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Bibliometric analysis of single-cell RNA sequencing in tumor microenvironment

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Abstract

The tumor microenvironment (TME) has emerged as a pivotal determinant of tumor progression, immune evasion, and therapeutic response, making it a central focus in modern oncology. Single-cell RNA sequencing (scRNA-seq) has revolutionized our ability to dissect the cellular heterogeneity and dynamic interactions within the TME at unprecedented resolution, enabling precise identification of cell types, functional states, and intercellular communication networks. In this study, we conducted a comprehensive bibliometric analysis of 187 publications of scRNA-seq in TME, indexed in the Web of Science Core Collection from January 1999 to July 2024. Our analysis reveals that China and the United States are the dominant contributors in terms of publication volume and citation impact, with Peking University and Harvard University leading institutional output. Keyword analysis revealed that the most frequent terms were "tumor microenvironment," "single-cell RNA sequencing," "gene expression," and "cancer". Journals with highly cited articles mainly include Nature Genetics, Cancer Research, Annual Review of Immunology, Nature Communications, Science Translational Medicine, Genome Biology, Journal of Hepatology, and Nature Cell Biology. Current studies primarily focus on using scRNA-seq to identify cell types and subtypes within the TME, as well as to investigate cell-cell interactions and communication. These findings offer valuable insights into advancing our understanding of tumor genetics, immune evasion mechanisms, diagnostic and prognostic biomarkers, and treatment resistance. While the field demonstrates rapid growth, enhanced international collaboration remains an urgent unmet need. Future research will likely to center on roles of immune and stromal cells within the TME revealed by scRNA-seq, as well as the integration of scRNA-seq with spatial transcriptomics and machine learning. It represents a promising direction for developing novel tumor therapies.

Keywords Bibliometric analysis, Single-cell RNA sequencing, Tumor microenvironment, Immunotherapy, Cancer immunity

1 Introduction

Tumors represent complex ecosystems composed of cells with diverse phenotypes, genotypes, and epigenetic states [1]. The tumor microenvironment (TME) plays a pivotal role in tumor initiation, progression, metastasis, and therapeutic response, catalyzing

a paradigm shift from tumor-centric to TME strategies. Angiogenesis within the TME supplies oxygen and nutrients essential for tumor growth and influences responses to anti-cancer therapies. Vascular endothelial growth factor (VEGF) is a key mediator that promotes the formation of a disorganized and primitive vascular network in various tumor tissues [2]. Consequently, drugs targeting the VEGF signaling pathway, often combined with cytotoxic agents, are clinically employed to suppress tumor neovascularization and delay disease progression [3]. The therapeutic mechanism of tumor-infiltrating lymphocytes (TIL) therapy is grounded in a deep understanding of the TME [4]. The extraction, expansion, and re-injection of TIL have demonstrated remarkable efficacy in treating solid tumors [5]. Additionally, tumor-associated fibroblasts (TAFs), tumor-associated macrophages (TAMs), and immune checkpoints such as CTLA-4, PD-1, and PD-L1 are critical targets in cancer immunotherapy [6, 7]. TME is a general term for the genesis and survival environment of tumor cells, consisting mainly of tumor cells, non-tumor cells, extracellular matrix (ECM), vascular system, and soluble products [8, 9]. These components profoundly influence immune responses against tumors, the efficacy of immunotherapies, and patient prognosis. Analyzing cell-cell communication within the TME helps decipher the mechanisms underlying cancer progression and drug resistance [10, 11]. The composition and functions of TME not only vary among different individuals but also differ within regions of the same tumor and can even change in development stages [12]. Such variability directly impacts biopsy strategies, diagnostic accuracy, and treatment personalization [13]. Therefore, elucidating cellular phenotypes within the TME is crucial for understanding the mechanisms of individualized disease progression and response to immunotherapy.

Advances in cancer genomics have established bulk RNA sequencing (bulk RNA-seq) and single-cell RNA sequencing (scRNA-seq) as vital tools in oncology research. However, bulk RNA-seq measures average gene expression across cell populations, masking cellular heterogeneity [14]. In contrast, scRNA-seq enables high-resolution profiling of gene expression at the single-cell level, providing a powerful approach for characterizing the heterogeneity of the TME. This technology has been instrumental in identifying lineage-specific transcriptional programs in tumor and stromal cells, delineating the precise cellular composition of the TME, and tracking dynamic transcriptional rewiring during tumor initiation, progression, and treatment response. Research integrating TME and scRNA-seq holds fundamental significance for improving clinical management and personalized therapy in oncology. Although progress has been made, the field remains complex, underscoring the need to systematically map research landscapes, identify key challenges, and define emerging hotspots to guide future investigations.

Bibliometrics employs quantitative methods to analyze the research field of literature production and dissemination by mathematical statistics [15]. Through statistical and network analyses of publications, it uncovers structural distributions, evolutionary trajectories of research outputs, and inherent scholarly connections, thereby facilitating the identification of emerging trends and future research priorities [16]. With the advancement of tumor biology and single-cell technologies, the application of scRNA-seq in TME research has garnered increasing attention. However, no bibliometric study has yet comprehensively explored the intersection of scRNA-seq in TME. Therefore, this study aims to perform a bibliometric analysis of literature indexed in the Web of

Science (WOS) Core Collection to systematically map the global progress, key research hotspots, and evolutionary directions of scRNA-seq applications in TME research.

