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Single-cell sequencing reveals the complexity of cutaneous T-cell lymphoma

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Abstract

Cutaneous T-cell lymphoma (CTCL) is a type of lymphoma that affects the skin and has various subtypes with different features. It can be either slow-growing or aggressive and currently has limited treatment options, causing a significant impact on patients' quality of life. However, more research is needed to fully understand the underlying causes of the disease. Single-cell sequencing technology shows promise in comprehending the complex genome and microenvironment of CTCL. By analyzing the molecular status of each tumor cell, this approach can provide insights into tumor heterogeneity, microenvironment composition, and cell state transitions, tailoring treatment strategies to individual patients. In this review, we highlight recent research progress in CTCL via single-cell sequencing, focusing on gene alterations and related biomarkers, disease origin and progression, and the tumor microenvironment. We aim to provide examples of single-cell studies that can help inform new drug discovery and contribute to improved treatment strategies for patients with CTCL.

Keywords CTCL, Single-cell sequencing, Gene, Tumor microenvironment

1 Background

Cutaneous T-cell lymphoma is a complex disease caused by malignant clonal mutations in skin memory T lymphocytes, resulting in various types of cancers [1]. Regrettably, it has a poor prognosis, and effective treatment options are limited [2]. However, the genomic variability of CTCL has led to the identification of many subtypes, including the most common type, mycosis fungoides (MF), which typically presents as patches or plaques and progresses slowly, and rare and aggressive Sézary syndrome (SS), which has a poor prognosis [3]. It is worth noting that even histologically similar subtypes can exhibit different clinical behaviors and prognoses, as evidenced by numerous studies. Moreover, CTCL progression is often accompanied by changes in the genome and immune microenvironment [4–6] (Fig. 1 in page 20). Therefore, gaining a thorough understanding of CTCL patient heterogeneity is crucial in uncovering the molecular mechanisms underlying disease progression.

Nowadays, single-cell sequencing has been widely used in cancer research to characterize the cellular and molecular composition of tumors [7], revolutionizing our

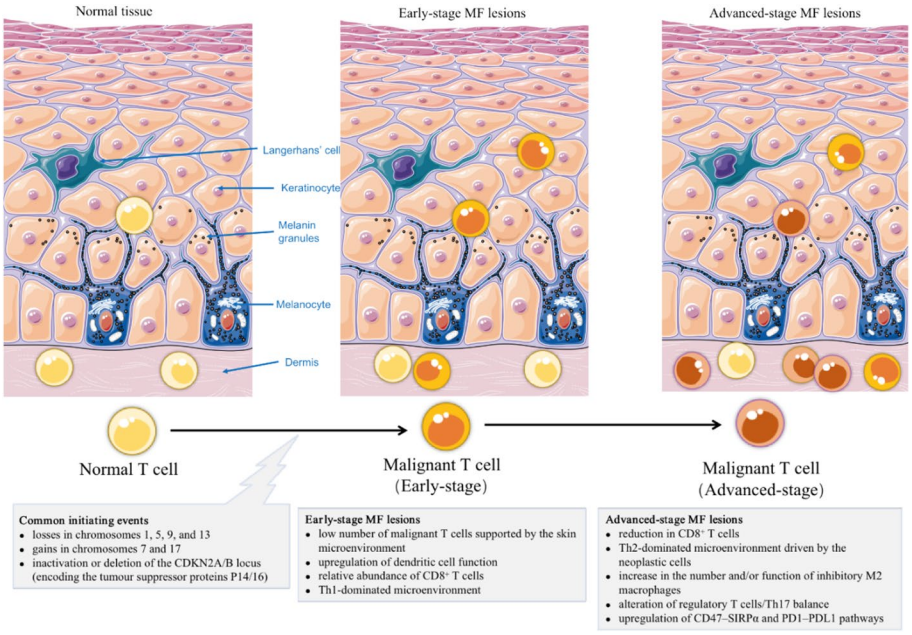

Fig. 1 Progression of CTCL (Take MF, for example). As MF progresses from normal to early malignant to advanced malignant cells, it is accompanied by chromosomal deletion/duplication, gene mutation, abnormal signaling pathway activation/inactivation, and changes in the immune microenvironment

understanding of normal human tissue anatomy, ontogeny, and diseases. This technique makes resolving tumor heterogeneity an increasingly achievable goal in cancer care [8, 9]. Single-cell sequencing has made some progress in the study of lymphoma. Single-cell spatial analysis of diffuse large B-cell lymphoma (DLBCL) revealed that tumor structures are closely associated with clinicopathological features, identifying candidate biomarkers and therapeutic targets [10]. Single-cell RNA sequencing of mantle cell lymphoma (MCL) bone marrow cells identified malignant cell subsets and explored the mechanism of immune escape and drug resistance caused by genetic alterations in specific subsets [11]. The above practice of single-cell sequencing in the clinical application of lymphoma shows that single-cell sequencing can provide unprecedented insights into the complex cell composition and tumor microenvironment and plays an important role in immunotherapy and drug development.

