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Review Article

Research Progress of Single-Cell Sequencing Technology in Giant Cell Tumor of Bone

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Abstract: Giant Cell Tumor of Bone (GCTB) is a common intermediate tumor, and the specific molecular mechanisms of this disease have not been fully elucidated. It exhibits strong heterogeneity in terms of targets, regulatory mechanisms, cell types, states, and subset distributions in the immune microenvironment. Traditional collective-level analyses cannot accurately reveal these differences. Single-cell sequencing technology is a technique that sequences the genome, transcriptome, and epigenome of diseases at the single-cell level. Single-cell sequencing can utilize a higher pixel resolution to reveal characteristic states of different cell subpopulations, contributing to broadening new perspectives for the study of GCTB heterogeneity. It holds significant value for the precise diagnosis of GCTB, identification of potential immunotherapy targets, and prognosis assessment. This study primarily reviews research related to GCTB, single-cell high-throughput sequencing technology, GCTB immune microenvironment, GCTB heterogeneity, and the construction of GCTB cell maps. It aims to provide theoretical reference for research on single-cell sequencing technology in GCTB and offers a theoretical basis for in-depth exploration of the mechanisms and treatment of GCTB.

Keywords: Giant Cell Tumor of Bone, Single-Cell Sequencing, Tumor Microenvironment, Heterogeneity

1. Introduction

Giant Cell Tumour of Bone (GCTB) is a tumour with a tendency to grow aggressively in the affected area, primarily observed in the Asian population [1]. The occurrence of this phenomenon is prevalent among individuals in their early adulthood, often ranging from 20 to 40 years of age, without any notable variations based on gender [2]. Lesion curettage surgery is the main treatment for GCTB [3]. Nevertheless, GCTB demonstrates significant local invasiveness, a proclivity for recurrence, and the possibility of malignant change [4]. From a histological perspective, this condition is defined by the existence of cells with multiple nuclei and cells with a single nucleus [5, 6]. Multinucleated giant cells display both functional and physical traits that are indicative of osteoclasts [7, 8]. In addition, mononuclear cells consist of actively dividing spindle-shape stromal cells (tumour components) and osteoclast precursors, which are part of the mononuclear macrophage lineage [9, 10].

The primary presentation of GCTB is characterised by localised and aggressive bone resorption, mostly driven by osteoclasts. This commonly results in the recurrence of the condition following surgical removal [11, 12]. Gaining insight into the aetiology of this locally invasive primary bone neoplasm is crucial for the development of novel therapeutic approaches. By utilising advanced sequencing techniques like single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq) in combination with single-cell RNA sequencing (scRNA-seq) technology, we can uncover regulatory patterns of gene expression at the chromatin level in individual cells. This strategy offers a viable means of studying the particular gene regulatory mechanisms of different cell types, revealing the reasons behind differential gene expression related to diseases, and providing new insights for early illness diagnosis and therapy [13]. Hence, this paper will largely examine the utilisation and present

research progress of single-cell high-throughput sequencing technique in investigating GCTB.

2. Single-Cell Sequencing Technology

2.1. The Principle of Single-Cell Sequencing Technology

Single-cell sequencing technique involves the use of high-throughput sequencing to analyse the genome, transcriptome, or epigenome of an individual cell. This approach provides a higher level of detail, allowing for the identification of specific properties of a Single-cell and enabling a more accurate understanding of the disease's underlying condition [14]. The conventional high-throughput sequencing technology solely captures the average gene expression of heterogeneous cells across numerous tissue samples. Single-cell sequencing technology surpasses traditional sequencing techniques by accurately quantifying gene expression levels and identifying non-coding RNA expression. Additionally, it maximises the benefits of sequencing unique samples, thereby compensating for the limitations associated with small and challenging-to-obtain sample sizes [15–17]. Acquire a more profound comprehension of the mechanisms that regulate gene expression during the processes of cell growth and differentiation. This technology has the capability to monitor the progression of individual cells, unveil the genetic characteristics of individual cells, and effectively examine the diversity among different cells and cell subgroups. Early illness diagnosis, targeted therapy, and researching the unique mechanisms of cell differentiation and growth and development are highly significant [18]. Single-cell transcriptome sequencing is currently extensively employed in various fields including reproductive development [19], nerve growth [20], cell differentiation [21], and tumour heterogeneity [22]. In the domain of immune microenvironment [23].

2.2. The Process of Single-Cell Sequencing Technology

The process of Single-cell sequencing mostly involves the following steps: collection of tissue samples, creation of single-cell suspension, capture of individual cells, amplification of the entire genome, high-throughput sequencing, and subsequent analysis of the acquired data. Although the specific processes may vary depending on the platform used, the overall procedure remains largely consistent. Specialised service firms typically offer single-cell sequencing technology due to the expensive nature of sequencing platforms and the requirement for trained individuals to operate them. Typically, researchers must select the suitable technical platform and carry out the duties of sample collection and data analysis.

