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Review Article

Single-cell RNA sequencing in ovarian cancer: revealing new perspectives in the tumor microenvironment

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Abstract: Single-cell sequencing technology has emerged as a pivotal tool for unraveling the complexities of the ovarian tumor microenvironment (TME), which is characterized by its cellular heterogeneity and intricate cell-to-cell interactions. Ovarian cancer (OC), known for its high lethality among gynecologic malignancies, presents significant challenges in treatment and diagnosis, partly due to the complexity of its TME. The application of single-cell sequencing in ovarian cancer research has enabled the detailed characterization of gene expression profiles at the single-cell level, shedding light on the diverse cell populations within the TME, including cancer cells, stromal cells, and immune cells. This high-resolution mapping has been instrumental in understanding the roles of these cells in tumor progression, invasion, metastasis, and drug resistance. By providing insight into the signaling pathways and cell-to-cell communication mechanisms, single-cell sequencing facilitates the identification of novel therapeutic targets and the development of personalized medicine approaches. This review summarizes the advancement and application of single-cell sequencing in studying the stromal components and the broader TME in OC, highlighting its implications for improving diagnosis, treatment strategies, and understanding of the disease's underlying biology.

Keywords: Ovarian cancer, single-cell sequencing, tumor microenvironment, complementary treatment

Introduction

Ovarian cancer (OC) is an example of a tumor that may be amenable to personalized medicine [1]. The overall survival rate of OC patients has gradually increased from 37% to 50% due to advancements in diagnosis and treatment technology [2]. The pathogenesis of ovarian cancer is complex and involves the interaction of genetic, environmental, and lifestyle factors [3-5]. As tumor research progresses, it has become apparent that unique phenotypic characteristics of tumor cells, as well as the tumor microenvironment (TME) in which they reside, may serve as targets for developing more effective treatment strategies for individual patients [6, 7].

The TME is a highly variable and heterogeneous environment that surrounds tumor cells and contains numerous cellular and non-cellular components [8]. Currently, the components of the TME are generally divided into immune infiltrating cells (IIC), stromal components, endothelial cells (EC), and non-cellular components which mainly including extracellular matrix and cell signaling molecules [9]. The presence of these intricate elements collectively impacts the development, invasion, dissemination, and other malignant processes of tumors by facilitating tumor growth, aiding tumor immune evasion, promoting angiogenesis, and enhancing tumor drug resistance, and it influences tumor response to various treatments (such as chemotherapy, radiotherapy, and immunotherapy) [9-11]. Given this complexity, research has shifted its focus from solely on tumor cells and tissues, towards studying tumor cells and the TME as a functional unit. It is worth noting that many immune cells are also present in the stromal component of the TME [12]. Strictly speaking, the concept of IIC examines how immune cells enter and play a role in tumor tissue, while that of immune cells in the cell matrix emphasizes the presence and distribution of immune

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cells in the tumor cell matrix. However, in specific tumor research, the boundary between the two is not always clear, and researchers are more concerned about the interaction between these immune cells after entering the TME. Therefore, in this article, we treat immune cells in the TME uniformly as part of the stromal component. Immune cells and non-immune cells in stromal components (mainly fibroblasts, endothelial cells, and mesenchymal cells) are an important research sub-direction and the main target of tumor immunotherapy. Previous studies have shown that the phenotype and function of matrix components in the TME are jointly affected by multiple factors [13-15]. High levels of diversity and heterogeneity still pose challenges to traditional research methods. Therefore, new technologies are urgently needed.

The development of genomics technology has led to the emergence of various technologies such as batch RNA sequencing, single-cell DNA sequencing, single-cell RNA sequencing, single-cell proteome, and metabolomic sequencing. These technologies offer a great opportunity to study complex diseases, including tumors, from a more refined perspective [16, 17]. This, in turn, presents more possibilities for developing effective tumor treatment strategies [18, 19]. This article reviews the current application of single-cell technology in the research of stromal features of the TME in OC. It also summarizes the latest applications of this emerging technology in research regarding the stromal features in OC.

Comparison of several common sequencing methods

The TME is a highly dynamic and heterogeneous entity, where each species or even individual cell possesses distinct genomic, metabolomic, and proteomic profiles [20-22]. Such heterogeneity, a noted characteristic of OC, is further complicated by varying gene expression outcomes among cells with similar expression patterns, influenced by internal drives and cellular regulations.