Through single-cell sequencing, correlative studies have made some advances in the heterogeneity [4], single-cell atlas [12], clone evolution [13], and immune microenvironment [14] of the CTCL (Fig. 2 in page 21). In this review, we summarize the current findings in Cutaneous T-cell lymphoma revealed by single-cell sequencing. This study aims to provide the latest insight into CTCL at the single-cell level (Table 1 in Additional File 1.xlsx). Given that many single-cell studies aggregate samples from different subtypes or disease stages, interpretations of shared transcriptional signatures can be confounded by subtype- or stage-specific signals; therefore we explicitly indicate subtype and stage in Table 1. Furthermore, we add another column in Table 1 to demonstrate patients' treatmet history included in every study for prior systemic or skin-directed therapies (e.g., HDAC inhibitors, photopheresis, immunotherapies) can reshape malignant and microenvironmental cell states. As examples, and Borcherding et al. proved treatment-associated transcriptional shifts, including FOXP3 increases and AIRE upregulation [15]. Different scRNA-seq platforms and library strategies have different sensitivity and

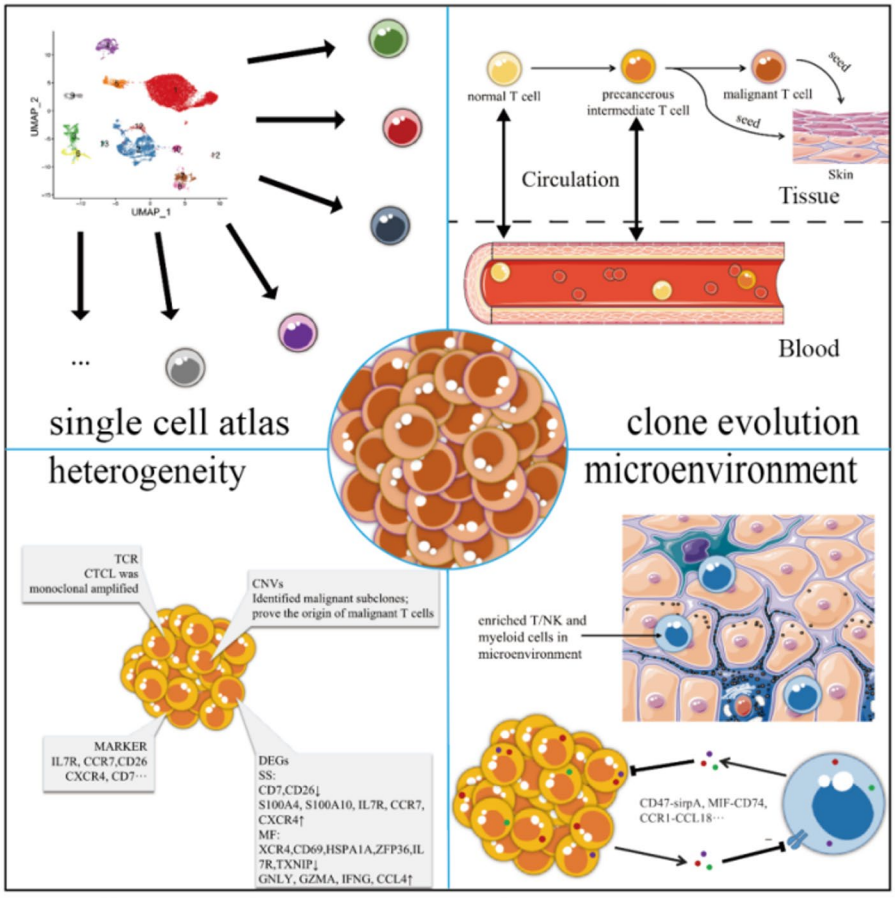

Fig. 2 Research progress of Single-cell sequencing in Cutaneous T-cell lymphoma. Numerous studies on CTCL utilizing single-cell sequencing have yielded noteworthy outcomes. The single-cell atlas furnishes us with useful insights into the subsets of CTCL and its clonal evolution. By analyzing copy number variations (CNVs), differentially expressed genes (DEGs), and various markers, we can shed light on the heterogeneity of CTCL. Additionally, by utilizing single-cell sequencing to perform component analysis of the immune microenvironment, we can discern the vital role different cells and signaling pathways play in maintaining the immune microenvironment of CTCL tumors

per-cell read requirements. Benchmarks show trade-offs between number of cells captured and per-cell sensitivity: droplet-based methods (e.g., 10x Chromium) allow large-scale profiling at moderate per-cell depth and are well-suited to detect major cell types and abundant markers, while full-length plate-based methods (e.g., Smart-seq2/3) provide higher sensitivity for low-abundance transcripts and isoform detection at the cost of lower throughput. Considering the significance of sequencing depth and plantform of each studym, we emphasize it in Table 1 despite some articles failing to provide relevant information.

