SPANDIDOS PUBLICATIONS

ONCOLOGY REPORTS 50: 184, 2023

Single-cell multi-omics advances in lymphoma research (Review)

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Received July 6, 2023; Accepted August 3, 2023

DOI: 10.3892/or.2023.8621

Abstract. The evolution of lymphoma is a multifactorial process that leads to unavoidable lymphoma heterogeneity in the form of genetic mutations, chromosomal translocations and other variations. Multi-omics analyses based on single-cell assays can reveal and characterize tumor components, enabling us to determine the timing of mutations and to profile disease progression. Increasing numbers of studies are using single-cell transcriptomics to unravel the mechanisms of lymphoma evolution, drug resistance and therapeutic approaches. Various single-cell multi-omics measurements involving genomics, transcriptomics and epigenomics have improved knowledge of the complex lymphatic system and made it possible to obtain individualized and precise tumor biological characteristics, which cannot be accessed from bulk cell analysis, and this can facilitate individualized treatment. In the present review, the advances in multi-omics analysis based on single-cell assays of lymphoma specimens were systematically discussed, including the sequencing of the single-cell from genomic and transcriptomic perspectives, the landscape of the lymphoma microenvironment, the development of single-cell histology biomarkers, the identification of lymphoma origin and evolution, as well as the current challenges and future prospects of single-cell multi-omics. The authors' insights may contribute to the exploration of novel lymphoma biomarkers and the discovery of efficient treatment combinations that target immunological checkpoints and underlying molecular mechanisms.

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Key words: single-cell multi-omics, lymphoma, single-cell RNA sequencing, biomarker, drug resistance

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1. Introduction

Lymphoma is a sizable collection of immunocyte-derived malignancies that exhibit molecular aberrations in various aspects (1). Variations in genotype, phenotype and metabolic events lead to changes in lymphoma cell morphology, progression rate and metastatic potential (2). However, conventional high-volume detection techniques, such as chromatin immunoprecipitation, real-time quantitative polymerase chain reaction, and western blotting, do not provide comprehensive analyses of the tumor cell landscape (3). Previously, novel techniques and analytic tools have been developed to promote research and understand lymphoma. These techniques and tools include capturing mRNAs from cell lysates using arrays of thousands of oligonucleotide probes (4), identifying differentially expressed genes and signaling pathways between two samples using Gene Set Enrichment Analysis (5), and inferring sample cell composition by deconvolution of the bulk transcriptome (6,7). Nevertheless, these techniques and tools fundamentally reflect the functions of lymphoma cells as a whole and do not describe the molecular mechanisms at the level of a single lymphoma cell. Presently, single-cell sequencing technologies involving genomics, transcriptomics, proteomics and epigenomics employ high-resolution characterization, which allows researchers to understand precisely tumor heterogeneity, the tumor microenvironment (TME), and tumor progression (8). Single-cell spatial transcriptomics is also used to understand these tumor events (9) (Fig. 1).

