Vol. 12 (2026): Proceedings of the 2026 International Conference on Life Sciences and Health Engineering
(IC-LSHE 2026)
E-ISSN: 3105-0522 P-ISSN：3105-0514
DOI: https://doi.org/10.70267/lshe.20260109

Progress in Single-Cell RNA Sequencing Data Analysis and Its Applications in the Study of Tumor Heterogeneity

Qingsong Cai*

College of Life Sciences, Sichuan University, Chengdu, Sichuan, 610000, China

**Corresponding author: Qingsong Cai*

Abstract

Tumors are heterogeneous diseases, and their transition to malignancy is often subtle; however, advanced computational tools can elucidate their development and drug resistance. Single-cell RNA sequencing offers high-resolution insights into cellular and molecular changes, enhancing our understanding of cancer dynamics. This review summarizes the important technological breakthroughs since the birth of single-cell sequencing technology and the recent research results concerning tumor heterogeneity and "precision medicine" through single-cell RNA sequencing. Overall, the future of single-cell RNA sequencing in tumor treatment is undoubtedly promising.

Keywords

application, research progress, single-cell sequencing, tumor heterogeneity

1. Introduction

Tumors are systemic diseases and major global challenges that have posed a threat to human health since the early twenty-first century. They undergo critical transformations—from premalignant to malignant states: from locally contained to metastatic disease and from treatment-responsive to treatment-resistant forms [1]. The complexity of treating tumors is exacerbated by their heterogeneity and the intricate composition of the tumor immune microenvironment (TIME), which significantly impacts tumorigenesis, progression, invasion, metastasis, and drug resistance [2-5]. Advances in sequencing technology have facilitated the analysis of data from individual cancer cells, ushering in the era of "precision medicine", which relies on precise molecular data [6, 7]. Single-cell RNA sequencing (scRNA-seq) offers considerable advantages over bulk RNA sequencing (RNA-seq), which provides only limited insights into tumor heterogeneity and average gene expression values of a sample. scRNA-seq is particularly advantageous because of its ability to characterize the frequency of cell types within each sample and to elucidate relationships between cells or cell types and its high-resolution cracking of the TIME [8, 9]. Owing to these advantages, it is significantly superior to previous sequencing techniques in advancing our understanding of the human biology of various cells, especially immune and tumor cells, and since the first single-cell mRNA sequencing in 2009 and the first human cancer cell sequencing in 2011, single-cell sequencing analysis based on next-generation sequencing has gained

attention and developed rapidly [5]. ScRNA-seq is becoming a popular tool in cancer research to explain disease heterogeneity [10]. ScRNA-seq provides biological information on individual tumor cells, analyzes determinants of gene expression heterogeneity within tumors, and determines the molecular basis of the formation of many tumors [11].

In this review, we describe the recent progress in scRNA-seq and summarize its application in the study of tumor heterogeneity, with a focus on how this technology can impact precision medicine in the future.

2. Advances in scRNA-seq

Single-cell RNA sequencing (scRNA-seq) has revolutionized the study of cellular heterogeneity, providing unprecedented high-resolution analytical capability for dissecting the tumor microenvironment and revealing the molecular diversity of cancer cells. The standard workflow mainly includes single-cell isolation, reverse transcription of RNA into cDNA, template preamplification, and high-throughput sequencing analysis [12].

Owing to the extremely low amount of RNA in individual cells, efficiently amplifying limited genetic material has become a core technical challenge in scRNA-seq [13]. Currently, commonly used amplification strategies include polymerase chain reaction (PCR)-based amplification (e.g., SMART-Seq2) and in vitro transcription-based amplification (e.g., CEL-Seq2). For cell isolation, mainstream methods include microfluidic systems, droplet-based technologies, and microwell arrays [14]. Although early representative platforms such as Fluidigm C1 could obtain high-quality gene expression data, constraints in throughput and cost limitations have led to their predominant application in small-scale sample studies [15-17].

The emergence of droplet-based technologies (e.g., Drop-seq and inDrop) marked a significant breakthrough in scRNA-seq. In 2017, 10× Genomics integrated related technologies to launch a commercial platform that greatly increased cell throughput and significantly reduced costs, thereby making large-scale tumor studies feasible [18-21].