Historically, bulk RNA sequencing was a dominant method for investigating the molecular intricacies of the TME [23]. This approach averages RNA expression across thousands of cells within a tumor, allowing comparisons with known tumor cell types and estimation of cell infiltration types and inter-cellular interactions within the TME. Tools like CIBERSORT [24], TIMER [25], and xCell [26] all emerged from this methodology. However, these algorithms provide only approximations, reliant on the quality and depth of gene expression data and the accuracy of reference cell types and expression profiles. Such bulk analysis also obscures the unique biologic characteristics and interaction modes of specific cell types and individual cells, masking the cellular-level heterogeneity [27, 28].

Single-cell sequencing technology was developed to address these limitations. Single-cell DNA sequencing represents another technique capable of providing genomic analysis at the individual cell level. This method discerns whole-genome variations in tumors at the single-cell level, aiding in understanding tumor clonal evolution [29]. However, it falls short in detecting expression differences among suppressor cells in the TME and is more complex and costly than single-cell RNA sequencing (scRNA-Seq), hindering widespread application. In contrast, scRNA-Seq effectively captures single nucleotide-level cellular heterogeneity and functional state changes at the single-cell level [16]. This technology enables the construction of high-resolution cellular expression spectra, uncovering new cell subtypes and functional states. This is crucial for elucidating complex cell interactions within the TME and identifying novel therapeutic targets for tumor treatment [30, 31].

scRNA-Seq technology overview

Overall, ScRNA-Seq, a significant advancement in molecular biology, offers detailed insight into transcriptional patterns at the single-cell level. This discussion primarily focuses on transcriptomics and spatial transcriptomics technologies. Single-cell research can be traced back to the early 20th century, but it was the introduction of polymerase chain reaction (PCR), single-cell DNA cloning, and microarray technologies in the 1980s that first enabled the analysis of gene expression patterns in individual cells [32, 33]. The advent of scRNA-Seq in the 21st century marked a new era in single-cell research, providing an unparalleled resolution for investigating intracellular gene expression. This

technology has revolutionized our understanding of cellular processes by allowing in-depth exploration of transcriptional activity within individual cells [34, 35].

Over the years, through numerous technical refinements, the experimental procedure of scRNA-Seq has matured, leading to a relatively standardized processing and analysis pathway. The key steps in the current scRNA-Seq protocol typically include sample collection, cell dissociation, single-cell isolation, reverse transcription, cDNA amplification, library construction, and sequencing analysis [36]. ScRNA-Seq technologies are diverse, each with its own set of strengths and limitations, largely influenced by the development companies and underlying technology platforms. Broadly, scRNA-Seq technologies can be categorized into three main types: 1. Microfluidic-based technologies, which primarily include the chip-based Fluidigm C1 and droplet microfluidic-based methods like Drop-seq and inDrops [37]. 2. Microwell-based technologies, such as the 10x Genomics Chromium system [38]. 3. Cell sorting-based technologies that combine fluorescence-activated cell sorting (FACS) with single-cell RNA sequencing [39].

Of these, the Drop-seq-based platform from 10X Genomics and the CytoSeq-based platform from BD Rhapsody have emerged as the most prominent and widely used commercial platforms. These advancements have significantly enhanced the precision and applicability of scRNA-Seq, making it a vital tool in contemporary molecular biology research.

A study in 2017 by Dagogo-Jack et al. highlights a crucial aspect of tumor progression: both the temporal dynamics and the spatial heterogeneity of cell distribution influence tumor outcomes [40]. While scRNA-Seq offers high-resolution genetic data at the individual cell level, its methodology often involves isolating cells from their native tissue environment, thereby losing critical spatial information. Consequently, cell communication analysis in scRNA-Seq largely relies on the expression of signaling molecules (like growth factors, cytokines, chemokines) and corresponding receptors across different cell types to infer and construct cell communication networks [41]. However, this approach is somewhat limited by data quality and sequencing depth, leading to analyses based on correlation models rather than on direct causal evidence. Furthermore, when multiple signals and receptors are involved in target cell populations, the existing Cell-Talk analysis methods may not adequately unravel these complex interactions [42].