Therefore, this review synthesizes recent advances in single-cell sequencing applied to CTCL, focusing on: (1) genomic alterations, (2) disease progression and biomarkers, (3) the tumor microenvironment, (4) clonal evolution and origin, and (5) conclusion. By addressing these areas, we aim to highlight how single-cell technologies are reshaping CTCL research and paving the way for personalized medicine.

2 Genomic alterations in CTCL

The diagnosis and treatment of cancer can be complicated by tumor heterogeneity. However, single-cell RNA sequencing provides a way to collect molecular profiles of individual cells, allowing for a quantitative understanding of cell heterogeneity. In the context of Cutaneous T-cell lymphoma (CTCL), the use of single-cell sequencing has greatly enhanced our comprehension of genetic heterogeneity, particularly in terms of differentially expressed genes (DEGs) and copy number variations (CNVs) [16]. Numerous studies have utilized samples from CTCL patients or their subtypes to illustrate the specific manifestations of intra-tumoral cellular heterogeneity in these individuals. Where reported, several studies validated scRNA-seq signatures using orthogonal methods such as immunohistochemistry (IHC), flow cytometry, or independent bulk cohorts. For example, Borcherding et al. validated AIRE protein expression by IHC across a panel of CTCL samples [15], and Song et al. cross-referenced single-cell signatures with independent cohorts/analyses [12]. Nonetheless, many reported single-cell markers remain to be confirmed across independent datasets and at the protein level.

2.1 Differentially expressed genes in MF revealed disease progression and phenotypic transformation

Mycosis Fungoides (MF) is the predominant clinical condition and accounts for approximately 60% of all cases [17, 18]. Biomarker monitoring is critical as the disease progresses from early indolent to progressive stages. A recent single-cell sequencing study revealed that lesion progression is consistently correlated with the downregulation of tissue-resident markers such as CXCR4 and CD69, the heat shock protein HSPA1A, tumor suppressors, the immunomodulatory mediators ZFP36 and TXNIP, and the interleukin-7 receptor (IL7R) in malignant clones. In contrast, malignant T-cells in clinically unaffected skin, that is, there is no significant change in the physical skin condition. from MF patients presented increased expression of these markers [19]. Furthermore, another single-cell RNA and T-cell receptor sequencing analysis of MF skin lesions in contrast to benign conterparts, including malignant T cells overexpressing keratins (KRT81, KRT86), galectins (LGALS1, LGALS3), S100 genes (S100A4, S100A6), while reactive T cells showed higher expression of IL7R, KLF2, KLF3, CD27, and SELL. The findings suggest potential biomarkers for distinguishing malignant from reactive cells and tracking MF progression [20]. These biomarkers could serve as indicators of MF disease progression, and the diverse expression of these genes may also account for the clinical phenotypic shift observed in advanced MF.

2.2 Differentially expressed genes in SS define molecular characteristics

Sezary Syndrome (SS) is a highly aggressive form of cancer that specifically affects T-cells in the skin with a median life expectancy of less than four years [21, 22]. Buus et al. conducted a study on the mRNA expression of malignant SS cells, focusing on a panel of 110 T-cell-related genes. To their surprise, they found significant heterogeneity in the expression of most genes within this panel across different malignant cells, including surface marker heterogeneity and transcriptomic heterogeneity, which highlight multiple axes of variation within malignant clones. The various manifestations of heterogeneity represent the complexity within the tumor. However, a cluster of five genes (S100A4, S100A10, CCR7, IL7R, and CXCR4) exhibited high expression levels in the majority of

malignant cells out of the 110 genes analyzed [5]. Additionally, a recent single-cell RNA sequencing study further elucidated subtype-specific gene expression in CTCL, identifying central memory markers (e.g., CCR7, SELL, LEF1) in CD4 + mycosis fungoides and cytotoxic markers (e.g., NKG7, GZMA) in TCR-γδ + MF and Berti lymphoma, highlighting distinct molecular profiles across CTCL subtypes [23]. Moreover, beyond these phenotypes, single-cell analyses have revealed a Treg-like transcriptional state ($FOXP3^+$, $IL2RA^+$), which represents a distinct axis of tumor adaptation [24]. These Treg-like malignant cells mimic regulatory T cells, suppressing anti-tumor immunity and complicating therapeutic strategies. This aspect, though underexplored in CTCL, highlights the functional diversity of malignant clones.

Despite the observed heterogeneity in SS, Borcherding et al. [15] identified certain common SS markers, including NEDD4L [25, 26], PLS3 [27], and TOX [28, 29], which may warrant further investigation. Furthermore, AIRE protein expression was detected in various CTCLs and emerged as the most significantly up-regulated gene in their analysis [15]. The expression of AIRE by CTCL cells could potentially attract and stimulate a wider range of regulatory T cells, leading to a better understanding and treatment of CTCL [15, 30]. Consequently, the differentially expressed genes (DEGs) identified through single-cell sequencing delineate the molecular characteristics of malignant T cells in SS patients. This underscores the presence of cellular heterogeneity among malignant T cells, which could also pose challenges in the development of novel therapeutic strategies targeting these proteins.