Particularly critical is that technical approaches capable of obtaining full-length transcripts, such as SMART-Seq2, demonstrate unique advantages in alternative splicing analysis, allele-specific expression detection, and RNA editing identification owing to their comprehensive coverage of cDNA sequences, greatly facilitating in-depth exploration of tumor heterogeneity [22-24]. Subsequent systems such as SMART-seq3 and BD Rhapsody have further achieved remarkable improvements in detection sensitivity and scalability [10, 25, 26].

In recent years, scRNA-seq technology has continued to advance in terms of increased throughput, multimodal integration, and spatial resolution. For example, the Stereo-cell technology released in 2025, which is based on high-density DNA nanoball arrays, enables in situ transcriptome capture without relying on droplet microfluidics, simultaneously integrating transcriptome, protein signals, and morphological information. This provides a novel perspective for multidimensional analysis of tumor heterogeneity and tissue architecture [27].

3. Some New and In-Depth Applications in Studying Tumor Heterogeneity

Tumor heterogeneity, including inter- and intratumor heterogeneity, is a key feature of malignant tumors and refers to molecular biological or genetic alterations in daughter cells during the proliferation of tumor cells, which is a significant obstacle to cancer treatment and research [28]. Recognizing tumor heterogeneity is key to further understanding and treating cancer, and the use of scRNA-seq for research has reached a consensus. scRNA-seq has been used to promote cancer diagnosis and prognosis prediction, increase comprehension of disease progression and cancer metastasis, and guide treatment [29-33]. In recent years, single-cell sequencing techniques have often been combined with other techniques, such as spatial transcriptomics, to deepen our understanding of tumor heterogeneity, from the tumor cells themselves to the various components of the TIME.

A study published in the *Journal of Cellular and Molecular Medicine* integrated single-cell and bulk RNA sequencing data with machine learning algorithms to construct an 11-gene (including ANXA3, APOE, CENPA, CKS1B) Prostate Cancer Meta-Program (PCMP) signature model. This model effectively predicts prognosis and revealed that the PCMP signature promotes epithelial cell malignant transformation by regulating the cell cycle and oxidative phosphorylation pathways. Experimental validation has shown that knocking down CENPA or CKS1B significantly inhibits PC3 cell proliferation [34].

Research published in *JECCR* by a team from Shanghai Pharmaceuticals in collaboration with Wenzhou Medical University combined single-cell sequencing with deep learning to develop a new method for accurately identifying tumor-reactive CD8+ T cells directly from tumor-infiltrating lymphocytes (TILs) without prior knowledge of neoantigens. The AUC of this deep learning model reached 0.958 in the training set. The study revealed that more than one-third of TR T cells were distributed in the GZMK+ effector memory cell subset, challenging the traditional belief that "TR cells exist only in exhausted populations". All types of TR CD8+ T cells commonly downregulated genes related to the mitochondrial respiratory chain pathway (e.g., MT-ATP6 and MT-ND1), suggesting that impaired mitochondrial function is a key feature. This study identified TIGIT-NECTIN2 as a previously overlooked important immune checkpoint in pancreatic cancer. TCR analysis revealed that the "clonal expansiveness" of the TCR alone could not determine tumor reactivity, and some TR CD8+ T cells simultaneously expressed multiple different TCR α/β chains. These multi-TCR combinations demonstrated stronger tumor-killing capacity. Clinical translational analysis revealed that pancreatic cancer patients with a high proportion of TR CD8+ T cells had a better prognosis after receiving neoadjuvant immunotherapy (e.g., GVAX vaccine ± nivolumab), suggesting that TR CD8+ T cells could serve as biomarkers for predicting immunotherapy efficacy [35].

A study published in *Hereditas* revealed the heterogeneity of cancer-associated fibroblasts (CAFs) in the glioblastoma (GBM) microenvironment by integrating scRNA-seq and bulk RNA-seq data and identified 5 CAF subsets, 3 of which were significantly associated with prognosis. Researchers constructed a three-gene risk model based on LITAF, OSMR, and TCF12, which showed good predictive performance in the TCGA and CGGA cohorts (1-year AUC=0.74). High-risk patients had a lower response rate to PD-L1 inhibitors, suggesting that CAFs might mediate immune escape through the IL6-JAK-STAT3 pathway [36].