2 Methods

2.1 Data sources and search strategy

Data for this bibliometric analysis were obtained from the WOS Core Collection. WOS is regarded as the most comprehensive source of scientific exploration and evaluation for bibliometric analysis with broad subject coverage, citation index, and rich analysis indicators [17, 18]. A comprehensive literature search was conducted to identify all publications related to scRNA-seq and TME, spanning the period from January 1999 to July 2024. Only original research articles and reviews published in English were included.

The search strategy is as follows: ((TS=(Tumor Microenvironments)) OR TS=(Cancer Microenvironments)) AND ((((((TS=(Transcriptome Analyses, Single-Cell)) OR TS=(Single Cell Gene Expression Analysis)) OR TS=(Single Cell Gene Expression Profiling)) OR TS=(Single Cell Transcriptome Analysis)) OR TS=(Single Cell RNA Seq)) OR TS=(ScRNA seq)). Two researchers independently screened the titles, abstracts, and keywords of the retrieved records. Any discrepancies were resolved through discussion or by consultation with a third researcher. Finally, a total of 187 articles were included in our study. Retrieved publications are exported as Plain text files with Full Record and Cited References. The process of data collection and analysis is shown in the flowchart (Fig. 1).

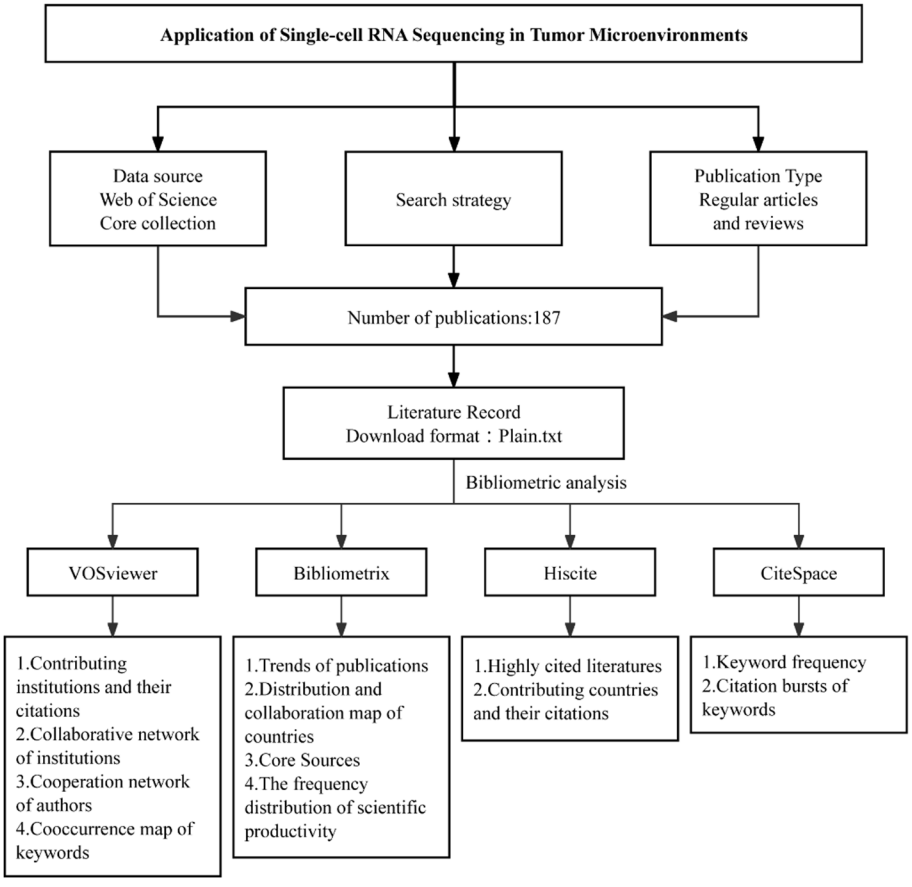

Fig. 1 Flowchart of literature selection for bibliometric analysis on scRNA-seq and TME

2.2 Bibliometric analysis

Using bibliometric software, we analyzed authors, countries, institutions, citation counts, and keywords to gain a comprehensive understanding of current research focuses and future directions in this field. We used VOSviewer software, leveraging probability theory-based data standardization methods, to construct visual representations of keyword co-occurrence relationships, as well as collaborative networks among authors, countries, and institutions. This approach effectively illustrates core research themes and collaborative trends across different entities within the field [19]. The results from VOSviewer are mainly presented by a co-occurrence diagram composed of nodes and lines. Each node represents different countries, institutions, authors, or keywords, with its size reflecting the number of related documents. The colors of the nodes denote the relevance and importance of these entities, delineating distinct clusters. For the display of time-related co-occurrence relationships, node colors reflect different time periods. Lines connecting nodes illustrate the collaborative or co-occurrence relationships related to publications [20]. CiteSpace is a web-based Java application for data analysis and visualization [21]. It uses synergetic analysis theory to draw a series of visual maps, analyze the literature in a specific field, and explore the development trend of related disciplines. Additionally, we utilized the "Bibliometrix" package in R and other tools such as Hiscite for complementary data analysis. To ensure analytical accuracy, we performed data cleaning to consolidate keywords with identical meanings, such as merging "single-cell analysis" and "single-cell RNA sequencing" into the unified term "scRNA-seq."