2.3 The comprehensive analysis identified common DEGs and essential pathways in patients with heterogeneous CTCL cohorts

Studies have shown that malignant T cells use various cancer-causing processes, like heightened oxidative phosphorylation (OXPHOS) and Myc, to become more aggressive and survive during transformation [12]. Ren et al. examined paired single-cell RNA from peripheral $CD4^+$ T cells of CTCL patients and found that these cells have differing transcriptomic profiles, with most exhibiting a mature Th2 differentiation and T-cell exhaustion phenotype [13]. They identified 66 genes that were upregulated and 13 that were down-regulated in CTCL cells compared with normal CD4 + T cells. Importantly, they observed increased CD82 expression in CTCL, which regulates CTCL proliferation and apoptosis via AKT/PI3K and JAK/STAT pathways [31, 32]. This finding suggests that new therapeutic approaches like bispecific antibodies could be developed to target CD82-expressing circulating T-cells or block CD82-dependent CTCL proliferation while increasing apoptosis.

2.4 Copy number variations (CNVs) in CTCL identify malignant subclones and tissue of origin

CNV, proliferation, and stemness are commonly used to determine the existence of malignant T cell subpopulations. T cell subsets that exhibit elevated levels of CNV, proliferation, and density are known to contribute to the malignancy of CTCL. It's worth noting that several of these malignant subpopulations showcase recurrent CNVs, with chromosome 7 gains and chromosome 13 losses being particularly prevalent [33–36]. Based on CNV accumulation, Herrera et al. established a complex and diverse phylogenetic landscape that revealed no clear unidirectional phylogenetic relationship between

skin and blood-derived subclones, giving a snapshot of the development of subclones in the skin and blood of CTCL patients and indicating the tissue origin of the disease [37]. Song X. et al. observed TCR monoclonality in CTCL, with monoclonal T cells displaying malignant CNV patterns, suggesting that TCR monoclonality is a crucial transformation feature [12]. We also compared single-cell observations with prior bulk genomic surveys like da Silva Almeida et al. did in 2015 [34] and find general concordance in recurrent copy-number events, while single-cell resolution reveals intra-patient subclonal CNV architectures and tissue-specific subclone distributions not evident from bulk assays. Collectively, the evolutionary relationship between the CNV-defined subclones in each patient provides a clone evolutionary atlas of CTCL, which makes it possible to identify the original tissue of malignant cells [12]. Here the CNV-defined subclones refer to malignant T-cell populations distinguished by recurrent copy number variations, such as chromosome 7 gains or chromosome 13 losses, which delineate clonal structure and evolutionary trajectory.

2.5 Single-cell transcriptome atlas of CTCL skin tumors show intra-tumor T lymphocyte heterogeneity

Traditional genome and gene expression profiles show the characteristics of patients' cancerous tissues, while the high heterogeneity within tumors has yet to be fully revealed. Therefore, studying the genome and gene expression at the single-cell level is particularly necessary. In recent years, studies on gene expression maps based on single-cell sequencing technology began to present the gene expression maps of CTCL at the single-cell level.

DEGs and CNVs mentioned above are the concrete expression of the heterogeneity of CTCL tumor cells without considering SNVs and epigenetic abnormalities despite their importance, while single-cell mapping is the process of presenting and visualizing this concrete embodiment in the form of a whole. Through the paired single-cell RNA sequencing of peripheral blood $CD4^+$ T cells, Ren et al. investigated that in addition to increases in several memory/activation markers in CTCL (NR4A2, TNFRSF25, REL, CCR7, TNFRSF4, BRD2, TSPAN2, ITGBB1, and SELL), the DEG atlas also showed significant depletion of immunomarker genes (PDCD1, CTLA4, TOX, TIGIT, LAG3) in CTCL cells, suggesting that chronic antigenic stimulation may affect CTCL patients [13].

By describing the transcriptome profiles of single-cell present in advanced CTCL skin tumors compared with those present in healthy control skin, Gaydosik et al. demonstrated the presence of a common gene expression signature in highly proliferative lymphocytes and concluded the gene expression characteristics of malignant lymphocytes in advanced CTCL skin tumors [4]. The results of the single-cell transcriptome atlas showed that the cancer cells shared characteristics within the tumor and showed intratumoral heterogeneity of the CTCL subtype and key tumor-related pathways, providing more possibilities for personalized treatment in the future.

2.6 Single-cell sequencing identified markers of disease progression in CTCL

Building on genomic alterations, single-cell sequencing has identified various biomarkers of CTCL progression, critical for tracking disease stages and phenotypic shifts. Surface antigen signatures are essential for building cells under varying conditions or unraveling molecular changes in cells to understand the disease better. Currently, there

are no dependable molecular biomarkers capable of predicting CTCL disease outcomes. Here, we present some potential markers of disease progression in CTCL based on existing research (Table 2 in Additional File 2.xlsx). Despite the highly heterogeneous expression of many classical T-cell markers in malignant populations, it has been suggested that alterations in the expression of biomarkers may underlie the clinical phenotypic shifts observed in advanced MF [19]. In conclusion, analysis of markers in patients with early and advanced CTCL has revealed differences between different lesion types, which may reflect mechanisms that promote disease progression and influence overall prognosis. Furthermore, targeting novel therapeutic strategies toward these markers may prove to be more effective in targeting CTCL cells across patients.