Spatial transcriptome sequencing technology addresses this limitation by integrating traditional transcriptomics with spatial positioning information. This technique allows researchers to obtain gene expression data and their specific spatial distribution in tissues, organs, or cells [43]. It typically involves fixing tissue sections onto specialized microarrays equipped with molecular probes, which capture mRNA from adjacent cells. These probes contain unique spatial barcodes that record the original location of each mRNA molecule. Sequencing and analyzing these spatially barcoded mRNAs enable the creation of detailed expression profiles, illuminating gene activity in various tissue locations [44, 45]. This approach partially restores the functional dynamics and real interactions of different cells within tumors, offering a more comprehensive understanding of cell-to-cell communication. Increasingly, studies are combining scRNA-Seq and spatial transcriptome sequencing to unravel the complex changes in the TME during tumor progression, providing a more holistic view of tumor biology [46-48]. The characteristics of common processes of bulk RNA sequencing, scRNA-Seq, and spatial transcriptome sequencing technology, are summarized in **Figure 1**.

Future work will delve deeper into OC research using these sophisticated sequencing technologies, offering insight, predictions, and guidance on emerging trends and developments in this field. This comprehensive approach promises to revolutionize our understanding and management of ovarian cancer, leading to more effective treatment and improved patient outcomes.

Special cells in the TME of OC under scRNA-Seq

IICs are crucial players in TME of OC. On the one hand, they are instrumental in tumor immune surveillance and response, exemplified by the cytolytic activities of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) against tumor cells [49, 50]. On the other hand,

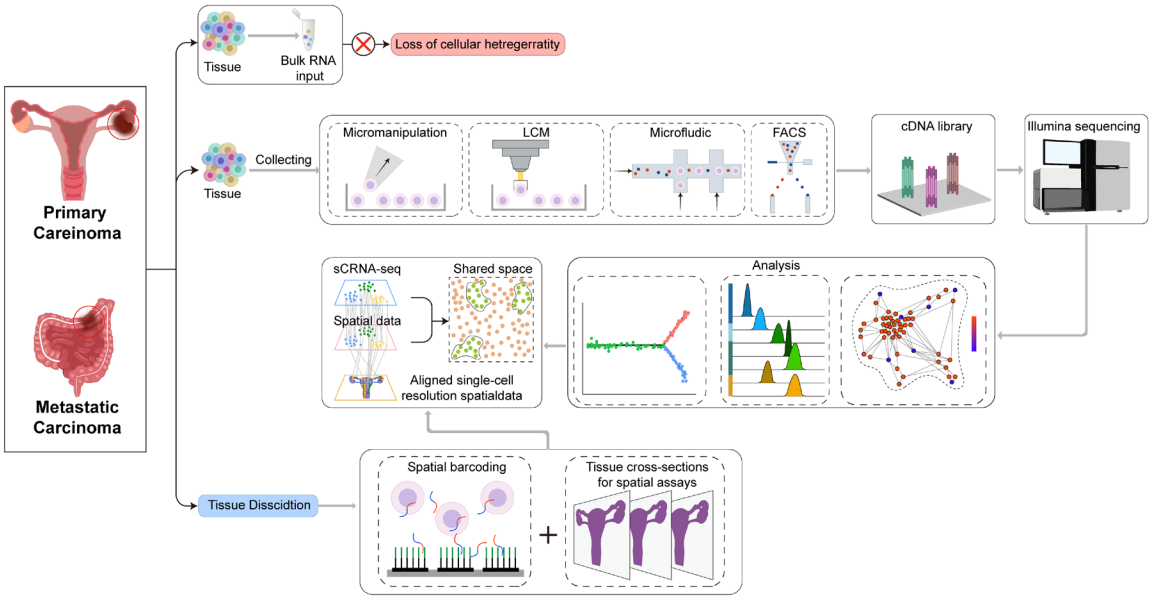

Figure 1. General process and characteristics of Bulk RNA sequencing, scRNA-Seq, and spatial transcriptome sequencing.

IICs contribute to malignancy through tumor growth, spread, and invasion. Notably, tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs), which have garnered increasing attention, release various pro-inflammatory and pro-angiogenic cytokines includingTNF, ILs, and VEGF, thereby promoting tumor progression [51, 52].

Additionally, emerging studies highlight the significance of IICs in the efficacy of tumor immunotherapy, marking them as key targets [53, 54]. Classic immune checkpoint inhibitors and the more recent CAR-T cell therapies, which enhance cancer cell targeting and destruction, exemplify this [55]. IICs in the TME also demonstrate considerable heterogeneity and differentiation, driven by intercellular communication with tumor and other cells in the TME, accounting for their functional diversity.