3 Results

3.1 Characteristics and trends of publication

After screening, duplicate removal, and eligibility assessment according to the predefined criteria, 187 articles were included in this bibliometric analysis. These articles were contributed by 1721 authors across 454 institutions in 29 countries, published in 103 journals, and cited a total of 10,657 references from 1622 journals. The publication time span of the included articles ranges from 1999 to 2024 (Fig. 2). In the initial stage of the field, research progress was relatively slow, with only 2 articles published in 1999 and 2002, followed by no relevant publications until 2014. Notably, a substantial increase in

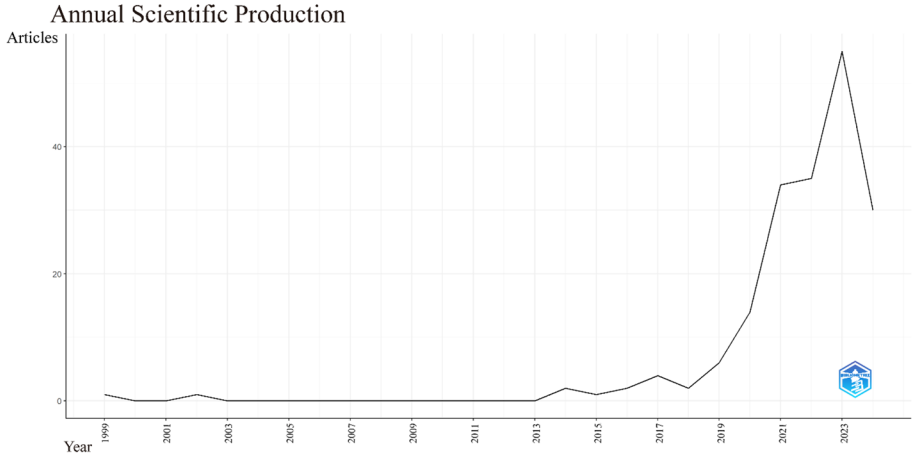

Fig. 2 Annual publication trends of scRNA-seq in TME research (1999–2024)

publications was observed between 2020 and 2021 and 2022–2023, reflecting the rapid expansion of research interest in this field.

Citations of articles serve as a key metric for gauging the degree to which scientific research papers are recognized and valued by peers, reflecting their influence within the field. Table 1 presents the top 10 most-cited papers in this field. These articles were published in high-impact scientific journals in this field, including Nature Genetics, Cancer Research, Annual Review of Immunology, Nature Communications, Science Translational Medicine, Genome Biology, Journal of Hepatology, and Nature Cell Biology. Notably, the paper titled "Reference Component Analysis of Single-cell Transcripts of Cells Elucidates in Human Colored Tumors", co-authored by Li and colleagues from Singapore, has garnered the highest number of citations. This study focuses on analyzing the transcriptional heterogeneity of colorectal tumors and their microenvironment through scRNA-seq of both colorectal tumors and matched normal mucosa, providing valuable insights into abnormal cell states within tumors [22]. Among the top 10 cited articles, 4 studies have corresponding authors from the United States and 2 studies from China, highlighting the significant contributions of these two countries to research in this field.

3.2 Analysis of countries and institutions

Researchers from 29 countries contributed to the 187 included articles. The top 10 countries by publication volume and their total citation counts are displayed in Fig. 3A. The top five countries by publication volume are China (101), United States (59), Germany (12), England (10), and Canada (9). The United States has the highest total citation count among all countries, reflecting the high academic influence of its research output. Despite the number of papers published in Singapore being 3, they have obtained a high number of citations, and one of them is the most cited paper with 708 citations. The country collaboration map was made by visualizing the outputs of the countries and the cooperation between different countries related to scRNA-seq and TME (Fig. 3B). In the country cooperation map, different colors of countries represent different outputs of articles. The darker of the color, the more output. China and the United States are the dominant contributors to publication output in this field, with the highest frequency of bilateral collaboration. In addition, researchers from the United States have more exchanges and cooperation with researchers from other countries. However, overall international collaboration in the field remains limited, highlighting an urgent need to strengthen international academic exchange and cooperation in this field.

The 187 articles in our collection were published by relevant researchers from 454 institutions. The publication volume and total citations of the top 10 research institutions were summarized in Fig. 3C. The results indicate that Peking University (China) and Harvard University (United States) have the highest publication volume and total citation counts, playing a leading role in this field. Of the top 10 most productive institutions, eight are represented by Peking Univ (10, 5.35%), Nanjing Med Univ (9, 4.81%), and Southern Med Univ (8, 4.28%) from China. The other two institutions are Harvard Univ (10, 5.35%) and MIT (5, 2.67%) from the United States. The institutions from China and the United States are acting as major driving forces in this field of research. In the co-occurrence diagram of institutions made by VOSviewer (Fig. 3D), it can be seen that most of the institutions with stronger cooperative relations come from China and the United States. Importantly, institutions with high publication volume typically have

