Advances in the Application of Single-Cell Sequencing Technology in the Treatment and Prognostic Assessment of Acute Myeloid Leukemia

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Abstract: Acute myeloid leukemia (AML) is a highly heterogeneous hematologic malignancy. Traditional treatment methods have proven inadequate, resulting in poor treatment outcomes. The rapid advancement of single-cell sequencing technology in recent years has provided a new perspective for AML research. This review summarizes the progress in the application of single-cell sequencing technology in the treatment and prognostic assessment of AML. In terms of treatment, this technology can uncover the heterogeneity of AML cells, provide a basis for personalized treatment strategies, monitor cellular responses, and assess treatment efficacy. In terms of prognostic assessment, single-cell sequencing technology can identify prognostic markers, construct prognostic models, and evaluate the impact of the immune microenvironment on prognosis. Despite challenges such as high costs and complex data interpretation, the integration of multi-omics analysis is expected to become a future trend, potentially enhancing AML treatment outcomes and patient survival rates through continuous technological innovation and optimization.

***Keywords*:** acute myeloid leukemia, single-cell sequencing, treatment, prognostic assessment

1. Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous hematologic malignancy, with its heterogeneity manifesting not only at the cellular level but also in the genomic and transcriptomic landscapes. Traditional AML treatments primarily include chemotherapy, targeted therapy, and hematopoietic stem cell transplantation. Chemotherapy regimens, such as the "3+7" protocol (cytarabine combined with anthracycline drugs), have limited efficacy in elderly patients or those unsuitable for intensive chemotherapy, with a median overall survival of only 9 months and a 5-year survival rate of 10% in patients over 60 years old[1]. Although targeted therapy has made progress, drugs targeting single molecular abnormalities are restricted in their effectiveness due to the heterogeneity of AML. For example, drugs targeting FLT3 mutations are ineffective against other subtypes[2]. CAR-T cell therapy is also limited in AML due to the lack of uniform targets and the complexity of the tumor microenvironment.

In recent years, the rapid development of single-cell sequencing technology has provided a new perspective for understanding the pathogenesis of AML and optimizing treatment strategies. Single-cell sequencing technology also offers a new approach for personalized treatment and prognostic assessment. This review will summarize the potential value of single-cell sequencing technology in clinical practice, exploring its latest applications in the treatment and prognostic evaluation of AML.

2. Applications of Single-Cell Sequencing Technology in the Treatment of Acute Myeloid Leukemia

2.1 Revealing AML Cell Heterogeneity

Each subpopulation of AML cells has unique gene expression profiles, reflecting the heterogeneity of AML cells at different stages of differentiation. Van Galen et al. (2019)[3] identified multiple cell subpopulations with distinct differentiation stages and functions in AML samples through single-cell RNA sequencing (scRNA-seq), including leukemia stem cell-like cells, progenitor-like cells, and various mature myeloid cell subpopulations. In the study by Wu et al. (2023)[4], single-cell RNA sequencing was performed on bone marrow samples from five NK-AML (M4/M5) patients and one healthy donor, identifying 18 distinct cell subpopulations, including early erythrocytes, monocytes, T cells, natural killer (NK) cells, and hematopoietic stem/progenitor cells. Fang et al. (2023)[5] used single-cell chromatin accessibility analysis to analyze 22 bone marrow samples from eight patients, finding that treatment-resistant AML cells exhibited stem cell and progenitor cell characteristics and covered a continuous spectrum from quiescent to activated and late-stage stem/

progenitor cell states. Additionally, treatment-resistant AML cells included cells differentiated into myeloid, erythroid, and even lymphoid lineages. This heterogeneity persisted in subsequent treatments and was associated with poor prognosis.

Petti et al. (2019)[6] integrated whole-genome sequencing (WGS) and single-cell RNA sequencing (scRNA-seq) to conduct an in-depth analysis of five cryopreserved AML samples. The study identified hundreds to thousands of cells carrying tumor-specific mutations and used these data to distinguish AML cells (including those with normal karyotypes) from normal cells. This integrated approach closely links genotype with phenotype, enabling high-resolution parsing of AML cell heterogeneity. The results showed that even in AML with normal karyotypes, there is significant clonal diversity, with clones exhibiting different characteristics in gene expression patterns. For example, some clones may have higher proliferative capacity, while others may be more resistant to treatment. By identifying these distinct clonal features, the study provides a theoretical basis for developing targeted therapies against specific subclones. The heterogeneity of AML cells is not only reflected in genetic mutations but also in the differentiation status and function of the cells. In AML, cell heterogeneity is manifested by some cells having stem cell characteristics, such as self-renewal ability and low proliferative activity, which may be resistant to treatment and cause disease recurrence[7].