In a 2017 study on OC TME, Cai et al. identified key IIC types including TAMs, T cell subsets, and tumor cell-associated dendritic cells (tDCs) [56]. This review will delve into the single-cell characteristics, phenotypic heterogeneity, and transcriptomic alterations among these immune cells. It is also important to note that, in addition to IIC, tumor-associated fibroblasts (CAF), as part of the TME matrix, also play an important role in the immune regulation of the TME, and current single-cell sequencing research also studies this type of cell intensively [57-59]. In this review, we also introduce and summarize this type of cells. Understanding these aspects is critical for optimizing immunotherapy strategies and identifying novel targets for tumor treatment. A comprehensive display of infiltrating cell types in the TME of OC, discussed below is shown in **Figure 2**.

TIMs and TAMs

In TME, tumor-infiltrating macrophages (TIMs) and tumor-associated macrophages (TAMs) are two pivotal types of immune cells. Numerous studies have highlighted their significant roles in tumor growth, invasion, and response to treatment [60, 61]. It is important to note that while there is an overlap in the types and functions of these cells, distinct differences also exist. Both TIMs and TAMs originate from monocytes. However, TIMs generally refer to macrophages that migrate from peripheral blood and infiltrate tumor tissue, with their functions often influenced by cytokines, metabolic status, and other factors within the TME [62]. This leads to varying polarization states that can either promote or inhibit tumor progression. TAMs, a primary immune cell type in the TME, tend to exhibit a more M2 polarization phenotype [63]. They interact with tumor cells and other cells in

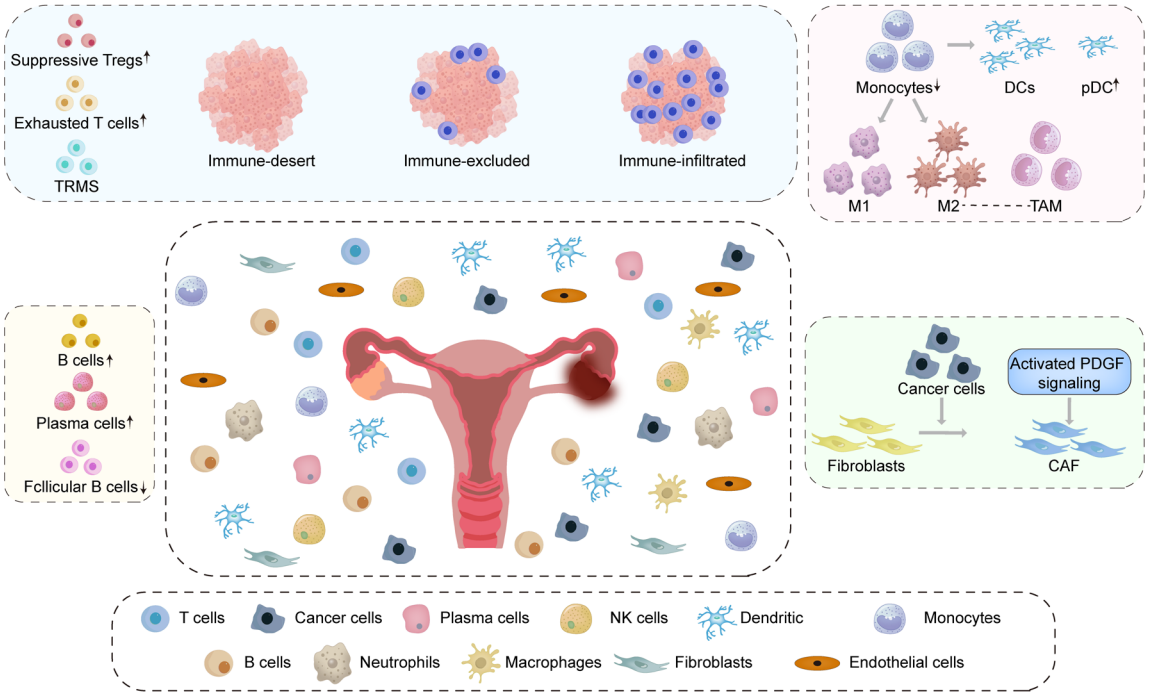

Figure 2. Some key cell subtypes in the TME of OC.

the microenvironment, secreting an array of cytokines, enzymes, and growth factors that collectively support tumor development [64]. Understanding the characteristics and roles of these cells in tumor progression is essential for research and therapeutic strategies, especially for those aimed at modulating immune responses within the tumor microenvironment.