Table 1 Top 10 most-cited articles in the field of scRNA-seq and TME

Rank	Article title	Journal	Country	Publication year	Citations
1	Reference component analysis of single-cell transcriptomes elucidates cellular heterogeneity in human colorectal tumors	Nature Genetics	Singapore	2017	708
2	Lineage-dependent gene expression programs influence the immune landscape of colorectal cancer	Nature Genetics	South Korea	2020	317
3	Modeling the Genetic Regulation of Cancer Metabolism: Interplay between Glycolysis and Oxidative Phosphorylation	Cancer Research	United States	2017	169
4	Insights Gained from Single-Cell Analysis of Immune Cells in the Tumor Microenvironment	Annual Review of Immunology	China	2021	154
5	HIF-1α and HIF-2α differently regulate tumour development and inflammation of clear cell renal cell carcinoma in mice	Nature Communications	Germany	2020	146
6	Dissociation of solid tumor tissues with cold active protease for single-cell RNA-seq minimizes conserved collagenase-associated stress responses	Genome Biology	Canada	2019	134
7	HBEGF+ macrophages in rheumatoid arthritis induce fibroblast invasiveness	Science Translational Medicine	United States	2019	130
8	Single-cell atlas of tumor cell evolution in response to therapy in hepatocellular carcinoma and intrahepatic cholangiocarcinoma	Journal of Hepatology	United States	2021	116
9	Dissecting esophageal squamous-cell carcinoma ecosystem by single-cell transcriptomic analysis	Nature Communications	China	2021	110
10	Subclonal cooperation drives metastasis by modulating local and systemic immune microenvironments	Nature Cell Biology	United States	2019	102

closer internal collaborative partnerships, with domestic collaboration being significantly more common than international collaboration.

3.3 Analysis of journals and authors

The 187 included articles were published in 103 journals. Based on Bradford's law, we analyzed the concentration and dispersion of the included publications across journals. Bradford's law categorizes journals into a high-output core area, moderately relevant area, and peripheral area solely according to the number of included publications [23, 24]. Based on the publication volume, there are 9 journals located in the core area, namely Frontiers in Immunology, Frontiers in Oncology, Nature Communications, Cancers, Briefings in Bioinformatics, Genome Medicine, International Journal of Molecular Sciences, Analytical Chemistry, and Cancer Cell International. Table 2 presents the detailed characteristics of journals in the Bradford high-output core area, with additional metrics of academic influence added to distinguish publication volume from scientific impact. Most of these 9 journals are ranked in Q1 of the 2024 JCR category quartile, with only one journal ranked in Q2. Two journals in the core area have 2024 impact factors greater than 10: Nature Communications (2024 IF = 15.7) and Genome Medicine (2024 IF = 11.2). Notably, the Bradford core area ranking is based on publication volume, which does not align with the academic influence of journals. Nature Communications (11 included publications) had a total of 418 citations from the included papers, with an average of 38.0 citations per paper, which was significantly higher than Frontiers in Immunology (15 included publications, 126 total citations, 8.4 average citations per paper) and Frontiers in Oncology (11 included publications, 106 total citations, 9.6 average citations per paper). This indicates that high-impact journals such as Nature Communications, and Genome Medicine are the main platforms for landmark high-quality research in this field.

A total of 1721 researchers participated in the research on scRNA-seq and TME. According to the analysis results of VOSviewer, the top three authors who published the most articles were Xianwen Ren, Zemin Zhang, and Lei Zhang, all of whom published three articles. Authors who published only 1 article accounted for 93.0% of all contributing authors, indicating that publication output in this field is still concentrated among a small number of core researchers, and the field requires further exploration by more investigators. Notably, Shyam Prabhakar from Singapore, despite only 2 included publications, received a total of 1025 citations, with both articles published in Nature Genetics (2024 IF = 29.0), representing landmark foundational studies in the field. The former article made a single-cell transcriptome analysis of tumor cells and stromal cells related to tumor heterogeneity, identified the possible tumor mechanisms and identified the genes that were out of tune [22]. The latter article explored the interaction between tumor cells and TME by scRNA-seq technology. Tumor cells are at the center of a network of tumorigenic cell interactions, and genetic changes in tumor cells may also trigger the formation of a unique TME [25]. The author collaborative network constructed by VOSviewer (Fig. 4) shows that among the 1721 contributing authors, stable, large-scale collaborative networks are still relatively rare, with most collaborations limited to small, internal research teams.

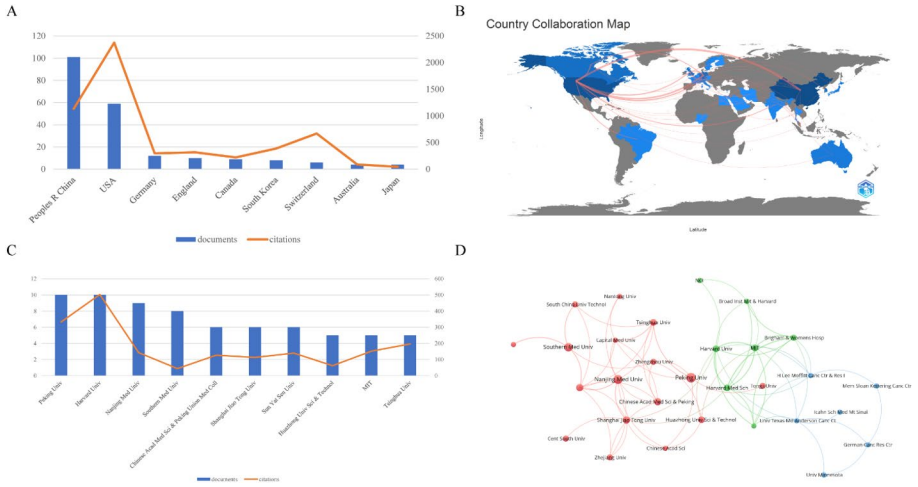

Fig. 3 Country and institutional analysis of scRNA-seq in TME research. **A** Top 10 contributing countries by publication volume and total citations. **B** Global distribution and collaboration network of countries/regions. **C** Top 10 contributing institutions by publication volume and total citations. **D** Collaborative network of research institutions