Single-cell sequencing technology can reveal the heterogeneity of antigen expression in AML cell populations, aiding in the selection of the most suitable antigen targets. Peroni et al. (2023)[8] pointed out that AML cells can evade immune attacks by expressing specific surface molecules such as CD33.

2.2 Assessing Treatment Efficacy and Explaining Mechanisms of Drug Resistance

Monitoring treatment responses using single-cell sequencing technology provides unprecedented precision in the efficacy assessment of acute myeloid leukemia (AML). Troy M. Robinson et al. (2023)[9] developed a single-cell minimal residual disease (scMRD) detection method that combines flow cytometry to enrich target precursor/mother cell populations with single-cell DNA sequencing and immunophenotyping. This method achieves high-sensitivity detection of residual leukemia cells after AML treatment. The study results show that the sensitivity of scMRD detection is approximately 0.01%, effectively deconstructing clonal structures and providing information on the clonal architecture of leukemia cells surviving after AML treatment. This method can assess the immediate treatment efficacy and predict the risk of disease recurrence, providing important evidence for timely adjustment of treatment plans. Wang et al. (2024)[10] used single-cell RNA sequencing (scRNA-seq) to analyze peripheral blood samples from 51 elderly AML patients before and after umbilical cord blood (UCB) infusion as an adjuvant therapy. The results showed that the proportions of cytotoxic CD8+ T cells (such as CD8T_01_GZMK and CD8T_06_STMN1) and natural killer (NK) cells (such as NK_02_XCL1) in patients' bodies increased significantly after treatment, while the proportion of CD14+ monocytes (Mo_01_CD14) decreased significantly. These changes were directly related to the extension of overall survival (OS) and event-free survival (EFS) in patients. Specifically, with a median follow-up of 27.3 months, the median OS had not been reached, and the median EFS was 72.2 months. The 2-year OS and EFS rates were 76.9% and 62.8%, respectively. Additionally, gene ontology (GO) analysis showed that biological processes related to antitumor and anti-aging effects were significantly enriched after treatment, such as antigen processing and presentation, activation of immune responses, and positive regulation of T cell activation.

Additionally, there was heterogeneity within AML cells, with some progenitor cells proliferating and differentiating and expressing an oxidative phosphorylation (OxPhos) signature, while others were OxPhos (low) miR-126 (high) cells, showing enhanced stemness and quiescence[11]. miR-126 (high) LSCs were enriched in chemotherapy-resistant AML and recurrence, and their transcriptional signature strongly stratified patient survival in large AML cohorts[12]. These findings not only explained the mechanisms of treatment resistance but also provided a theoretical basis for developing new targeted therapies.

2.3 Developing Personalized Treatment Strategies

Single-cell sequencing technology not only helps researchers understand the co-occurrence and mutual exclusivity of gene mutations at the single-cell level but also reconstructs clonal evolutionary patterns, including linear and branched evolution, including convergent evolution[13]. For example, in patients with FLT3-mutated AML, this technology can precisely track the evolution of clones during FLT3 inhibitor treatment and identify the emergence and expansion of drug-resistant clones[14]. Researchers found that drug-resistant clones often carry additional NRAS or KIT mutations that may mediate drug resistance by activating the RAS signaling pathway[15]. NRAS and KIT thus become potential targets for combination therapy. Mechanistically, NRAS and KIT mutations can counteract the inhibitory effects of FLT3 inhibitors, promoting the proliferation and survival of leukemia cells by activating the downstream MAPK signaling pathway[16]. Single-cell sequencing technology helps develop personalized treatment strategies by tracking clonal evolutionary patterns. It can precisely track the evolution of drug-resistant clones during FLT3 inhibitor treatment, providing scientific evidence

for overcoming drug resistance.

3. Applications of Single-Cell Sequencing Technology in the Prognostic Assessment of Acute Myeloid Leukemia

3.1 Identifying Prognostic Markers

Zou et al. (2023)[17] pointed out that single-cell RNA sequencing (scRNA-seq) technology can parse the transcriptional heterogeneity within AML cells and identify key genes related to disease progression and recurrence. For example, through scRNA-seq technology, researchers identified that high expression of the HOXA3-10 gene in AML cells is closely related to poor prognosis. The high expression of these genes may affect the survival rate and recurrence rate of AML patients by regulating biological processes such as cell proliferation, differentiation, and apoptosis[18].