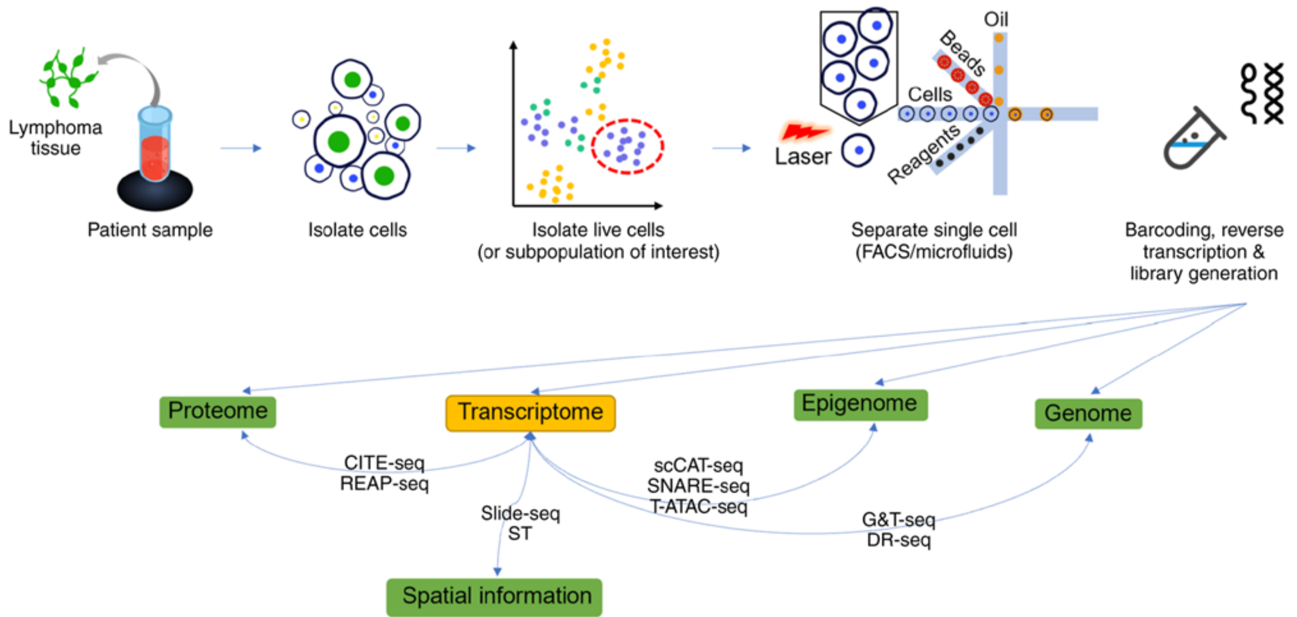

Figure 1. Representative single lymphoma cell multi-omics method. Representative method for sequencing single lymphoma cells. The standard single lymphoma cell acquisition process is shown, where the cells are studied in a suspension. In the second step, individual cells are isolated by flow cytometry or a microfluidic technique. In the third step, each cell is assigned a barcode to allow pooling, and different cell fractions can be subjected to parallel experimental workflows such as reverse transcription and RNA sequencing or genome sequencing. Finally, single cells are sequenced, and genomic, epigenomic and proteomic information can be analyzed simultaneously with the transcriptome. Spatial transcriptomic information can also be obtained simultaneously by tissue sectioning.

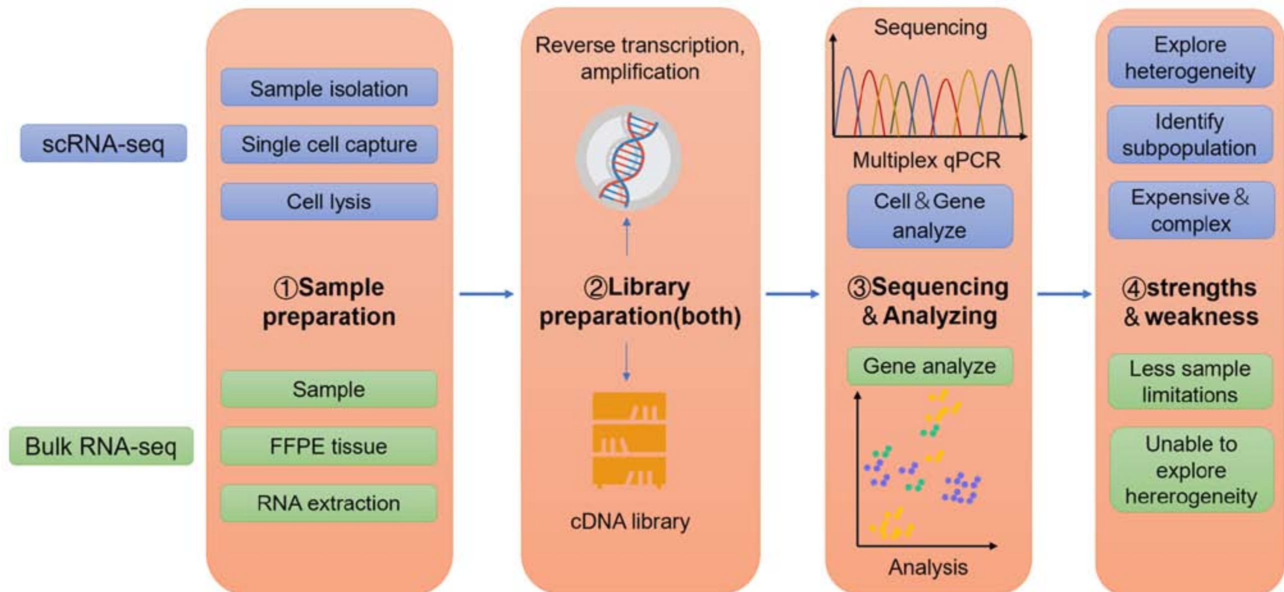

Figure 2. Workflow of classic scRNA-seq vs. typical bulk RNA-seq. Comparison of scRNA-seq workflow and typical bulk RNA-seq method, mainly focusing on the sample preparation phase. In contrast to the bulk RNA-seq method, which obtains the sample from formalin-fixed paraffin-embedded tissue or peripheral blood, scRNA-seq requires that individual cells be isolated from the sample at the beginning of the experiment. In addition, the advantages and disadvantages of the aforementioned two strategies are also shown. scRNA-seq, single-cell RNA sequencing.

By utilizing the rapidly-evolving single-cell multi-omics techniques to identify the different types of lymphoma, it is possible to reveal heterogeneity (1,10,11) and drug resistance (12), identify presumptive precancerous groups (13) and evolutionary processes (14,15), identify new biomarkers (16,17), and explore TMEs (18,19). Lymphoma research has advanced to a more sophisticated level with the existence of single-cell multi-omics approaches. Using single-cell RNA sequencing (scRNA-seq) as an example, in Fig. 2 the differences and similarities between the workflow of scRNA-seq and bulk RNA seq are illustrated.