Recent research on macrophage phenotypic diversity primarily categorizes them into two polarized groups: M1 and M2. M1 macrophages, known as classically activated macrophages, are considered protective factors against OC, whereas M2 macrophages, also referred to as alternatively activated macrophages, are seen as risk factors for OC [65, 66]. Wang et al. developed a scoring mechanism based on the M1/M2 ratio using scRNA data from OC tumor nests [67]. This mechanism helps identify immune subtypes in OC patients and has prognostic value. Further advancing this research, Chang Liu et al. found that most macrophages in OC tumor nests are M2-type TAMs [68]. They identified SLAMF7 and GNAS as key genes in these cells that contribute to OC's resistance to cisplatin, providing a deeper understanding of Wang's findings. Additionally, Jinye Ding's research indicated that, apart from phenotypic heterogeneity, M2 macrophages exhibit higher autophagic states [69]. These highly autophagic M2 macrophages show a positive correlation with cisplatin resistance in OC.

When discussing OC, of note, peritoneal metastasis to the omentum is a common clinical manifestation, particularly in advanced stages of OC [70]. The omentum, characterized by its milky spots, serves as a congregation of immune cells, functioning analogously to lymph nodes. Research has shown that OC cells preferentially colonize these spots, creating a distinct TME compared to that of the ovary [71, 72]. Susan Olalekan et al. utilized Drop-seq technology to analyze peritoneally metastasized OC, identifying T cells, B cells, and macrophages as the predominant immune cell types [73]. Their study revealed variations in macrophage phenotypes within different TMEs, particularly in their anti-tumor capabilities. Notably, macrophages in TMEs with robust immune responses were predominantly M1-type, exhibiting anti-tumor activity. However, these cells also express CD274, deviating from the traditional M1 macrophage profile [74]. Furthermore, gene expression patterns akin to myeloid-derived suppressor cells (MDSCs) were

observed in these immune-reactive clusters, indicating a potential transitional state of these cells.

These findings underscore the significant role of macrophages in the progression of ovarian cancer and the intricacies involved in M1 and M2 macrophage functions within tumor biology. They also challenge the conventional binary classification of macrophages into M1 and M2 types as overly reductive. The discovery of intermediate states between M1 and M2 macrophages demands a more detailed and comprehensive approach to understand the diversity and complexity of macrophages in the TME.

Building upon these insights, Junfen Xu et al. provided a more detailed characterization of macrophages in ovarian cancer using scRNA-Seq technology [75]. Analyzing high-grade serous ovarian cancer (HGSOC) samples, they meticulously categorized OC macrophages into 10 distinct subpopulations. Their findings revealed a dynamic progression in macrophage behavior: initially, macrophages display anti-tumor activity, but as the tumor advances, their ability to recruit other immune effector cells diminishes. Concurrently, there is upregulation in the expression of growth factor genes that facilitate tumor growth. Importantly, the study highlighted that the proportions of these various macrophage clusters significantly influence patient prognosis, underscoring the complex role of macrophages in the tumor microenvironment and their impact on the course of ovarian cancer.

In addition to phenotypic heterogeneity, another important property of macrophages is their plasticity. Previous studies have shown that Chemokine (C-C motif) Ligand 2 (CCL2) [76], Colony Stimulating Factor 1 (CSF-1) [77], Chemokine (C-C motif) Ligand 18 (CCL18) [78], Tumor Necrosis Factor alpha (TNF-α) [79] and even some non-coding RNAs [80] may induce macrophage phenotypic shifts, in addition to the overall metabolic state of the space in which the macrophage resides, which also influences its phenotype [81, 82]. More specific macrophage morphologies are now being identified by scRNA technology. For example, a study by Chen Zhang et al. pointed out that C5aR1 can be specifically expressed on macrophages in OC and inhibit the killing function of T cells, promoting processes such as tumor angiogenesis [83]. A study by Anjali Geethadevi et al. pointed out an even more unique phenomenon of macrophage phenotypic remodeling, whereby macrophages release oncostatin M (OSM) under the influence of IL-6, the binding of which to OSMR leads to OSMR-IL6ST dimerization, a dimer that further initiates STAT3 signaling in tumor cells and promotes OC progression and invasive processes [84]. Several previous studies have also pointed out the critical role of IL-6 for the phenotype formation of TAMs [85, 86], and scRNA technology has enriched this pathway.