Table 2 Journals based on the publication volume of Bradford's law

Rank	Journal	Documents	Citations	JCR category quartile (2024)	Impact factor (2024)
1	Frontiers in Immunology	15 (8.0%)	126	Q1	5.9
2	Frontiers in Oncology	11 (5.9%)	106	Q2	3.3
3	Nature Communications	11 (5.9%)	418	Q1	15.7
4	Cancers	8 (4.3%)	65	Q2	4.4
5	Briefings in Bioinformatics	4 (2.1%)	80	Q1	7.7
6	Genome Medicine	4 (2.1%)	5	Q1	11.2
7	International Journal of Molecular Sciences	4 (2.1%)	70	Q1	4.9
8	Analytical Chemistry	3 (1.6%)	35	Q1	6.7
9	Cancer Cell International	3 (1.6%)	5	Q1	6.0

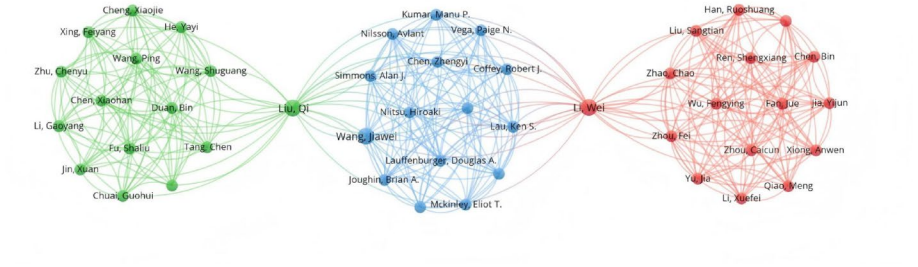

Fig. 4 The cooperation network of authors of scRNA-seq in TME research

3.4 Analysis of research hotspots and frontiers

Keyword analysis serves as an effective bibliometric approach to identify core themes and knowledge structures within a scientific field. The node size represents the frequency of keyword occurrence, and the color represents the time of occurrence in CiteSpace's keyword network analysis (Fig. 5A). The most frequent keywords related to scRNA-seq and TME include tumor microenvironment, gene expression, expression,

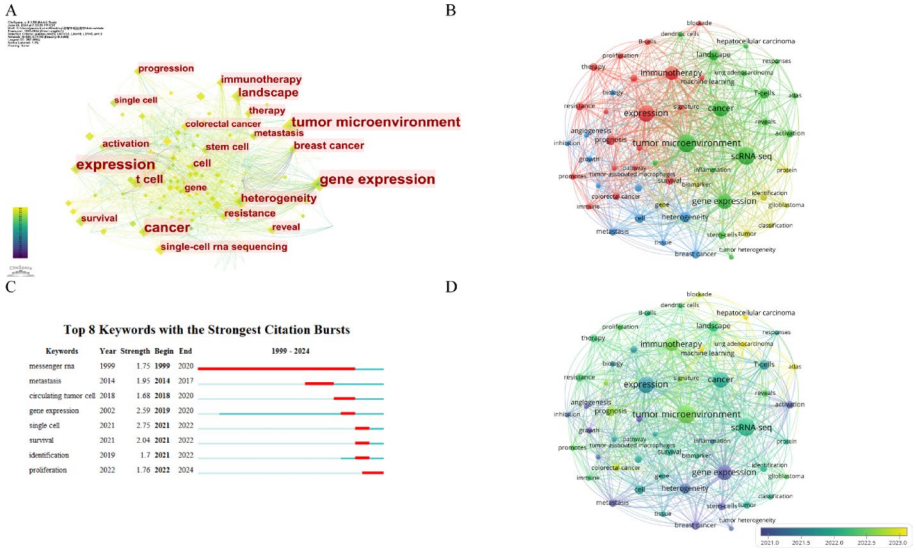

Fig. 5 Keyword and research hotspot analysis. **A** Keyword frequency related to scRNA-seq and TME. The size of the node represents the frequency of keyword occurrence, and the color represents the year of occurrence. **B** Cooccurrence map of keywords. Nodes with different colors represent clusters with different connections. There are four clusters focusing on different research topics in the figure. **C** Top 8 keywords with the strongest citation bursts. The red part of the blue timeline indicates the duration of the burst keyword. **D** Cooccurrence map of keywords indicates the period of the keywords