A research team from Tongji University published a study in *Nature Cancer* 7. They integrated scRNA-seq data from over 4 million single cells from 746 samples across 36 cancer types in public datasets, along with spatial transcriptomics data from 62 samples of 6 cancer types, to establish a pancancer atlas of tumor microenvironment heterogeneity—the TabulaTIME. This atlas defines six major cell lineages and 56 cell subtypes in the TME. CTHRC1 is a marker for extracellular matrix-related CAFs, which are enriched in various cancer types and may be located at the interface between malignant and normal areas, preventing immune cell infiltration. Furthermore, SLPI+ macrophages exhibit a profibrotic phenotype and colocalize with CTHRC1+ CAFs to form unique spatial ecotypes [37].

The study of tumor heterogeneity has revealed that modern medicine has a strong pursuit of "precision medicine", and single-cell sequencing technology, which focuses on the subtle differences between cells, undoubtedly plays an important role in this process. Therefore, deciphering tumor heterogeneity is not only fundamental to understanding cancer biology but also critical for developing effective targeted therapies and overcoming treatment resistance.

4. Conclusions

Since its introduction in 2009, single-cell sequencing technology has developed rapidly, and its powerful analytical capabilities have provided unprecedented help for tumor research. It provides high-resolution transcript sequencing, analyzes intra- and intertumor heterogeneity, and studies the tumor immune microenvironment, making it an increasingly indispensable tool for studying tumor initiation, progression, treatment response, and drug resistance.

However, it is important to recognize that scRNA-seq still has many drawbacks. First, scRNA-seq can capture only a transient "snapshot" of the transcriptome, suffering from transcriptional bursting and insensitivity to low-abundance transcripts. More critically, it fails to provide multiomics information (e.g., genomic variations, protein expression, and epigenetic states), leading to a fragmented and incomplete interpretation of cellular states. Second, the technology heavily relies on live cells. The tissue dissociation process (enzymatic digestion, mechanical force) can induce stress responses, kill cells, degrade RNA, and completely obliterate crucial spatial and morphological contexts. Furthermore, not all cells are captured equally, and differences in platforms or processing dates introduce stubborn batch effects, compromising data quality and comparability. Third, the technology remains expensive, time-consuming, and complex in terms of data analysis, hindering its application in large-scale clinical cohorts. Individual patient differences and the use of varying platforms across studies further limit the generalizability and reliability of findings.

Consequently, current discoveries require cautious validation in clinical trials, and there is still a gap before routine clinical diagnostic application [5, 38].

Therefore, future research directions should be carried out in the direction of higher throughput, higher sensitivity, and lower cost technologies (such as the development of stereo-cells to trillion-level cell fluxes [27]) and should be more deeply integrated with other technologies, such as spatial multiomics, which will help breakdown technical barriers. In addition, in this era of increasingly advanced artificial intelligence technology worldwide, AI and machine learning will play important roles in building cell RNA libraries so that single-cell sequencing technology will play a greater role in cell type identification, gene regulation, disease state prediction, drug response prediction and neoantigen identification. In terms of applications, single-cell sequencing will be used more deeply to promote "precision medicine", such as discovering more biomarkers of disease and tracking tumor evolution at the cellular level to find evidence of drug resistance. We believe that single-cell sequencing technology can be better developed in the future to help humans find cures for more diseases.

References

[1] Rozenblatt-Rosen, O., Regev, A., Oberdoerffer, P., Nawy, T., Hupalowska, A., Rood, J. E., Ashenberg, O., Cerami, E., Coffey, R. J., Demir, E., et al. The Human Tumor Atlas Network: Charting Tumor Transitions across Space and Time at Single-Cell Resolution. Cell. 2020, 181(2), pp. 236-249. https://doi.org/10.1016/j.cell.2020.03.053.

[2] Vitale, I., Shema, E., Loi, S. and Galluzzi, L. Intratumoral heterogeneity in cancer progression and response to immunotherapy. Nature Medicine. 2021, 27(2), pp. 212-224. https://doi.org/10.1038/s41591-021-01233-9.