2.7 Single-cell sequencing can inform drug discovery and therapeutic development for CTCL

Single-cell technologies have to some degree advanced our understanding of cellular heterogeneity, enabling precise characterization of the molecular pathways involved in CTCL pathogenesis. This high-resolution approach has allowed the identification of novel therapeutic targets by uncovering druggable vulnerabilities and analyzing drug mechanisms in malignant T-cell populations. In 2022, Van de Sande noted that scRNA-seq can inform decision-making by facilitating the identification of improved biomarkers for patient stratification and enabling more precise monitoring of drug response and disease progression [38]. For example, a study using single-cell RNA sequencing of primary PBMCs from an advanced CTCL patient before and after anti-PD-1 treatment found that PD-1 monoclonal antibodies led to significant proliferation and activation of tumorigenic T cells, whereas there was no significant change in the gene expression profile of non-tumorigenic CD8 + T cells. This suggests that a PD-1 monoclonal antibody relieves the inhibitory effect of the PD-1 pathway on downstream signaling pathways and promotes the proliferation and activation of tumorigenic T cells, leading to hyper-progression of CTCL [39]. In addition, the technology has identified subtype-specific putative therapeutic targets, such as CCR4 and KIR3DL2 in CD4 + mycosis fungoides, which are absent in TCR-γδ + MF and Berti lymphoma, guiding the development of targeted therapies like mogamulizumab [23, 40]. Furthermore, CD82 has been implicated in several single-cell studies as upregulated in malignant CTCL cells and functionally linked to proliferation/survival through AKT/PI3K and JAK/STAT signaling; experimental data suggest CD82 modulation alters CTCL cell proliferation and apoptosis [13]. Table 3 (in Additional File 3.xlsx) presents several possible useful drugs for CTCL developed by single-cell sequencing [2, 40–51]. Given that inter- and intra-patient variability in CTCL are complicated, leveraging single-cell data to design more targeted therapies may significantly enhance drug screening and MoA analysis and improve patient outcomes. In conclusion, the integration of single-cell sequencing into drug discovery and development pipelines has the potential to contribute to personalized medicine approaches for CTCL, providing tailored strategies to address disease progression and resistance mechanisms [50]. It should be noted that the findings reported in refs. 48–50 (cited in Table 3) have not yet been independently validated by other groups, and therefore remain contentious. While these pioneering studies provide intriguing hypotheses about new drug development like PD-L1 blockade, their conclusions should be interpreted with caution until confirmed by larger, multi-center cohorts.

3 Tumor microenvironment (TME) of CTCL

CTCL refers to a form of cancer that involves various elements interacting with tumor cells. These elements include tumor-associated macrophages, fibroblasts, dendritic cells, mast cells, chemokines, and cytokines, which collectively form the tumor microenvironment (TME). The TME can either directly or indirectly regulate tumor cell migration and proliferation, and may significantly contribute to cancer progression [52, 53]. With single-cell sequencing techniques, researchers are able to explore the TME heterogeneity on high resolution. Some Research has shown that the molecular diversity of malignant T cells in skin and blood is influenced by the CTCL TME [37, 54, 55]. Single-cell sequencing techniques provide better resolution than conventional gene sequencing methods, enabling a better understanding of the molecular mechanisms of immune cells in the TME and how they affect tumor genesis, development, metastasis, drug resistance, and immune escape. This knowledge can significantly aid in clinical diagnosis, treatment, and prediction of solid tumors.

3.1 The diversity of malignant T-cell sources determines the heterogeneity of TME

Liu, X. et al. conducted a study to analyze the single-cell data of TME in the T_{CyEM} (effector memory cytotoxic T cell) group and T_{CM} (central memory T cell) group, focusing on tumor-infiltrating lymphocytes (TILs) [14]. As expected, $CD8^+$ TILs were found to be the central antitumor effector cells in CTCL (SS). However, they were more exhausted in the T_{CM} group, which is consistent with previous findings that malignant T cells confer anti-tumor immunity to damaged normal T helper cells in CTCL (SS) [56]. Then, they interestingly found that the T_{CyEM} group showed a relatively higher fraction of macrophages. At the same time, the abundance of B cells and plasma cells was significantly higher in the T_{CM} group [14]. Further, they propose that malignant T_{CyEM} cells appear to form a tumor-supporting environment in T_{CyEM} cases by producing high levels of CCL5 and CCL4 to recruit and polarize M2 TAM expressing CD163, while malignant T cells activate B cells in TCM cases via the CD40L/ CD40 axis, that highly infiltrated B cells may promote tumor progression through activation of Tregs. This study emphasizes the need to personalize anti-CTCL immunotherapies based on the molecular subtype of each patient, as the heterogeneity of TME is determined by the variety of malignant T-cell sources.