Presently, the generally acknowledged platforms of high-throughput single-cell sequencing are Drop-Seq (20), inDrop (21), and 10X Genomics (22). The scRNA-seq

Table I. Common scRNA-seq techniques for lymphoma analysis.

Techniques	Platform	Measure	Characteristics	(Refs.)
IFCs	The Fluidigm C1 System	RNA sequence	• produce high-quality gene expression readouts; • unstable capture efficiency; • unavoidable size bias;	(23-26)
Seq-well, Cytoseq	Microwell Platforms		• cheap; • portable; • environmentally friendly;	(27-30)
10X Genomics	Chromium System by 10X Genomics		• high efficiency; • prohibitive cost;	(31,32)
MARS-seq, SMART-seq3	Fluorescence activating cell sorter (FACS)		• large scale; • high sensitivity and precision;	(33)
CITE-seq	Existing scRNA-seq platforms		• not limited to a certain size or a certain number of cells to be tested; • high precision;	(34,35)

technologies commonly used in lymphoma analysis are listed in Table I.

2. Single-cell multi-omics to characterize immune cells

The multiparametric classification approach from the World Health Organization and the revised European and American Lymphoma classification (36) have tremendously aided lymphoma clinical and translational research, which serves as a foundation for discovering the causes of molecular changes in these tumors (37). Although a diagnosis of Hodgkin's lymphoma according to the existence of Reed-Sternberg (R-S) cells has been proposed for a long time, the lymphomagenesis of Hodgkin's lymphoma remains unclear (38). Given that malignant R-S cells derived from germinal center (GC) B cells (39,40) represent a minority of lymphoma cells, an inadequate biopsy may fail to identify these cells and make an accurate diagnosis (41). Non-Hodgkin's lymphoma accounts for ~3% of all cancer occurrences and deaths worldwide, making it the most common malignant tumor in the hematological field (42). Each of its >40 subtypes has specific driver genetic mutations and distinct risk factors (43). Thus, it is the extensive and remarkable heterogeneity of lymphoma cells that requires to finely characterize lymphoma cell molecules.

Currently, there is rapid progress in the accurate characterization of single-cell immune cells, which allows for comprehensive detection of lymphoma and precise clinical diagnosis. For instance, phenotyping CD molecules ($CD3^+$, $CD4^+$ and $CD8^+$) on T cells can help identify the specific cellular status and advance the development of immunotherapy (44). It has been shown that using just 6 biomarkers (CD95, CD73, RB, CD39, CD38 and CD27) can define tonsillar (and also lymph node) B cells and characterize B cells (45). Furthermore, single-cell sequencing analysis of NK cells from patients with acute myeloid leukemia (AML) revealed similar patient-specific patterns of NKp30 and CD27 expression, and downregulation of CD160 transcription on NK cells is related to the reduced survival of patients with AML, suggesting that CD160 is promising precision medicine biomarker (46). A single-cell histological assay of a subpopulation of T cells associated with Hodgkin's lymphoma revealed prominent expression of the immunosuppressor receptor LAG3, which offers a new approach targeting immune checkpoints in Hodgkin's lymphoma, second only to PD-1 (47). High-resolution clustering and combinatorial gene characterization using large scRNA-seq datasets of human γδ T cells allow in-depth characterization of these cells, which can specifically identify T cell receptor (TCR) Vδ1 and TCR Vδ2 subpopulations (48). Repertoire and Gene Expression by Sequencing (RAGE-seq), which combines short-read transcript analysis based on single-cell libraries of barcodes with long-read sequences and targeted capture of B cell receptor (BCR) or the TCR mRNA transcriptome, can identify the full-length antigen receptor profiles of B or T cells (49).

These single-cell multi-omics techniques have been applied to study various cells in the human immune cell population, and they have great potential in identifying new subpopulations of lymphoma cells or in exploring new biomarkers.

3. Single-cell multi-omics to define the transcriptomic, proteomic and epigenomic features of lymphoma

In the field of tumor research, single-cell multi-omics techniques have transformed the existing knowledge of the biological features of lymphoma lesions (3). Different technologies, such as scRNA-seq, scDNA-seq, single-cell chromatin immunoprecipitation-seq, and methylation sequencing, have been widely used in lymphoma research, and their specific features have been well characterized.