Furthermore, Miao et al. (2024)[19] used scRNA-seq technology to construct a prognostic scoring model for AML based on the tumor immune microenvironment. They found that high expression of genes such as ETS2, CCL5, and IL2RA is significantly associated with low survival rates in AML patients. These genes regulate the infiltration and activation of immune cells, affecting immune evasion and disease progression in AML patients. Qian Xiong et al. (2020)[20] identified 31 differentially expressed genes through single-cell RNA sequencing analysis of bone marrow or peripheral blood samples from t(8;21) AML patients, of which 13 were related to leukemia. Further validation confirmed ARID2, MLL, and SYNCRIP as potential prognostic biomarkers. The expression levels of these genes can predict the overall survival outcomes of t(8;21) AML patients, with high expression of ARID2 and MLL associated with poor prognosis, while high expression of SYNCRIP is related to better prognosis.

Single-cell sequencing technology plays a key role in the construction and validation of prognostic models, with identified prognostic markers aiding physicians in more accurate prognostic evaluations of patients.

3.2 Assessing the Impact of the Immune Microenvironment on Prognosis

The state of the immune microenvironment plays an indispensable role in the prognosis of AML patients. Guo et al. (2021)[21] used single-cell sequencing technology to depict the diversity and dynamic changes of immune cells in the AML bone marrow microenvironment, revealing how AML cells evade immune surveillance by altering the functional state of immune cells. For example, inhibitory cells in the immune microenvironment, such as Treg cells and MDSCs, secrete inhibitory cytokines (e.g., IL-10 and TGF-β) and express immune checkpoint molecules (e.g., PD-1 and CTLA-4), suppressing the activation and function of CD8+ T cells and weakening the antitumor immune response. Meanwhile, AML cells themselves also express inhibitory ligands (e.g., PD-L1) and secrete cytokines (e.g., IL-6 and IL-10), further promoting the formation of an immunosuppressive microenvironment. This immunosuppressive state not only helps AML cells evade host immune surveillance but also increases their resistance to chemotherapy drugs, leading to poor prognosis.

Based on these findings, the development of immunotherapies targeting the immune microenvironment has become a new treatment strategy for AML. For example, immune checkpoint inhibitors (e.g., PD-1 and PD-L1 inhibitors) that block immunosuppressive signals and re-activate T cell-mediated killing of AML cells have shown some efficacy in clinical trials (Daver et al., 2021)[22]. Additionally, cell immunotherapies such as CAR-T cell therapy, which genetically engineer T cells to specifically recognize and kill AML cells, have provided a new treatment option for AML patients[23].

4. Conclusions

Single-cell sequencing technology has demonstrated significant value in the treatment and prognostic assessment of acute myeloid leukemia (AML). It can reveal the heterogeneity of AML cells, monitor treatment responses, assess treatment efficacy, and predict the likelihood of disease recurrence, thereby providing a basis for developing personalized treatment strategies and timely adjusting treatment plans. Many related clinical trials have been conducted in this area.

However, current research still faces certain limitations and challenges. First, single-cell sequencing technology is costly, making it difficult to widely implement in clinical practice. Second, data interpretation and analysis require higher levels of professional knowledge and skills from researchers. Additionally, the process of sample acquisition and processing is complex, which may affect the accuracy and reliability of the results. Finally, although single-cell sequencing technology can provide a wealth of information, how to translate this data into specific treatment decisions in clinical practice still needs further exploration.

Future research directions will focus on technological innovation and optimization to reduce costs, increase throughput, and improve accuracy, thereby promoting the clinical application of single-cell sequencing technology. The integration of multi-omics analysis will become a new trend in research. By comprehensively analyzing the pathogenesis of AML, including

genomic, transcriptomic, proteomic, and metabolomic data at multiple levels, a more comprehensive understanding of the disease can be achieved, thereby more effectively supporting precision medicine. Additionally, new targets and markers identified through single-cell sequencing technology will promote the exploration and validation of new treatment strategies, including immunotherapy, targeted therapy, and combination therapies. The treatment outcomes and patient survival rates of AML are expected to be further improved. In short, with continuous technological progress and in-depth research, single-cell sequencing technology will play an increasingly important role in the treatment and prognostic assessment of AML, bringing more hope and possibilities to patients.