2.3. The Classification of Single-Cell Sequencing Technologies

Single-cell sequencing technology covers a series of multidimensional and multi-level technologies. In recent years, various single-cell sequencing methods have emerged, including whole genome sequencing (scWGS), whole transcriptome sequencing (scRNA seq), whole DNA methylation sequencing (scM seq), whole exome sequencing (scWES), transposase accessible chromatin region sequencing (scATAC seq) [24]. Various sequencing technologies possess distinct attributes and benefits, enabling the acquisition of cellular characteristic data from diverse levels and dimensions at various phases. ScATAC-seq is a technique used to examine the accessibility of chromatin at the single-cell level [25]. ScWGS for screening copy number abnormalities and individual nucleotide mutations. ScRNA seq is the most advanced single-cell sequencing technology currently accessible. It allows for the identification of cell types, analysis of cell states, pathway analysis, analysis of expression subtypes, analysis of transcription factors, inference of tumour lineages, and analysis of cell type specific differential expression [26, 27].

3. Value of Single-Cell Sequencing in GCTB

3.1. Single-Cell Sequencing Technology Research on GCTB Heterogeneity

The presence of diverse characteristics within tumours is a significant factor contributing to the emergence of drug resistance during tumour medication therapy. Additionally, it poses a major obstacle in achieving precise medical treatment for tumours. Giant cell tumour of bone, being a tumour with intermediate characteristics, exhibits significant local invasiveness, a propensity for recurrence, and a potential for malignancy. The clinical features and molecular causes of GCTB are intricate, and conducting cell grouping analysis on GCTB tumour tissue can aid in identifying drug-resistant cell clusters, thereby enabling targeted treatment of GCTB. Single-cell sequencing technology enables precise and impartial grouping of GCTB cells. Feng et al. [28] created a thorough human GCTB cell atlas using both Single-cell and spatial resolution techniques. The researchers collected a total of 8033 cells from a patient diagnosed with GCTB using single-cell transcriptome sequencing. They identified eight distinct cell types, which included macrophages, osteoblasts, NK/T cells, osteoclasts, pericytes, proliferating cells, endothelial cells, and chondrocytes. Upon conducting a more detailed examination of the transcriptome of osteoclasts, it was discovered that there are three distinct subgroups of osteoclasts. Among these subgroups, the chemotactic cytokine CKLF was found to be present in the C2_ subgroup, which is predominantly composed of mature osteoclasts. This suggests that mature osteoclasts in the C2_ subgroup exhibit a high level of migration and indicates the involvement of CKLF in this process. The development and migration of mature osteoclasts are regulated by four primary signalling pathways: the RANKL signalling pathway network, CD137 signalling pathway network, PARs signalling pathway network, and SMAA3 signalling pathway network. This finding establishes

a theoretical foundation for future investigations into the osteoclastogenic impact of GCTB. Additionally, it offers rich data resources for analysing the diversity, prognosis, and migratory development of GCTB.

3.2. Single-Cell Sequencing Technology Research on GCTB Immune Microenvironment

The tumor immune microenvironment (TME) encompasses the diverse components, including immune cells, inflammatory cells, stromal cells, blood vessels, and extracellular matrix, that coexist within tumor tissue. These components interact with tumor cells and play a crucial role in the genesis, growth, invasion, metastasis, and response to treatment of malignancies. The tumor microenvironment (TME) primarily consists of many types of immune cells, including T cells, B cells, natural killer cells, macrophages, dendritic cells, and inflammatory cells. CD8+T cells and natural killer cells has a dual role. Firstly, they have a cytotoxic effect on tumor cells, leading to their destruction. Secondly, they have the ability to facilitate the growth and progression of tumors [29]. An analysis of the tumor immune microenvironment can provide insights into the mechanisms by which tumors evade the immune system and develop resistance to immune responses. This understanding can inform the development of therapeutic strategies, such as immune checkpoint inhibitors and CAR-T cell therapy, to enhance the efficacy of tumor treatment. Consequently, the investigation of the tumor microenvironment has gained significant attention as a focal point for precise cancer treatment, emerging as a crucial domain in the study of tumor biology [30]. The utilization of Single-cell sequencing technology allows for the thorough examination of the diversity among immune cell populations in the tumor microenvironment (TME) and the identification of distinct subgroups of immune cells within tumors [31]. In order to examine the possible therapeutic targets and novel immunological markers of GCTB, Yang et al. [32] collected tumor tissue from 2 patients diagnosed with GCTB who received or did not get DMAB treatment. Single-cell transcriptome analysis was performed, and Single-cell transcriptome was acquired from 13857 cells. Cellular heterogeneity was observed in cells of identical phenotype derived from distinct organs. The variation among these cells mostly lies in the tumor immunological microenvironment, with a notably larger proportion of tumor-associated macrophages (TAM) in samples with DMAB compared to those without DMAB. Especially, most TAMs have an M2 like phenotype, with considerably higher M2 polarization and anti-inflammatory scores; It was found that the function of T cells was diminished, leading to a transition from an activated state to depleted T cells. The potential mechanism behind this T cell depletion was also identified. This discovery greatly aids in comprehending tumor biology and holds significant value in identifying potential therapeutic targets and immune markers for GCTB. Giant cell tumor of bone (GCTB) arises from the uncontrolled growth of mononuclear spindle stromal cells, an imbalance in the bone metabolism cytokines RANKL/OPG, the stimulation of malignant transformation within GCTB, the excessive activation of multinucleated giant cells, and finally leads to the progression of GCTB. The precise alterations in TME cells during the advancement of GCTB remain uncertain. The scRNA sequencing technology identifies distinct cell populations and transcriptional characteristics to investigate the precise regulatory mechanisms in TME cells in GCTB.