At the genetic level, the rather mature single-cell combinatorial indexing RNA sequencing can provide information on lymphoma copy number variation (50). In addition, genomic mutations can also be identified in different types of lymphoma with single-cell precision. Stratified clustering of NK cells showed that a total of 56 genomic mutations based on the JAK-STAT pathway and TP53 were present in 102 EBV patient samples (51), which is in line with previous bulk analysis. Single-cell exome sequencing and protein profiling (SNV and

small insertions) of GI-DLBCL and non-GI-DLBCL exhibited changes in gene mutation frequency, among which ID3, CCDN3 and TP53 were increased, while BCL2, CREBBP and MYD88 were significantly decreased (52). In an exploration of lymphoma molecular drivers, researchers identified signature mutations in CD79B and MYD88 L265P as well as a CDKN2A deletion in BCR and NF-kB pathways (53). As for the clinical application, based on gene mutation data from the NF-κB pathway, CCND1, as well as ATM according to scRNA-seq, researchers classified mantle cell lymphoma into four genetic subgroups as C1-C4 for the first time (54). In another study, single-cell genome and exome sequencing showed that CDKN1B, SMARCB1 and DAZAP1 expression has a negative impact on patient prognosis, suggesting that they could be used as biomarkers for patient prognosis (55). Lymphoma heterogeneity has been investigated among primary BM specimens by applying single-cell targeted DNA sequencing through the Fluidigm C1 system (56). As for the molecular mechanism, single-cell gene transcription analysis of IG-non MYC translocations in Burkitt's lymphoma, the most common type of childhood lymphoma, revealed MYC oncogene dysregulation and demonstrated that at least one BL subgroup precursor could be expressed in each patient (57). Furthermore, KIR2DL4 was reported to be overexpressed in malignant NK cells by scRNA-seq, and KIR2DL4 promotes lymphoma pathogenesis by mediating NK cell proliferation and apoptosis, which are associated with certain signaling pathways such as AKT and NF-κB (58). After analyzing the transcriptome of tens of thousands single tumor cells from 6 primary FL patients, the results revealed that malignant B cells showed more pronounced expression of the BCL2 gene and absence of expression of MHC-II and CD52 genes. Moreover, the results also identified the co-expression of B2M and CEBPA genes in Treg cells with immune checkpoint molecules, which helps further map the network of genes included within the immunoregulatory mechanism (59). Thus, single-cell analysis could help expand tumor genomic studies in different classes of lymphoma and accelerate the transition from genetic analysis to pharmacodynamic therapy in the future.

Targeted and functional proteomic analysis enables bulk and thorough studies of proteins, particularly for high-throughput screening of promising biomarkers from complex tumor biological microenvironments. Currently, proteomics has become one of the key fields of lymphoma research, and it is considered as the most suitable approach to discovering new biomarkers and personalized therapies, as well as it being a high-throughput technique for revealing genotype-phenotype uncoupling in lymphoma and tracking proteomic dynamics in relapsed patients (60). For example, through the E1A-binding protein p300, mice with knockdown BCL6 expression on the surface of lymphomas can block oncogenic transformation by inhibiting the acetylation of Lys132 in the p53 gene, which upregulates cystein-1 to reduce BCL6 stability (61).

Single-cell epigenomic analysis of lymphoma has also greatly aided the analysis lymphoma diagnostic and prognostic processes. The epigenome of lymphoma is defined by the regulation of normal gene expression in cells, modified by histone proteins and DNA, plus the action of non-coding RNA (62). Previous studies have demonstrated strong associations between the differentiating expression of miRNAs and the pathogenic progression of NKTCL tumors by single-cell techniques targeting P53, cell-cycle related genes and MAPK pathways (63,64). The reduced functions of miR-26 and miR-101 cause EZH2 overexpression, whereas the over-activation of miR-223 contributes to PRDM1 suppression (65,66). Hypermethylation in the promoter region was studied by whole methylation assay and methylation site-specific validation, and the results demonstrated that the functions of TET2, PTPRK, SOCS6 and PTPN6 can increase the expression of methylated genes (67,68). Functionally, TET2 inactivation may lead to hypermethylation of lymphoma promoters, and the negative regulation of JAK-STAT by PTPRK, SOCS6 and PTPN6 suggests an alternative mechanism associated with the activation of the JAK-STAT signaling pathway (67).

Besides gene transcription, protein expression and epigenetic inheritance, single-cell technology also has roles in other areas. For example, scATAC-seq can identify specific chromatin motifs (69), whereas scNGS can provide information about somatic mutations and cellular heterogeneity (70). It is even possible to reveal the mutation profiles of B cells in the entire life cycle of a human by single-cell whole genome sequencing, thus identifying potential cancer-causing mutations (71)

Taken together, single-cell multi-omics techniques provide the productive characterization of the biological features and intrinsic dynamics of lymphoma, which may widen our knowledge of lymphoma and accelerate the pace of clinical diagnosis and treatment.

4. Single-cell multi-omics reveal lymphomagenesis and clonal evolution

A comprehensive understanding of tumorigenesis and clonal evolution can help to unravel tumor heterogeneity as well as targeted therapies for tumors. Although protein-altering lesions have always been detected in lymphoma diagnosis, the tumor's clonal structures and the initial progress remain unclear. Quantitative single-cell genomics based on mass spectrometry is used specifically to understand the pathogenesis of lymphomas, and its incremental value in deciphering the complexity of lymphoma entities to regulate the heterogeneity of molecular mechanisms is currently a popular diagnostic approach and therapeutic strategy (72). Single-cell multi-omics techniques can reveal the clonal specificity of lymphoma and establish the order of genetic events in lymphoma.