[3] Liu, J., Xu, T., Jin, Y., Huang, B. and Zhang, Y. Progress and Clinical Application of Single-Cell Transcriptional Sequencing Technology in Cancer Research. Frontiers in Oncology. 2021, 10, p. 593085. https://doi.org/10.3389/fonc.2020.593085.

[4] Erfanian, N., Derakhshani, A., Nasseri, S., Fereidouni, M., Baradaran, B., Jalili Tabrizi, N., Brunetti, O., Bernardini, R., Silvestris, N. and Safarpour, H. Immunotherapy of cancer in single-cell RNA sequencing era: A precision medicine perspective. Biomedicine & Pharmacotherapy. 2022, 146, p. 112558. https://doi.org/10.1016/j.biopha.2021.112558.

[5] Lei, Y., Tang, R., Xu, J., Wang, W., Zhang, B., Liu, J., Yu, X. and Shi, S. Applications of single-cell sequencing in cancer research: progress and perspectives. Journal of Hematology & Oncology. 2021, 14(1), p. 91. https://doi.org/10.1186/s13045-021-01105-2.

[6] Amelio, I., Bertolo, R., Bove, P., Buonomo, O. C., Candi, E., Chiocchi, M., Cipriani, C., Di Daniele, N., Ganini, C., Juhl, H., et al. Liquid biopsies and cancer omics. Cell Death Discovery. 2020, 6(1), p. 131. https://doi.org/10.1038/s41420-020-00373-0.

[7] Malone, E. R., Oliva, M., Sabatini, P. J. B., Stockley, T. L. and Siu, L. L. Molecular profiling for precision cancer therapies. Genome Medicine. 2020, 12(1), p. 8. https://doi.org/10.1186/s13073-019-0703-1.

[8] Bühler, M. M., Martin-Subero, J. I., Pan-Hammarström, Q., Campo, E. and Rosenquist, R. Towards precision medicine in lymphoid malignancies. Journal of Internal Medicine. 2022, 292(2), pp. 221-242. https://doi.org/https://doi.org/10.1111/joim.13423.

[9] Ding, J., Sharon, N. and Bar-Joseph, Z. Temporal modelling using single-cell transcriptomics. Nature Reviews Genetics. 2022, 23(6), pp. 355-368. https://doi.org/10.1038/s41576-021-00444-7.

[10] Li, P.-H., Kong, X.-Y., He, Y.-Z., Liu, Y., Peng, X., Li, Z.-H., Xu, H., Luo, H. and Park, J. Recent developments in application of single-cell RNA sequencing in the tumour immune microenvironment and

cancer therapy. Military Medical Research. 2022, 9(1), p. 52. https://doi.org/10.1186/s40779-022-00414-y.

[11] Hong, M., Tao, S., Zhang, L., Diao, L.-T., Huang, X., Huang, S., Xie, S.-J., Xiao, Z.-D. and Zhang, H. RNA sequencing: new technologies and applications in cancer research. Journal of Hematology & Oncology. 2020, 13(1), p. 166. https://doi.org/10.1186/s13045-020-01005-x.

[12] Gawad, C., Koh, W. and Quake, S. R. Single-cell genome sequencing: current state of the science. Nature Reviews Genetics. 2016, 17(3), pp. 175-188. https://doi.org/10.1038/nrg.2015.16.

[13] Picelli, S. Single-cell RNA-sequencing: The future of genome biology is now. RNA Biology. 2017, 14(5), pp. 637-650. https://doi.org/10.1080/15476286.2016.1201618.

[14] Zweifel, P., Felder, S. and Meiers, M. Ageing of population and health care expenditure: a red herring? Health Economics. 1999, 8(6), pp. 485-496. https://doi.org/https://doi.org/10.1002/(SICI)1099-1050(199909)8:6<485::AID-HEC461>3.0.CO;2-4.

[15] Potter, S. S. Single-cell RNA sequencing for the study of development, physiology and disease. Nature Reviews Nephrology. 2018, 14(8), pp. 479-492. https://doi.org/10.1038/s41581-018-0021-7.