3.3. Single-Cell Sequencing Technology for Targeted Therapy of GCTB

Tumor targeted therapy has gradually become a hot topic in cancer treatment, but the biomarkers used for clinical applications are still difficult to determine, and targeted therapy for tumors still faces many challenges. Single-cell sequencing technology has the potential to be a valuable tool for investigating targeted therapy for tumors, anticipating possible anti-tumor targets, and has significant clinical application value in the targeted therapy of GCTB. In 2022, Mesalie et al. [33] performed transcriptome analysis utilizing single-cell RNA sequencing (scRNA-seq) on tumor tissue obtained from one patient with giant cell tumor of bone (GCTB) and six patients with osteosarcoma (OS). The study revealed the presence of epidermal growth factor-like domain 7 (EGFL7) in GCTB. Studies in tumor biology have demonstrated that EGFL7 plays a role in controlling tumor development, metastasis, and proliferation [34]. The EGFL7 protein is also thought to facilitate tumor angiogenesis, as indicated by studies [35, 36]. Simultaneously, it was discovered that the angiogenic gene VEGF exhibited a substantial increase in expression, suggesting that these genes and their expression patterns are particular to certain cell types. It is hypothesized that VEGF is excessively produced in GCTB tissues and maybe contributes to the invasion and spread of GCTB [37]. The elevated expression of VEGF in GCTB may be associated with the advanced stage of malignancies [38]. GCTB can benefit from the identification of potential prognostic biomarkers and the development of cell type focused therapy.

CD8+ T cells are essential in the immune response as they are primarily responsible for recognizing and eliminating infected or aberrant cells, such as those infected by a virus or cancer cells. Nevertheless, persistent immunological activation can result in compromised functionality of CD8+T cells, diminishing their ability to eliminate pathogens. This can manifest as symptoms like weariness, reduced efficacy, and the adoption of an inhibitory phenotype, which is referred to as CD8+T cell depletion. Tumor immune suppression is caused by the depletion of CD8+ T cells within tumors [39]. By employing variable promoters, a single gene can generate several mRNA transcripts, hence facilitating more intricate control over gene expression. This phenomenon is crucial in producing a wide range of transcriptome variations, distinct tissue expression patterns, and diverse protein functions originating from a single gene [40]. Yang [32] validated a decline in T cell activity levels in GCTB using scRNA seq analysis and discovered notable expression of distinctive

markers associated with immunological exhaustion. Single-cell sequencing technology has uncovered the immune depletion mechanism of GCTB, offering a pathway for immunotherapy in the clinical management of GCTB patients.

4. Conclusions

The development of GCTB is intricate, and its identification and management pose significant difficulties. Single-cell sequencing technology enables the identification of cellular heterogeneity at more detailed levels, and serving as valuable reference materials for the diagnosis and prognosis of GCTB. Nevertheless, single-cell sequencing technology currently exhibits numerous limitations. The method is constrained by the challenges of isolating and capturing individual cells, as well as the difficulty in preserving the integrity and functionality of cell membranes. As a result, the genuine condition of cells in their natural environment is compromised. Recent research has demonstrated that the most recent developments in spatial transcriptomics (ST), can address the constraints of single-cell RNA sequencing (scRNA seq) by providing unbiased recordings of the entire tissue slice [41]. The task of quantifying a substantial quantity of genomic data from individual cells in a single experiment, as well as interpreting and managing the resulting extensive data, is a significant obstacle in understanding biological information. Furthermore, the capture efficiency imposes restrictions on the process of preparing samples, and as a result of the destructive or inefficient characteristics of current technologies, it is probable that uncommon cell populations will be disregarded. Hence, it is imperative to enhance the effectiveness of transcriptome and genome coverage throughout the amplification process, thereby diminishing mismatch rates and noise effects. Moreover, it is imperative to develop more accurate and thorough bioinformatics techniques for evaluating the developmental path of cell populations. Therefore, promoting interdisciplinary cooperation between bioinformatics and computational biology will significantly accelerate the progress of single-cell sequencing experiments. This collaboration will be instrumental in identifying the variations among tumor cells and gaining a comprehensive comprehension of the tumor microenvironment. Consequently, it will yield more dependable data for clinical treatment and serve as a theoretical foundation and guiding framework for precise personalized therapy.

Conflicts of Interest

The authors declare no conflicts of interest.

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