3.2 The interaction between malignant T and bone marrow cells suggests new therapeutic strategies

This interaction is particularly relevant in advanced Sézary syndrome (SS), where bone marrow infiltration is common, whereas early-stage Mycosis fungoides (MF) rarely demonstrates such involvement [1]. A recent study conducted by Du and colleagues compared single-cell RNA-seq data from patients with advanced-CTCL (one SS and four patients with large cell transformation) to that of healthy individuals. The findings indicated that patients with CTCL had elevated T/NK cells, myeloid cells, $CCL13^+$ monocytes/macrophages, and $LAMP3^+$ cDC cells [33]. Myeloid cells can promote tumor growth and metastasis or inhibit host immunity, making them crucial in cancer development [57]. Additionally, $CCL13^+$ Mono/Macro cells and $LAMP3^+$ cDC cells can encourage myeloid migration to the tumor microenvironment, restore immunosuppression, and hasten carcinogenesis [58].

Moreover, the study revealed that malignant T cells and bone marrow cells in CTCL expressed higher levels of S100 A9 and its receptor TLR 4. The interaction between S100 A9 and TLR4 can activate the NF-kβ signaling pathway, promote tumor growth, and suppress cytotoxic T cells by bone marrow cells [59–61]. Prior research has suggested that blocking the S100A9 signaling pathway can hinder S100A9 and TLR4 signals in myeloid-derived suppressor cells (MDSC) tumor infiltration. Consequently, using tasquinimod, a small molecule that inhibits S100A9 signaling, may be an effective way to target the tumor microenvironment and treat patients with CTCL. Thus, treatments that target the interaction between malignant T cells and bone marrow cells, such as pharmacologically targeted therapy of S100 A9-TLR4, may be a promising strategy for treating patients with CTCL.

In a separate investigation, Song and colleagues conducted a comparison between malignant T cells found in transformed tumors (TT) and benign CD4 + T cells present in the tumor microenvironment (TME). Additionally, they utilized single-cell sequencing to analyze the different types of benign immune cells in transformed Cutaneous T-cell Lymphoma (tCTCL) TME [12]. The results of the study revealed that B cells were found to be significantly more prevalent in TT and could potentially contribute to the growth of tumors [62]. Their deeper investigation suggested that B cells may interact with malignant T cells through MIF-CD74 signaling, thereby indicating a possible tumorigenic role in tCTCL [12]. Similarly, Li, R. et al. revealed a predominance of TH2-like malignant T cells supported by a B cell-rich tumor microenvironment, including enriched MHC-II + fibroblasts and dendritic cells that promote TH2 polarization via cytokines (e.g., IL33, CCL5) compared with benign skin sample. The study identified B cell aggregations potentially forming tertiary lymphoid structures associated with disease progression and poor prognosis, along with CTCL-specific markers (e.g., TOX, CD9, CXCL13) for diagnosis, suggesting B cell-directed therapies as a promising approach [63].

Taken together, studies of TME through single-cell sequencing will help reveal previously unrecognized T-cell and non-T-cell heterogeneity and intercellular communication and delineate the overall immune landscape of CTCL (Fig. 3 in page 22). These research findings carry substantial significance in our understanding of how malignant T cells reshape and modify the composition and features of the multicellular environment within the tumor microenvironment. They offer insights into CTCL, shedding light on its intricacies and pave the way for novel avenues in the advancement and utilization of immunotherapeutic approaches.

4 Origin, clone evolution, and progress of CTCL

Despite significant progress in comprehending the clone evolution of CTCL over the past few decades, the source and characteristics of malignant T cells remain undefined. Unlike solid tumors, CTCL starts as multiple skin lesions, leading to debates regarding its tumor source and development pattern. Although one study posits that CTCL emerges from monoclonal mature memory T cells present in the skin [64], current findings suggest that malignant T cells originate from circulating immature precursor cells that infiltrate the skin and transform into heterogeneous lymphomas [65–67].

Liu, X. et al. conducted a thorough investigation utilizing single-cell RNA analysis, revealing the monoclonal nature of CTCL and presenting a multi-step tumor evolution model. Their research proposes that monoclonal malignant T cells undergo a multi-step