[16]Xin, Y., Kim, J., Ni, M., Wei, Y., Okamoto, H., Lee, J., Adler, C., Cavino, K., Murphy, A. J., Yancopoulos, G. D., et al. Use of the Fluidigm C1 platform for RNA sequencing of single mouse pancreatic islet cells. Proceedings of the National Academy of Sciences. 2016, 113(12), pp. 3293-3298. https://doi.org/10.1073/pnas.1602306113.

[17] DeLaughter, D. M. The Use of the Fluidigm C1 for RNA Expression Analyses of Single Cells. Current Protocols in Molecular Biology. 2018, 122(1), p. e55. https://doi.org/https://doi.org/10.1002/cpmb.55.

[18] Klein, Allon M., Mazutis, L., Akartuna, I., Tallapragada, N., Veres, A., Li, V., Peshkin, L., Weitz, David A. and Kirschner, Marc W. Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells. Cell. 2015, 161(5), pp. 1187-1201. https://doi.org/10.1016/j.cell.2015.04.044.

[19] Macosko, Evan Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, Allison R., Kamitaki, N., Martersteck, Emily M., et al. Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell. 2015, 161(5), pp. 1202-1214. https://doi.org/10.1016/j.cell.2015.05.002.

[20] Zheng, G. X. Y., Terry, J. M., Belgrader, P., Ryvkin, P., Bent, Z. W., Wilson, R., Ziraldo, S. B., Wheeler, T. D., McDermott, G. P., Zhu, J., et al. Massively parallel digital transcriptional profiling of single cells. Nature Communications. 2017, 8(1), p. 14049. https://doi.org/10.1038/ncomms14049.

[21] Papalexi, E. and Satija, R. Single-cell RNA sequencing to explore immune cell heterogeneity. Nature Reviews Immunology. 2018, 18(1), pp. 35-45. https://doi.org/10.1038/nri.2017.76.

[22] Ding, S., Chen, X. and Shen, K. Single-cell RNA sequencing in breast cancer: Understanding tumor heterogeneity and paving roads to individualized therapy. Cancer Communications. 2020, 40(8), pp. 329-344. https://doi.org/https://doi.org/10.1002/cac2.12078.

[23] Hochgerner, H., Lönnerberg, P., Hodge, R., Mikes, J., Heskol, A., Hubschle, H., Lin, P., Picelli, S., La Manno, G., Ratz, M., et al. STRT-seq-2i: dual-index 5′ single cell and nucleus RNA-seq on an addressable microwell array. Scientific Reports. 2017, 7(1), p. 16327. https://doi.org/10.1038/s41598-017-16546-4.

[24] Ramsköld, D., Luo, S., Wang, Y.-C., Li, R., Deng, Q., Faridani, O. R., Daniels, G. A., Khrebtukova, I., Loring, J. F., Laurent, L. C., et al. Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. Nature Biotechnology. 2012, 30(8), pp. 777-782. https://doi.org/10.1038/nbt.2282.

[25] Fan, H. C., Fu, G. K. and Fodor, S. P. A. Combinatorial labeling of single cells for gene expression cytometry. Science. 2015, 347(6222), p. 1258367. https://doi.org/10.1126/science.1258367.

[26] Shum, E. Y., Walczak, E. M., Chang, C. and Christina Fan, H., (2019). Quantitation of mRNA Transcripts and Proteins Using the BD Rhapsody™ Single-Cell Analysis System. In Suzuki, Y. (ed.) *Single Molecule and Single Cell Sequencing*. Singapore: Springer Singapore, pp. 63-79.

[27] Liao, S., Zhou, X., Liu, C., Liu, C., Hao, S., Luo, H., Hou, H., Liu, Q., Zhang, Z., Xiao, L., et al. Stereo-cell: Spatial enhanced-resolution single-cell sequencing with high-density DNA nanoball-patterned arrays. Science, 389(6762), p. eadr0475. https://doi.org/10.1126/science.adr0475.

[28] Liu, J., Dang, H. and Wang, X. W. The significance of intertumor and intratumor heterogeneity in liver cancer. Experimental & Molecular Medicine. 2018, 50(1), pp. e416-e416. https://doi.org/10.1038/emm.2017.165.