Lymphoma cells may have translocation mutations in gene bases, although few types of lymphoma can result from recurrent translocations involving specific genes. For example, the IGH translocation on 14q32 is responsible for a certain proportion of mature B-cell tumors (73). Meanwhile, regulatory chromatin genes (CREBBP, EZH2 and KMT2D) have also been shown to mutate in the early drivers in lymphoma (74). These mutations indicate the initiation phase of lymphoma development.

The clonal evolution of lymphoma is inevitably accompanied by changes in the transcriptome of genes, and to clarify genotyping, numerous experiments have been performed using single-cell sequencing techniques to describe the clonal progression of lymphoma. Fluorescence *in situ* hybridization results identified that lymphoma cells had MYC

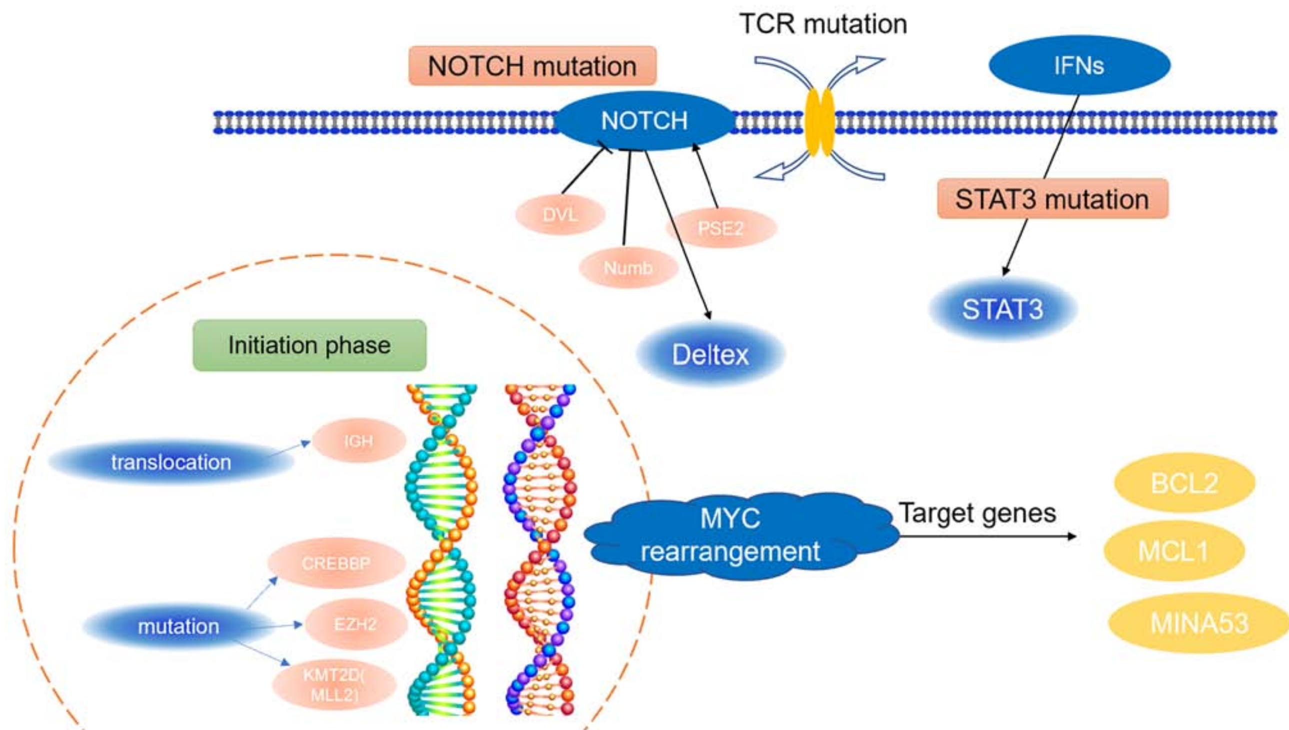

Figure 3. Simple genetic process of lymphoma development. Lymphoma undergoes genetic-level changes, such as chromosomal translocations and gene mutations, accompanied by significant MYC gene rearrangements, leading to transcriptional-level changes, while mutations in pathways, such as STAT3 and NOTCH, are important factors of lymphoma deterioration.

rearrangements that were not BCL2 or BCL6, but involved the immunoglobulin light or heavy chain gene and the 8q24 region, which are markers of lymphoma lesions (75). This finding is remarkable because the prognosis of lymphoma with MYC translocation is often worse than that of lymphoma alone (76). However, this is a limited ordering study of lymphoma using bulk samples, and it did not explore the tumor evolution. Single-cell capture and WGA of microfluidic specimens have proved RAG-mediated structural vibrations to precede lymphoma (77). The acquisition of fusion genes and the loss of 9p21 (CDKN2A/B) accounts for the intermediated clonal evolution of lymphoma (56). Gene rearrangements and chromosomal translocations such as these play a non-negligible role in promoting lymphoma development.

