to be significantly associated with better patient survival and improved response to immunotherapy in triple-negative breast cancer. Unlike bulk sequencing, which might only show an increase in general T-cell markers, single-cell analysis allowed for the precise distinction between exhausted T cells, effector T cells, and these crucial TRM cells. This distinction is vital for developing personalized immunotherapies, as it suggests that strategies aimed at expanding or reactivating this specific T-cell subset could be more effective than broad immune stimulation.

6. Discussion on other therapeutic aspects of breast cancer via single-cell sequencing

Single-cell sequencing has also recently been used to characterize changes in tumors after treatment. This technology is also used to determine how these changes are related to drug resistance, the suppression of tolerant cell populations, and combination strategies. A single-cell study in breast cancer tissue indicated the existence of preexisting genotypes resistant to chemotherapy. These genotypes have the capacity for further adaptation after exposure to chemotherapy. This contrasts with transcriptional profiles that can adapt to chemotherapy. scRNA-seq studies promise to provide information on each cell type in the tumor whose alterations might be associated with patient demographics, diagnosis, treatment, and prognostic factors. In the near future, scRNA-seq might be used clinically to develop personalized medical regimens for treating each patient. Tumours can be analysed on the basis of their cellular composition and expression of specific targetable proteins, which could ultimately lead to an optimal response for each patient.

6.1. Evolution of chemoresistance in TNBC

A critical application of scRNA-seq is dissecting the evolutionary history of chemoresistance. Kim et al. performed single-cell DNA and RNA sequencing on triple-negative breast cancer (TNBC) patients receiving neoadjuvant chemotherapy [8]. Their analysis conclusively resolved a long-standing debate regarding the origin of resistance: did the drugs induce new mutations (acquired resistance), or did they select for pre-existing rare clones (adaptive selection)? The data showed that in the majority of patients, resistant genotypes were already present at low frequencies in the primary tumor before any treatment was administered. Chemotherapy acted as a selection pressure that eliminated sensitive clones, allowing the pre-existing resistant subpopulation to expand and become dominant. This implies that early detection of these rare, pre-existing resistant clones via deep single-cell sequencing could guide the use of combination therapies upfront, rather than waiting for treatment failure to occur.

Single-cell sequencing has also generated large-scale single-cell atlases of the breast cancer ecosystem to identify new precision medicine approaches. Another study used scRNA-seq data to identify unique breast epithelial clusters from healthy breast tissue. Interestingly, certain genes were coexpressed with the estrogen receptor (ER) in bulk breast cancer tissue samples, suggesting clinically relevant subclasses within ER-positive breast cancer. Furthermore, understanding the mechanism of tumor dormancy is crucial. Albrengues et al. discovered that inflammation-induced neutrophil extracellular traps (NETs) can awaken dormant cancer cells, triggering metastasis [9]. Additionally, Wagner et al. generated a large-scale single-cell atlas of the breast cancer ecosystem, identifying unique epithelial clusters and immune cell phenotypes associated with different clinical outcomes [10].

7. Conclusion

This article discusses single-cell sequencing in breast cancer with respect to the origin of DTCs, heterogeneity, metastasis, etc. I believe that the development of single-cell sequencing technology has advanced breast cancer research, enabling us to seek answers to many previously unknown questions. However, the field of single-cell data science still faces challenges, including cost, throughput, and data interpretation. Clinical translation is still limited by difficulties in sample acquisition, data standardization, and the threshold for bioinformatics analysis.

7.1. From single cells to spatial transcriptomics

While scRNA-seq provides a catalog of cell types, it requires tissue dissociation, which leads to the loss of spatial information—knowing “where” a cell is located is often as important as knowing “what” it is. To address this, the field is moving towards spatial transcriptomics. Ståhl et al. [11] introduced a method to visualize and analyze gene expression in tissue sections without disrupting the tissue architecture. In the context of breast cancer, this technology allows researchers to map the precise location of immune cells relative to the tumor core and the invasive margin. Understanding this spatial organization is crucial because the same immune cell type may have a tumor-suppressive function when infiltrating the tumor but a pro-tumorigenic function when located in the stroma. Integrating single-cell resolution with spatial coordinates will be the next frontier in constructing a truly holistic 3D model of the breast cancer ecosystem. Furthermore, better methods are needed to allow for the integration of single-cell data across experiments and measurement types. Given the difficulty of sample acquisition, I suggest integrating this technology with regional healthcare systems to reduce collection challenges. In summary, an analysis of the origin, metastasis, and heterogeneity of DTCs will likely contribute to the discovery of new treatment strategies and cell research in the near future, thereby strengthening the implementation of precision medicine and

enhancing therapeutic outcomes.

Disclosure statement

The author declares no conflict of interest.

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