landscape, cancer, immunotherapy and single-cell RNA sequencing. These keywords show the core topics and research contents in this field. To further investigate relationships among keywords, we constructed a keyword cluster analysis map using VOSviewer (Fig. 5B). The five most frequent keywords were tumor microenvironment (59 occurrences), scRNA-seq (58), expression (50), cancer (45), and gene expression (43). A total of 54 keywords occurring more than five times were selected to generate the keyword co-occurrence network. Nodes with the same color reflect research topics with strong correlation. Our analysis mainly includes four research clusters, including green, red, yellow and blue. The keywords of the green cluster are tumor microenvironment, scRNA-seq, cancer, gene expression and T-cells, which indicate that the related research of the cluster focuses on the analysis and identification of gene expression of related cells in TME by using scRNA-seq technology to guide the prognosis characteristics and drug resistance of patients with tumor. The main keywords included in the red cluster are expression, immunotherapy, prognosis, resistance and survival. We believe that the research focus of the red cluster is mainly to explore prognostic indicators based on TME and the application of scRNA-seq in TME immune infiltration through the analysis of scRNA-seq data. Biomarker, protein, gene, and identification in yellow cluster are mainly used to analyze TME-related characteristics and identify genetic markers by scRNA-seq. The topics presented by heterogeneity, cell, metastasis, and progression in the blue clusters focus on the application of scRNA-seq in the TME and studies related to tumor heterogeneity.

The analysis of keywords in the time dimension can reflect the research focus of the research field in different time periods and the possible research direction in the future. The burst detection of keywords is seen as an indicator of the advance of research frontiers or emerging topics in a particular field over time to predict research trends. CiteSpace is used to detect burst keywords. The blue line represents the timeline, and

the red part of the blue timeline represents the duration of the burst keywords (Fig. 5C). The keyword with the longest duration of outbreak is messenger RNA, lasting from 1999 to 2020, and the keywords include single cell, survival, identification, proliferation, and emergence in the latest years. Of these, proliferation has continued to this day. In this VOSviewer map, color represents the time when keywords are concentrated, and the deeper the color of lines and nodes, the farther away from the present time. Inversely, the lighter the color of lines and nodes, the closer it is to the present time, and the more novel the keyword theme displayed. Figure 5D shows the keyword analysis of the time dimension displayed in VOSviewer. The keywords that appeared earlier include gene expression, heterogeneity, metabolism, growth, inhibition, activity, angiogenesis, and differentiation, which may be related to the application of early scRNA-seq in exploring TME in tumor growth, metastasis, and differentiation and related mechanism research. With the deepening of research on TME and the advancement of tumor immunotherapy technology, keywords that appear recently are mainly machine learning, hepatocellular carcinoma and lung adenocarcinoma.

4 Discussion

Our systematic bibliometric analysis of 187 studies focused on scRNA-seq in TME research reveals that, following an initial period of slow development, annual publication output has grown substantially since 2020, driven by the expanded application of scRNA-seq technologies and increasing research focus on the TME. China and the United States dominate research output and serve as core collaborators internationally. Among the top ten most productive institutions, eight are from China and the other two are from the United States. The output of papers in this field is still concentrated among authors who publish fewer articles. While the field continues to grow and deepen, close international collaboration among authors, countries, and institutions remains limited, representing a key gap to be addressed in future research. Transitioning from this macro-bibliometric landscape to specific research themes, it is evident that the application of scRNA-seq in TME research advancing our understanding of tumor initiation, progression, and metastasis, while facilitating clinical translation in biomarker discovery, prognostic stratification, and therapeutic response prediction.

At the mechanistic level, scRNA-seq has provided unprecedented resolution into the cellular heterogeneity of specific tumors. The keywords in the time dimension reveals the progress of scRNA-seq in exploring specific tumors. Lung adenocarcinoma is the most common type of lung cancer. The scRNA-seq has been used to explore mast cells, sphingolipid metabolism, and inflammatory responses in the TME, revealing its value in identifying biomarkers and clarifying TME complexity in lung adenocarcinoma [26–29]. Notably, recent integrative analyses have elevated the utility of scRNA-seq from descriptive profiling to predictive modeling. For instance, a mitochondrial pathway signature (MitoPS) derived from scRNA-seq and bulk transcriptomics has demonstrated superior performance in predicting immunotherapy response in lung adenocarcinoma. This approach identified NDUFB10 as a pivotal immune regulator, where its deficiency correlates with increased CD8 + T cell infiltration and improved efficacy of immune checkpoint inhibitors, offering a precise metabolic-based strategy for patient stratification [30]. In hepatocellular carcinoma, scRNA-seq has elucidated the roles of T cells, hepatocytes, epithelial cells, and monocytes in patient survival and immunotherapy response

[31–34]. This insight is further supported by landmark pan-cancer studies, including the work by Barkley et al., which systematically defined recurrent malignant cell states across tumor types and revealed how these conserved states shape TME composition, immune evasion, and treatment response [35]. In melanoma specifically, scRNA-seq has identified distinct microphthalmia-associated transcription factor (MITF)-high and AXL receptor tyrosine kinase (AXL)-high cell populations, the latter of which is directly linked to drug resistance and shaped by the surrounding TME [36]. Furthermore, the persistence of the keyword "proliferation" in burst detection analysis underscores the relevance of scRNA-seq in studying tumor cell replication and the influence of non-tumor cells during carcinogenesis [37, 38].