Multi-step mutations in NOTCH, the STAT3 signaling pathway, and TCR are always late events in the evolution of lymphoma and can further accelerate the proliferative potential of tumor cells, leading to the development of highly malignant clones that can lead to disease onset and progression (9).

Single-cell sequencing techniques allow the complete clonal evolutionary structure of tumor cells to be reconstructed, thus providing insights into the evolution of lymphoma and revealing synergistic combinations that promote clonal expansion and dominant mutations, which offers information for therapeutic decisions (Fig. 3). For example, according to 2017 World Health Organization criteria, lymphomas with mutations in MYC and BCL2 or BCL6 during clonal evolution, as mentioned above, and with concurrent DLBCL histological features are no longer classified as DLBCL, but are referred to as advanced B cell lymphomas with MYC and BCL2 or BCL6 rearrangements (41). These sub-entities tend to have a worse prognosis for common DLBCL and often require the application of different treatments (78-80).

To further dissect the heterogeneity of the origin of lymphomas, the studies on the origin of lymphoma at the cellular level were also summarized, as detailed below (Single-cell multi-omics for modeling B-cell GCs).

5. Single-cell multi-omics to explore the mechanism of drug resistance in lymphoma

Drug resistance in lymphoma can be broadly divided into the development of resistance at genetic and transcriptional levels within cancer cells and the development of resistance at tissue and cellular levels. According to the widely accepted theory of clonal evolution, the pre-emptive generation of heterogeneous drug-resistant clonal mutations through therapeutic selection is the most critical biological process (81). The ability to detect populations of cells that survive under a period of anticancer treatment is essential for drug resistance prediction or even reversal. It is difficult for conventional genetic studies of bulk samples to identify small numbers of drug-resistant cells. Today, through gene expression analysis under single-cell resolution, these cells and their mechanisms of drug resistance can be characterized (82).

Changes in gene levels are responsible for drug resistance in certain cancers, including lymphoma. Transcriptome sequencing analysis targeting the BTK gene [encodes a signaling protein vital for B cell development, differentiation and signaling (83)] found that after the inhibition of BTK, the FC receptors (FCGR2A, FCGR2B and FCGR3A) on the surface of B cells exhibited a higher level of expression (84),

and the FC receptors promoted the internalization of the CD20 monoclonal antibody rituximab [core drug against malignant B lymphocyte proliferation (85)], thus reducing its clinical efficacy (86). In addition, through mutation or deletion, TP53, ATM, CDKN2A, KMT2D, and other key lymphoma suppressor genes can be inactivated, leading to genomic instability and promoting secondary mutations and drug resistance (87).

At the cellular level, lymphoma resistance in the macroscopic sense is mostly manifested by the presence of inherent molecular mechanisms of genotoxic drug resistance (88), hypoxia (89) and DNA damage (90). Previous studies have used single-cell transcriptomics techniques to deeply explore the mechanisms of acquired drug resistance and the differential alterations in the lymphoma microenvironment. Early experimental manipulations of whole-genome sequencing and DNA microarray of murine lymphoma have been performed to analyze CpG methylation, mRNA expression and DNA sequences that are related to increased drug resistance (91). Another transcriptional analysis revealed that lymphomas with low GAPDH expression predominantly adopt bovine/phosphorus metabolism and are dependent on mTORC1 signaling and glutaminyl solubilization to generate ATP (92), which indicates a large metabolic reprogramming response and reflects the acquisition mechanism of drug resistance (93). In another pan-cancer analysis of lymphoma, the results revealed differences in mRNA expression using data from The Cancer Genome Atlas and concluded that targeting the high expression of TRPV channel-associated genes can enhance drug resistance, and they may be promising biomarkers of patient prognosis (94). In addition to lymphoma, there is a single-cell muti-omics study showing that the levels of MSH2, MSH6 and MLH1, together with homologous recombination effectors such as BRCA2 and RAD51, are reduced in rectal cancer, suggesting that just like single-cell organisms, tumor cells can also promote drug-resistant persistent cells through continuous mutation derivation (95).

Although lymphoma has multiple mechanisms of drug resistance development, targeted single-cell studies have been used for molecular sequencing and outcome assessment of drug resistance in lymphoma. The single-cell multi-omics techniques not only explore and detect, but also show therapeutic potential to drug resistance in lymphoma. According to a single-cell analysis, KP772 can induce apoptosis of BCL-2-independent cells and upregulate the Harakri gene, making KP772 a promising candidate for anti-multidrug resistance of malignant lymphoma (96). Meanwhile, splicing modulators have been clinically tested for the treatment of drug resistance in lymphoma given their ability to interfere with drug metabolism or absorption of gene expression such as FPGS, dCK and SLC29A1 (97).

In addition to changes at the genetic and cellular levels, the mechanisms by which lymphomas develop drug resistance are complex and also include changes in the TME.

6. Single-cell multi-omics to profile the lymphoma microenvironment

The heterogeneity of lymphoma cells includes malignant cells and the TME (98). Although the relationship between cellular subpopulations and the TME remains not fully understood, it is most likely associated with lymphoma drug resistance and recurrence (99). As a result, research has focused on targeting the lymphoma microenvironment to achieve research breakthroughs.