[29] Ruan, H., Wang, Z., Zhai, Y., Xu, Y., Pi, L., Zheng, J., Zhou, Y., Zhang, C., Huang, R., Chen, K., et al. Single-cell transcriptome analysis of diffuse large B cells in cerebrospinal fluid of central nervous system lymphoma. iScience. 2021, 24(9), p. 102972. https://doi.org/10.1016/j.isci.2021.102972.

[30] Wang, R., Dang, M., Harada, K., Han, G., Wang, F., Pool Pizzi, M., Zhao, M., Tatlonghari, G., Zhang, S., Hao, D., et al. Single-cell dissection of intratumoral heterogeneity and lineage diversity in metastatic gastric adenocarcinoma. Nature Medicine. 2021, 27(1), pp. 141-151. https://doi.org/10.1038/s41591-020-1125-8.

[31] Borcherding, N., Voigt, A. P., Liu, V., Link, B. K., Zhang, W. and Jabbari, A. Single-Cell Profiling of Cutaneous T-Cell Lymphoma Reveals Underlying Heterogeneity Associated with Disease Progression. Clinical Cancer Research. 2019, 25(10), pp. 2996-3005. https://doi.org/10.1158/1078-0432.CCR-18-3309.

[32] Davis, R. T., Blake, K., Ma, D., Gabra, M. B. I., Hernandez, G. A., Phung, A. T., Yang, Y., Maurer, D., Lefebvre, A. E. Y. T., Alshetaiwi, H., et al. Transcriptional diversity and bioenergetic shift in human breast cancer metastasis revealed by single-cell RNA sequencing. Nature Cell Biology. 2020, 22(3), pp. 310-320. https://doi.org/10.1038/s41556-020-0477-0.

[33] Cao, X., Wang, Y., Zhang, W., Zhong, X., Gunes, E. G., Dang, J., Wang, J., Epstein, A. L., Querfeld, C., Sun, Z., et al. Targeting macrophages for enhancing CD47 blockade–elicited lymphoma clearance and overcoming tumor-induced immunosuppression. Blood. 2022, 139(22), pp. 3290-3302. https://doi.org/10.1182/blood.2021013901.

[34] Wu, J., Chen, Z., Wu, W., Qin, J., Zhong, R., Meng, J., Yin, Y., Guo, P. and Fan, S. Single-Cell and Bulk RNA Sequencing Highlights Intra-Tumoral Heterogeneity and Malignant Progression Mechanisms in Prostate Cancer. Journal of Cellular and Molecular Medicine. 2025, 29(16), p. e70806. https://doi.org/https://doi.org/10.1111/jcmm.70806.

[35] Sun, H., Shi, C., Fang, G., Guo, Q., Du, Z., Chen, G., Wu, Y., Chen, Z.-S., Hua, J., Zhang, Y., et al. Functional tumor-reactive CD8 + T cells in pancreatic cancer. Journal of Experimental & Clinical Cancer Research. 2025, 44(1), p. 253. https://doi.org/10.1186/s13046-025-03517-1.

[36] Zhang, W., Li, Y., Li, C. and Huang, Q. Integrated single-cell and bulk transcriptomic profiling reveals cancer-associated fibroblast heterogeneity in glioblastoma and establishes a clinically actionable prognostic model and preliminary experimental validation. Hereditas. 2025, 162(1), p. 173. https://doi.org/10.1186/s41065-025-00548-8.

[37] Han, Y., Zhang, L., Sun, D., Cao, G., Wang, Y., Yue, J., Hu, J., Dong, Z., Li, F., Li, T., et al. Spatiotemporal analyses of the pan-cancer single-cell landscape reveal widespread profibrotic ecotypes

associated with tumor immunity. Nature Cancer. 2025, 6(11), pp. 1880-1898. https://doi.org/10.1038/s43018-025-01039-5.

[38] Huang, D., Ma, N., Li, X., Gou, Y., Duan, Y., Liu, B., Xia, J., Zhao, X., Wang, X., Li, Q., et al. Advances in single-cell RNA sequencing and its applications in cancer research. Journal of Hematology & Oncology. 2023, 16(1), p. 98. https://doi.org/10.1186/s13045-023-01494-6.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgment

This paper is an output of the science project.

Open Access