References

[1] Kantarjian, H.M., et al., Acute myeloid leukemia management and research in 2025. CA Cancer J Clin, 2025. 75(1): p. 46-67.
[2] Shimony, S., M. Stahl, and R.M. Stone, Acute myeloid leukemia: 2023 update on diagnosis, risk-stratification, and management. Am J Hematol, 2023. 98(3): p. 502-526.
[3] van Galen, P., et al., Single-Cell RNA-Seq Reveals AML Hierarchies Relevant to Disease Progression and Immunity. Cell, 2019. 176(6): p. 1265-1281.e24.
[4] Wu, J., et al., A single-cell survey of cellular hierarchy in acute myeloid leukemia. J Hematol Oncol, 2020. 13(1): p. 128.
[5] Fang, D.D., et al., MDM2 inhibitor APG-115 exerts potent antitumor activity and synergizes with standard-of-care agents in preclinical acute myeloid leukemia models. Cell Death Discov, 2021. 7(1): p. 90.
[6] Petti, A.A., et al., A general approach for detecting expressed mutations in AML cells using single cell RNA-sequencing. Nat Commun, 2019. 10(1): p. 3660.
[7] Karantanos, T. and R.J. Jones, Acute Myeloid Leukemia Stem Cell Heterogeneity and Its Clinical Relevance. Adv Exp Med Biol, 2019. 1139: p. 153-169.
[8] Peroni, E., et al., Acute myeloid leukemia: from NGS, through scRNA-seq, to CAR-T. dissect cancer heterogeneity and tailor the treatment. J Exp Clin Cancer Res, 2023. 42(1): p. 259.
[9] Robinson, T.M., et al., Single-cell genotypic and phenotypic analysis of measurable residual disease in acute myeloid leukemia. Sci Adv, 2023. 9(38): p. eadg0488.
[10] Wang, J., et al., A phase 2 pilot study of umbilical cord blood infusion as an adjuvant consolidation therapy in elderly patients with acute myeloid leukemia. Signal Transduct Target Ther, 2024. 9(1): p. 358.
[11] Zhai, X. and X. Jiang, Properties of Leukemic Stem Cells in Regulating Drug Resistance in Acute and Chronic Myeloid Leukemias. Biomedicines, 2022. 10(8).
[12] Zhou, J. and W.J. Chng, Unveiling novel insights in acute myeloid leukemia through single-cell RNA sequencing. Front Oncol, 2024. 14: p. 1365330.
[13] Liu, J., et al., Decoding leukemia at the single-cell level: clonal architecture, classification, microenvironment, and drug resistance. Exp Hematol Oncol, 2024. 13(1): p. 12.
[14] Levis, M. and A.E. Perl, Gilteritinib: potent targeting of FLT3 mutations in AML. Blood Adv, 2020. 4(6): p. 1178-1191.
[15] McMahon, C.M., et al., Clonal Selection with RAS Pathway Activation Mediates Secondary Clinical Resistance to Selective FLT3 Inhibition in Acute Myeloid Leukemia. Cancer Discov, 2019. 9(8): p. 1050-1063.
[16] Tecik, M. and A. Adan, Therapeutic Targeting of FLT3 in Acute Myeloid Leukemia: Current Status and Novel Approaches. Onco Targets Ther, 2022. 15: p. 1449-1478.
[17] Zou, Y.X., H.Y. Zhang, and H.B. Hu, Big data and single-cell sequencing in acute myeloid leukemia research. Medcomm-Oncology, 2023. 2(3).
[18] Chen, S.L., et al., The Role of the HOXA Gene Family in Acute Myeloid Leukemia. Genes (Basel), 2019. 10(8).
[19] Miao, P., et al., Establishment and verification of a TME prognosis scoring model based on the acute myeloid leukemia single-cell transcriptome. Sci Rep, 2024. 14(1): p. 19811.
[20] Xiong, Q., et al., Single-cell RNA sequencing of t(8;21) acute myeloid leukemia for risk prediction. Oncol Rep, 2020. 43(4): p. 1278-1288.
[21] Guo, R., et al., Single-cell map of diverse immune phenotypes in the acute myeloid leukemia microenvironment. Biomark Res, 2021. 9(1): p. 15.
[22] Daver, N., et al., T-cell-based immunotherapy of acute myeloid leukemia: current concepts and future developments. Leukemia, 2021. 35(7): p. 1843-1863.
[23] Marofi, F., et al., Novel CAR T therapy is a ray of hope in the treatment of seriously ill AML patients. Stem Cell Research & Therapy, 2021. 12(1).