Transcriptomic analyses from multiple independent lymphoma cohorts were performed to describe four microenvironment subtypes based on clinical behaviors and biological aberrations, and this approach identified the ECM proteins DCN and BGN as novel potential therapeutic targets for the TME (100). In another assessment of gene expression profiles, it was revealed that SPARC expression in the TME has fair inter-observer reproducibility and is a strong prognostic reference (101). In terms of treatment, the increased expression of $CD8^+$ co-receptors (PTRPC and FYB) was found to enhance LCK and FYN expression, thereby activating the signaling of T cells and greatly contributing to immunocidal effects (84). A comprehensive transcriptomic atlas from over 100,000 lymphoma non-hematopoietic cells (NHCs; mesenchymal stromal and endothelial cells) at single-cell resolution demonstrated that NHC heterogeneity in LNs can be detected even in aggressive lymphomas, indicating the powerful practicability of single-cell analysis of the NHC profile in characterizing various TME subtypes (102).

It is well established that angiogenesis contributes to the inevitable progression of lymphoma through signaling transducers, cytokines and other components in the TME (103,104). Studies have shown that STAT3 is overexpressed in primary central nervous system lymphoma tissues vs. normal brain cells and vessels (105), and chromatin modifications occur when PCNSL forms (106). Single-cell histological analysis have shown that ROBO1, KAT2B and KMT2D regulate the lymphoma microenvironment by disrupting the functions of keratinocytes and stromal cells via non-histone acetylation (107), including histone deacetylase and DNA methyltransferase inhibitors are under investigation, suggesting they may be a new therapeutic option for lymphoma (108). Lysine acetyltransferase inhibitors enable the activation of KAT2B transcription to affect lymphoma angiogenesis (109). Recently, the study of gene impact associated with epigenetic modifications and angiogenetic events to fully profile the lymphoma microenvironment has gained research momentum.

The TME is also closely related to immune escape and drug resistance development in lymphoma, and studies at single-cell resolution reveal several novel mechanisms, suggesting that the microenvironment can suppress the host's antitumor immune activity (110). The immune escape of tumors is also a topic of research and an approach of clinical care.

Taken together, single-cell multi-omics techniques allow for the comprehensive characterization of the lymphoma microenvironment and the application of the gained information to a wider range of research.

7. Single-cell multi-omics to model B-cell GCs

GCs formed in secondary lymphatic organs undertake the binding of antigens to mature primitive B cells. GC B cells can produce numerous types of non-Hodgkin's lymphoma, such as DLBCL and Burkitt's lymphoma, which develop through different pathogenic mechanisms due to their origin in different stages of GC B cells (111). Transcriptional profiling revealed

that DZ and LZ cells express RNA and the surface proteins CXCR4 and CD83, respectively, reflecting a heterogeneity that distinguishes them from other types of cells (112).

Single-cell multi-omics analysis revealed that reduced TNFRSF14 expression and STAT6 activation improves the inhibitory effect on GC B-cell signaling (113,114). The results showed that the epigenetic modifiers EZH2 and CREBBP can directly impact on GC B-cell's function. For example, upon CREBBP inactivation, MHC-II and CD40 are suppressed, which disturbs the presentation of antigens from lymphoma cells to CD4$^+$ T cells (115).

Utilizing single-cell multi-omics techniques to model GCs can improved the characterization of the origin of lymphoma and provide more constructive insight into lymphomagenesis.

8. Single-cell multi-omics contribute to lymphoma treatment

The extensive heterogeneity of lymphoma is a major obstacle to the clinical management and the pharmacological treatment of this disease. As aforementioned in the sections pertaining to the development of novel biomarkers and the investigation of drug resistance and immune mechanisms in lymphoma, single-cell technology is rapidly evolving and may be of great value in the diagnosis and treatment of lymphoma. For example, scRNA-seq can identify several suitable entities for targeted therapy. Combined with capture technology, scRNA-seq can identify transcriptomic profiles related to the efficacy and toxicity of certain drugs, which has also been used to study the biomarkers of toxicity (116). Similarly, another study used scRNA-seq data to identify CD19 expression in brain wall cells and reveal the mechanism of neurotoxicity in CD19-targeted therapy (117). And it is promising that combining single-cell multi-omics techniques would be beneficial in the diagnosis of lymphoma, especially if the pathology is poorly characterized, as scRNA-seq identified immune cell biomarkers and determined essential biomarkers such as CD31, CD84 and CD226, which were hugely over-regulated and are promising to be the diagnostic biomarkers for lymphoma (118).