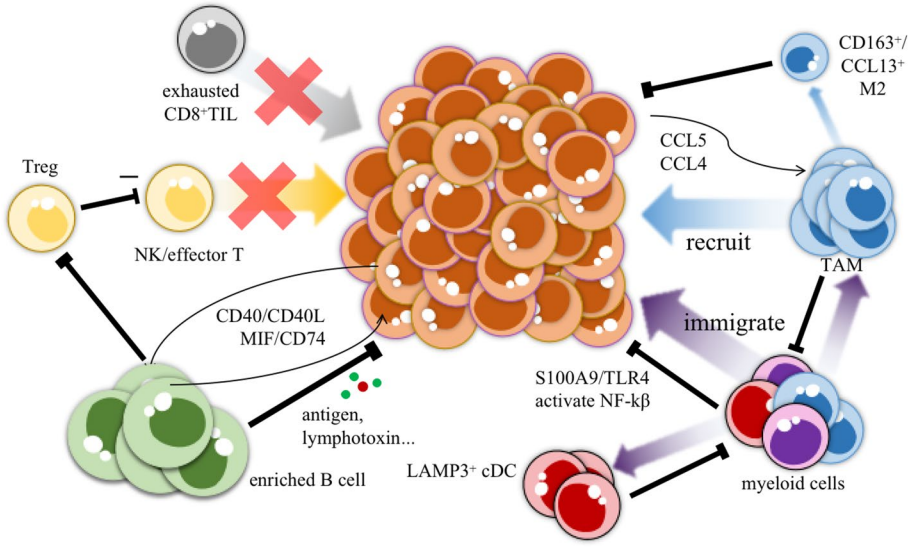

Fig. 3 Interactions in CTCL tumor microenvironment revealed by single-cell sequencing. This schematic illustrates generalized interactions observed across CTCL subtypes, including Mycosis Fungoides (MF) and Sézary Syndrome (SS), informed by multiple single-cell studies. Within the tumor microenvironment of CTCL, the CD8 + Tumor-Infiltrating Lymphocytes (TIL) undergo exhaustion, while the Natural Killer (NK) and effector T cells are inhibited. Enriched B cells and myeloid cells are essential for the composition and maintenance of the immune microenvironment in CTCL. The formation of this environment relies on intricate signaling pathways between cells, with variations in cellular composition and interactions possible between subtypes (e.g., higher macrophage abundance in effector memory cytotoxic T cell-like cases and elevated B cells in central memory T cell-like cases). TAM, Tumor-Associated Macrophages; M2, M2 macrophages; cDC, conventional dendritic cells

seeding process, resulting in the observed divergence between CTCL lesions, with parallel subclonal evolution [14]. These intriguing findings shed light on the origin, clonal evolution, and progression of CTCL.

4.1 The potential of heat-diffusion for affinity-based trajectory embedding (PHATE) analysis and pseudotime constructs the trajectory of the cells' transition from normal CD4$^+$ t cells to CTCL

Pseudotime analysis focuses on temporal relationships during cell development or differentiation, while PHATE analysis focuses on visualizing and discovering cell types and transformation processes. Ren J. et al. utilized PHATE analysis to reveal a transitional population between normal CD4$^+$ T cells and established CTCL cells in CTCL patients [13]. Meanwhile, Song et al. utilized Monocle, a machine-learning algorithm, to order the trajectory of t-CTCL(transformed CTCL) constituents in pseudotime, demonstrating that the expression levels of MHC I genes consistently decreased from benign CD4$^+$ T cells to malignant cells [12]. In light of the foregoing statements, single-cell profiling across cohorts indicates that malignant CTCL clones frequently occupy continuous transcriptional landscapes rather than discrete, immutable phenotypes: the same dominant clonotype can display TCM-like, TCyEM-like or Treg-like transcriptional programs depending on tissue (skin vs. blood), disease stage and prior therapy. Because trajectory and pseudotime reconstructions are descriptive, they should not be taken alone as proof of unidirectional lineage progression; instead, they point to transcriptional plasticity that requires functional validation. To resolve cause–effect relationships we therefore recommend combining single-cell atlases with lineage-tracing or perturbation experiments (for example, Perturb-seq or CRISPR-based single-cell screens) and spatial assays to

confirm whether state transitions reflect true lineage changes or tissue-induced reprogramming [12, 14]. In general, PHATE and Pseudotime do contribute to the understanding of the origin, clone evolution, and progress of CTCL, providing an overall picture of CTCL evolution.

4.2 The intrinsic features and transcriptional trace analysis of malignant T cells suggest distinct tumor origins and subtypes of CTCL

Due to the diversity of its subtypes and classifications, classifying CTCL is a complex task. Currently, classification is mainly based on clinical features, cell morphology, and T cell type rather than the molecular characteristics of malignant T cells [42]. In an attempt to address this, Liu et al. conducted an unsupervised cluster analysis of DEGs, which separated all samples from the 10x genomic dataset into two groups: the cytotoxic effector memory T cell (T_{CyEM}) group and the central memory T cell (T_{CM}) group, regardless of disease subtypes [14]. Further trajectory analysis revealed that starting with the benign, naive T cell subpopulation, malignant T_{CyEM} and T_{CM} cells move to nearly opposite branches, representing two distinct tumor origins.

Herrera et al. conducted an insightful analysis on the transcriptional traces of malignant T cells derived from both skin and blood samples. Their findings highlighted a distinctive transcriptional bias in the malignant T cells from skin tissue. Notably, subclones originating from skin samples had a tendency to align towards the end of the branches of blood-derived subclone trajectories, which resulted in a masking of subclonal disparities [37].