The power of scRNA-seq is most prominently displayed in deconstructing the multicomponent nature of the TME. TME is composed not only of tumor cells but also immune cells and stromal cells, which have a significant impact on tumorigenesis, pathogenesis, progression, and metastasis. Tumor-infiltrating immune cells are involved in complex interactions during tumor development. Immune cells not only have anti-tumor effects but also play a role in promoting tumor through different phenotypes or secreting cytokines. TAMs and tumor-associated neutrophils (TANs) are key components of the TME and affect tumor progression by directly regulating tumor cell proliferation or interfering with the pro-tumor or anti-tumor immune responses of other immune cells [38, 39]. Macrophages include M1-type macrophages and M2-type macrophages. A large number of activated macrophages infiltrate tumor stroma and are called TAMs. M1 macrophages have anti-tumor effects, while M2 macrophages promote the proliferation and invasion of tumor cells. TAMs can impair the tumor-killing capacity of T cells and NK cells, and also foster tumorigenic inflammation by generating inflammatory T helper subsets such as T follicular helper cells [40]. Similarly, TANs can be categorized into anti-tumor neutrophils (N1) and pro-tumor neutrophils (N2) subsets [41], functioning through chemotaxis, phagocytosis, degranulation, and neutrophil extracellular trap formation [42, 43]. Neutrophils promote tumor progression through multiple mechanisms: (a) inducing VEGF overexpression to stimulate angiogenesis [44]; (b) interacting with immune cells to suppress anti-tumor responses, such as by inducing T cell apoptosis [45]; and (c) releasing enzymes that stabilize integrins and facilitate cancer migration and invasion [46]. Additionally, neutrophils recruit other immune cells like Tregs via chemokine secretion, which may either inhibit or promote tumor growth depending on TME polarization [47]. A major future challenge lies in developing therapies that selectively inhibit the pro-tumor functions of immune cells while preserving their anti-tumor capacity, which highlights the complexity and importance of scRNA-seq for the study of cell subsets.

Beyond the immune landscape, stromal components represent another critical frontier elucidated by single-cell technologies. Through scRNA-seq of stromal cells such as fibroblasts, endothelial cells, smooth muscle cells and epithelial cells in the TME, the researchers further elucidate the TME to provide substantial help for tumor clinical treatment. Notably, TAFs modulate anti-tumor immunity through the secretion of cytokines, growth factors, chemokines, and exosomes. Their immunosuppressive mechanisms include: (a) inducing abnormal polarization or trans-differentiation of immune cells into pro-tumorigenic subtypes; (b) enhancing the recruitment, infiltration, and activation of immunosuppressive cells; and (c) impairing the cytotoxic activity and cytokine

production of natural killer cells and cytotoxic T lymphocytes [48–50]. Additionally, TAFs remodel the ECM via production of fibronectin, collagen, and other components, further shaping the TME [51–53]. Emerging therapeutic approaches targeting stromal cells involve depleting specific stromal subsets through surface markers or blocking their activation and effector functions [54]. Fibrotic progression can also be blocked by limiting the remodeling of ECM, including therapies that target fibrotic activation signaling pathways and their fibrotic products [55]. Therapeutics that neutralize stromal-derived immunosuppressive signals may help restrain tumor growth and improve survival. Thus, the application of scRNA-seq in TME stromal cells represents a promising direction for developing novel tumor therapies.

While scRNA-seq excels at defining cellular identity, its inherent limitation has necessitated the integration of multi-dimensional technologies. The scRNA-seq improves our understanding of cell subsets, but the spatial information of cells in the original tissue environment is destroyed during the process of single-cell extraction of the dissociated tissue [56, 57]. However, spatial information in the primordial tissue environment is critical to understanding cell-cell interactions [58]. The combination of scRNA-seq and spatial transcriptome can improve the understanding of TME. ScRNA-seq is used to detect the types and subtypes of cells in TME. Through the knowledge of ligand-receptor complex, scRNA-seq can also understand the interaction and communication between cells, so as to master the development, differentiation and inflammation process of cells [59, 60]. To accurately model these interactions, advanced tools like CellChat provide a framework that accounts for the multi-subunit structure of receptors and the influence of various cofactors [61]. Furthermore, the expansion of databases like CellPhoneDB v5 now allows for the inclusion of non-peptidic signals, such as hormones and neurotransmitters, offering a more comprehensive map of the signaling landscape within the TME [59]. Spatial transcriptome can detect cell composition and original gene expression in different regions, directly obtain differential gene expression information in different functional regions, and conduct a more comprehensive synthesis of gene expression, spatial location and histological information. The integration of scRNA-seq and spatial transcriptome data will improve the understanding of cell type identification and corresponding functional properties of the TME, and gradually clarify the physical location of each component [62]. For example, tissue samples from patients with esophageal squamous cell carcinoma were used to identify different cell subpopulations using scRNA-seq. The spatial transcriptomics method based on microarray was used to characterize the spatial landscape of the expression data by an array of spots [63]. Data from scRNA-seq combined with spatial transcriptomics defined cutaneous squamous cell carcinoma tumor and stromal cell subpopulations, the spatial niches in which they interact, and their communication gene networks involved in cancer [64]. The combination of scRNA-seq and spatial transcriptomes applied to the exploration of the TME is a novel approach to dynamically and multidimensional understanding of the molecular mechanisms of disease and the development of new classes of spatial biomarkers. The portfolio provides a powerful resource for diagnosing disease, monitoring disease progression and treatment effects, and discovering new classes of space-targeted drugs [65].