At the same time, numerous new assays based on single-cell sequencing are emerging, and investigators worldwide are working toward an easier and a more accurate application of single-cell technologies for lymphoma detection, which may offer constructive options for lymphoma treatment. For example, transient transfection of short barcode oligonucleotides allows the estimation of drug targets by transcriptional patterns of multiple scRNA-sequences to assess the efficacy and cytotoxicity of different drugs (119). Currently, a study is underway on the identification of targeted neoantigens by applying scRNA-seq combined with TCR sequencing for single-cell genomics (120). In the sequencing of paired single-cell RNA and TCR in patient tumors before and after PD-1 immunotherapy, the researchers identified the co-expression of biomarkers of chronic T cell apoptosis (121). The profiles of various T cells and TCR populations from normal adjacent tissues of tumors and peripheral blood was investigated by in-depth scRNA-seq and TCR sequencing from common types of tumors (122). RNA velocity can predict the future changes of each cell on a timescale of hours through the direct estimation of unsprayed and spliced mRNA from common scRNA-seq protocols, and it will greatly assist in the analysis of developmental lineages and cell dynamics in the future, which has been shown experimentally to be also applicable to human lymphoma (123). Mostly natural sequencing-by-synthesis for scRNA-seq is a novel approach applicable to the Ultima genomics platform, which has been benchmarked against scRNA-seq technologies and has a broader application potential (124).

In addition to the aforementioned novel single-cell technologies that have been applied to lymphoma, numerous other technologies in the field of oncology may have application potential in lymphoma, including CITE-seq, which enables simultaneous comparison of protein abundance and mRNA expression in specimens (34). In addition, SUPeR-seq synchronously detects linear and circ-rRNA expression levels in the tumor cell and the other control cell (125), whereas G&T-seq describes the whole genome and transcriptome of any sample cell (126). scTrio-seq simultaneously profiles the genomic, transcriptomic and epigenomic status of a single tumor cell (127), whereas INS-seq, a synthesis technique that enables large-scale parallel collection of scRNA-seq data and detection of intracellular protein activity, holds promise in exploring new immune subpopulations by analyzing different intracellular immune signaling signatures as well as metabolic activity and transcription factor binding (128). Currently, SCT provides a favorable platform for cancer diagnosis through the development of specific tumor biomarkers and individualized tumor therapy, and this technology will revolutionize the prognosis and treatment of all types of cancer on a global scale (129,130). MULTI-seq involves the multiplexed analysis of scRNA-sequences and mononuclear RNA sequences via lipid-labelled indexing, which can barcode all cells and nuclei worldwide through accessible plasma membranes. This approach is currently being used in triple-negative breast cancer (131).

9. Future prospects

The future of single-cell technology is unlimited, as evidenced by the increasing number of new techniques and improvements that are emerging (132). For example, RAGE-seq reconstructs highly diverse sequences (49). To advance lymphoma research and address lymphoma heterogeneity, additional integrated single-cell multi-omics technologies are essential to understand the cellular (tumor, stromal and immune) information about transcriptomes, proteomes and epigenomes, and even predict the state of each cell via pseudo-time detection of abundant patient data. In the near future, it may be possible to routinely analyze millions of cells; as a case in point, a pilot study to characterize a human cell atlas with 35 trillion human cells has reportedly begun (133).

Through multiple fusion approaches and technological innovations, single-cell transcriptomics techniques can assess how individual genetic drivers differently contribute to the fitness of lymphoma cells and investigate the mechanisms of cellular mutational symbiosis, multiple lesion interactions and clonal evolution, as revealed by spatial transcriptomics and spatial proteomics (134). Single-cell spatial transcription technologies have confirmed the upregulated expression of the major ligands CCR4, CCL17 and CCL22, and the

downregulated expression of certain biomarkers in NK cells and CTL cells (135). Single-cell spatial analysis was applied to the DZ/LZ region of GCs in the tested lymph nodes, showing that MHC I/II expression and EZH2 mutation frequency were different within this microenvironment, which could assist in the diagnosis of GC lymphoma (136). And to reveal deeper analyses of tumor-environment interactions, the spatial analysis identified CXCR3 as biomarkers suggesting immune desert region, and also indicated therapeutic targets such as CCR4 and TIM-3, which are associated with combination treatment assays of lymphoma cellular therapies (137). Although knowledge of lymphoma spatial histology is currently in a state of infancy, there already exists a glimpse of the great potential of the combined application of single-cell multi-omics. Convergence and cross-analysis of multi-omics on top of different histologic assays may be a more important direction of development, and it is expected that multi-omics research will change the treatment paradigm of lymphoma in the future, and improve guiding the choice of clinical treatments. With the help of single-cell technology, this revolution is expanding throughout the field of immunology, not just lymphoma (34,138).

Feedback on the developed single-cell multi-omics platforms will be provided clinically through advanced cancer risk stratification, which is likely to deliver accurate lymphoma prognosis and detailed individual treatment.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Natural Science Foundation of Jilin (grant no. YDZJ202201ZYTS117).

Availability of data and materials

Not applicable.

Authors' contributions

CJ and DZ authored or reviewed drafts of the paper, and approved the final draft. JL provided figures and helped with proofreading of draft. LB and LL prepared tables, and approved the final draft. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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