Recent studies using single-cell TCR sequencing data have indicated that the monoclonality of the TCR is a well-established diagnostic hallmark of CTCL, useful for tracking malignant clones [12]. To better understand the origin of CTCL, it is necessary to conduct further research using single-cell TCR sequencing data from CTCL lesions at different disease stages, which will provide insights into the timing of TCR gene rearrangement.

The aforementioned studies shed light on noteworthy shifts in the source and advancement of CTCL. Suppose these shifts are confirmed to play a role in the emergence, upkeep, and alteration of the ailment. In that case, tracking them pre- and post-treatment may unveil their connection to the treatment's prolonged efficacy and the probability of disease reappearance.

5 Conclusions

In recent years, single-cell sequencing has made breakthroughs in technological advances and clinical applications. For example, Batch TCR-SEQ showed the diversity of TCR clonotypes in CTCL and showed that T cell tumors occurred before TCR gene rearrangement [66]. However, thanks to the higher resolution, single-cell TCR sequencing revealed the monoclonality of TCR [12]. With the development of single-cell sequencing, it has been increasingly used in sequencing studies of different tumor types, pushing molecular cancer studies such as tumor microenvironment and heterogeneity to new levels of precision and accuracy [68–70].

Although single-cell sequencing offers benefits in analyzing the transcription landscape of CTCL, it has limitations that must be acknowledged. It is important to note that integrating single-cell datasets from heterogeneous cohorts that differ in CTCL subtype,

disease stage, and prior treatment may obscure biologically relevant patterns. Stage-dependent immune microenvironment remodeling and therapy-induced transcriptional reprogramming can significantly influence results, necessitating careful stratification and reporting in future studies. The specificity of transcription in time and space makes it impossible for real-time sequencing to fully decode the transcription landscape. Besides, single-cell sequencing cannot correlate phenotypes alone, suggesting the need for low-cost, high-throughput multi-omics techniques to map the overall tumor tissue landscape.

One way to address these limitations is to utilize alternative high-dimensional transcriptomic profiling approaches, such as single-nucleus RNA sequencing and digital spatial transcriptomics. Additionally, combining single-cell sequencing with metabolomics and epigenomics, such as single-cell metabolic flux analysis and scATAC-seq, has dramatically enhanced the application prospects of single-cell sequencing. This provides the possibility to study molecular metabolism and chromatin accessibility at the single-cell level [71, 72]. To expand the scope of single-cell sequencing research significantly, a multidisciplinary research strategy may be adopted, which could become one of the new development directions in the future [73].

The issue of tumor heterogeneity has long posed a significant challenge in the field of tumor research. This phenomenon has a profound impact on the clinical treatment of various cancers due to complex immune heterogeneity, leading to varying responses among patients. However, the classification of CTCL subgroups based on single-cell sequencing has important implications for designing combination and novel therapies based on heterogeneity to combat drug resistance and treatment failure [5, 74, 75].

In this review, we delve into the latest developments in single-cell sequencing for CTCL, covering three key areas of progress: genomic alteration, tumor progression, and the tumor microenvironment. Owing to advancements in these areas, we now have a much deeper understanding of this complex and varied disease. This improved understanding holds promise for the future discovery of more effective therapeutic methods to improve survival rates and reduce cancer relapse. Moving forward, ongoing research will be crucial in obtaining a comprehensive understanding of CTCL single cells and tumor microenvironment, including the integration of single-cell analysis into clinical trials and assessing tumor susceptibility risk through early changes in the expression of individual cells. These breakthroughs will offer unprecedented solutions to address multiple mechanisms of drug resistance and morbidity.

Abbreviations

CTCL	Cutaneous T Cell Lymphoma
MF	Mycosis Fungoides
SS	Sézary syndrome
DEG	Differentially Expressed Genes
TCR	T-cell Receptor
CNVs	Copy Number Variations
TME	Tumor Microenvironment
TILs	Tumor-Infiltrating Lymphocytes
PBMC	Peripheral Blood Mononuclear Cells
NK	Natural Killer
TLR4	Toll-like Receptor 4
BCL2L12	B-Cell CLL/lymphoma 2 Like 12
AKT/PI3K	Protein Kinase B/Phosphoinositide 3-Kinase
ATF5	Activating Transcription Factor 5
CCR7	C-C Chemokine Receptor Type 7
GPCR	G Protein-Coupled Receptor
AIRE	Autoimmune Regulator

T_{CyEM} Effector memory cytotoxic T cells
T_{CM} Central memory T cells

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s12672-025-04063-0.

Supplementary Material 1. Includes a table about Single-cell sequencing in cutaneous T cell lymphoma

Supplementary Material 2. Includes a table about markers of disease progression in CTCL revealed by single-cell sequencing.

Supplementary Material 3. Includes a table about drug development for CTCL with single-cell sequencing.

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Jun Du conceived and oversaw the project, and revised the manuscript. Hao-Ran Yuan, Zi-Han Lin and Jun-Ting Lv draw the picture and wrote the manuscript. The rest authors were responsible for literature retrieval and helped with manuscript revision.

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Declarations

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The authors declare no competing interests.

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