To navigate the massive datasets generated by such multimodal approaches, the field is increasingly turning towards computational intelligence. With the rapid development of artificial intelligence, machine learning in the field of medicine is also providing

more information for medical practice. Machine learning is the application of multidisciplinary theory to use computers to simulate human learning behaviors and activities to acquire knowledge and skills, constantly update and rebuild the knowledge framework, and improve system performance [66]. Intercellular communication is mainly mediated by physical intercellular contact, shared ECM and secreted diffusion signaling molecules. Inference of intercellular communication helps to understand the molecular mechanism of tumor progression and metastasis, and provides guidance for anticancer drug design and tumor targeted therapy. Recent advances in scRNA-seq have enabled the analysis of intercellular signals from gene expression in single-cell datasets [67–69]. Sun et al. [70] combined machine learning algorithms with scRNA-seq datasets to validate the role of key genes in hepatocellular carcinoma progression. Liu et al. [71] evaluated the TME characteristics of hepatocellular carcinoma through a combination of single-cell level and machine learning to provide prognostic prediction and rational treatment for patients. Liao et al. [72] used machine learning to analyze immune cells in the TME by integrating scRNA-seq data for potential drug targets. Based on scRNA-seq data, researchers can also apply alternative machine learning methods to build a tumor risk assessment system that comprehensively evaluates the tumor immune microenvironment, tumor mutation load, and tumor immune checkpoints, thereby providing new and reliable biomarkers for tumor treatment and prognosis [73]. Furthermore, the integration of scRNA-seq with advanced computational frameworks has evolved from static profiling to a more dynamic and integrated paradigm. For instance, by employing an optimized dynamic network biomarker (DNB) method, researchers have deciphered high-resolution heterogeneity in differentiated thyroid cancer, identifying Stage II as a critical transition point and pinpointing the ASPH gene as a key regulator driving malignancy [74]. This systems-biology approach demonstrates the power of network-based algorithms in identifying precision biomarkers that are often overlooked by conventional analysis. Collectively, the launch of the 10X Genomics Chromium platform, a high-throughput droplet-based scRNA-seq technology, has dramatically lowered the technical threshold and accelerated the explosive growth of scRNA-seq applications in TME research [75]. To effectively support such computationally intensive research and the integration of massive multi-omics datasets, large-scale international collaborative projects such as the Human Tumor Atlas Network (HTAN) are now leading the standardization of multi-omics single-cell profiling of the TME, providing a unified, open-access resource for integrating scRNA-seq, spatial transcriptomics, and other omics data across tumor types [76]. Besides, innovative infrastructure like cloud-based GWAS platforms has been developed [77]. These platforms enable sub-second data acquisition and seamless cross-database analysis. The synergy between high-resolution algorithmic models and scalable computational platforms is becoming essential for translating complex single-cell landscapes into actionable clinical insights in precision oncology. These greatly promote the clinical translation of single-cell TME research. We highlight the urgent need for enhanced international cooperation, standardized analytical frameworks, and integrated multi-omics approaches to fully realize the translational potential of scRNA-seq in precision oncology. This bibliometric review provides a timely and systematic roadmap for researchers, funding agencies, and policymakers seeking to navigate and accelerate progress in this rapidly evolving domain.

However, this bibliometric analysis has several limitations. First, studies were only retrieved from the WOS Core Collection, which may lead to incomplete coverage of relevant studies published in other databases or non-English sources. In addition, due to the lag in database indexing, some recently published high-quality studies may not have been included. Finally, by focusing on general trends in scRNA-seq applications in the TME, this review does not fully capture heterogeneity among distinct cancer types or specific experimental designs. Despite these limitations, this study provides a reliable and systematic overview of the field, and the findings remain informative for future research directions.

5 Conclusions

This study provides a comprehensive, systematic bibliometric overview of the global research landscape at the intersection of scRNA-seq and TME research. Current research efforts primarily utilize scRNA-seq to characterize cellular heterogeneity and cell-cell interaction networks within the TME, offering critical insights into tumor biology and treatment response mechanisms. While the field has experienced rapid and sustained growth over the past 5 years, enhanced international collaboration between research teams, institutions, and countries remains an urgent unmet need. Future research will likely focus on defining the functional roles of immune and stromal cell subsets within the TME by scRNA-seq, as well as the integration of scRNA-seq with spatial transcriptomics and advanced machine learning frameworks. These directions represent a promising avenue for developing novel precision tumor therapies and advancing the clinical translation of single-cell oncology research.

Author contributions

Jing Ma and Tingting Chen contributed to the conception, data interpretation, and drafted the manuscript. Kefei Shen, Mengru Fang, and Lu Yang contributed to performing the literature retrieval, data interpretation, and Figure. Yanyan Zhang, and Longfei Guo revised the manuscript and coordinated the whole work. All authors contributed to the manuscript and approved the submitted version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All data used in this study were downloaded from public databases. Therefore, no ethical approval was required.

Consent for publication

Not applicable.

Institutional review board statement

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Competing interests

The authors declare no competing interests.

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