T cells

The role of T-cell populations within TME is critical yet intricate [87, 88]. Although previous research has established that not all T cells d exhibit active anti-tumor roles, T-cell-centered approaches remain central to immunotherapy in oncology [89-91]. In OC, T cells within the TME are broadly categorized into tumor-infiltrating lymphocytes (TILs), predominantly comprising CD8+ T cells and CD4+ helper T cells, and regulatory T cells (Tregs). Earlier studies indicate that a high infiltration of TILs combined with a lower proportion of Tregs generally correlates with a favorable prognosis in OC [92]. However, this classification appears inadequate to explain resistance to immunotherapy [93].

One hypothesis suggests that TILs may be hindered by inhibitory macrophages, which prevent their effective tumor cell killing [94]. Moreover, a significant proportion of CD8+ TILs in HGSC are unresponsive to autologous tumor cells or known tumor antigens, suggesting that not all TILs are actively anti-tumoral [95]. Identifying the specific subpopulations of TILs that are functionally capable of targeting and destroying OC cells is crucial for advancing T cell-based immunotherapeutic strategies. This nuanced understanding of T-cell subtypes and their functional roles is essential for the development of more effective, targeted immunotherapies in ovarian cancer.

The research conducted by Céline Laumont et al. made significant strides in the understanding of TILs in HGSOC by identifying three specific surface markers: CD39, CD103, and PD-1. By examining the expression patterns of these markers, they were able to categorize TILs into

more distinct subgroups. Notably, they discovered that CD8+ T cells expressing all three markers demonstrated enhanced cell-killing capabilities and more precise target specificity. Patients with a higher proportion of these particular T cells generally had a better prognosis [96].

Complementing this, the study by Susan Olalekan et al. added depth to the macrophage profile in OC, highlighting that the degree of T-cell infiltration markedly defines the immune subtype of OC. In TMEs with high T-cell infiltration, TILs exhibited a unique cellular phenotype characterized by TOX+ CD8+ and GNLY+ CD4+ T cells. Conversely, in low T-cell infiltration scenarios, GNLY expression was predominantly observed in CD8+ T cells. Additionally, the study identified the presence of plasma cells with high PRDMI and SDC1 expression, and plasmoblasts expressing IFNG in patients with significant T-cell infiltration. These nuanced distinctions open avenues for more personalized therapeutic approaches in treating ovarian cancer, tailoring treatments based on specific T-cell and macrophage profiles [73].

In a detailed study by Junfen Xu et al., a comprehensive temporal profile of CD8+ T cells in HGSOC was constructed. This study identified a total of nine distinct CD8+ T cell subtypes, each characterized by unique expression traits, with spatial specificity in their distribution [75]. Notably, tissue-resident memory CD8+ T cells (CD8+ TRM cells), marked by the CD8-C1-IFIT3 signature, were predominantly found in tumor tissues. Moreover, a substantial proportion of T cells exhibiting high expression of exhaustion markers such as CTLA4, HAVCR2, LAG3, PDCD1, SIRPA, and TIGIT (CD8+ TEX cells) were observed in the tumor milieu. Temporal analysis suggested a decline in the percentage of CD8+ TEX cells as the tumor progressed. Intriguingly, the study posited that CD8+ TEX cells in early-stage tumors represent a differentiation endpoint for both CD8+ TRM and central memory CD8+ T cells (CD8+ TCM cells). Further investigations into CD8+ TRM cells revealed their high expression of IL15, IL17, and NOTCH ligands, which contribute to local immune protection in early HGSOC tumors. The study emphasized the role of IL15 as a key driver in the induction of T cell exhaustion [97]. Of course, studies of the same type have also utilized scRNA technology to point out that CD47 has a similar effect [98]. Additionally, a critical insight from Junfen Xu's study was the interaction between macrophages and T cells in the TME. The research indicated that macrophages could recruit CD8+ TRM cells by chemokine secretion, possibly leading to HGSOC's evasion of immune clearance. This finding underscores the complexity of cellular interactions within the TME and highlights the potential for targeting these dynamics in therapeutic strategies for HGSOC. In summary, these studies targeting T cells using scRNAs in greater detail point us to the optimizable space for OC immunotherapy, which is significant for improving a highly effective technology like CAR-T.

CAFs

Cancer-associated fibroblasts (CAFs) are the most prevalent cell type within the TME matrix. Current understanding suggests that CAFs primarily originate from mesenchymal cells, though they can also transdifferentiate from other cell types including endothelial and epithelial cells [99]. Previous research has established that CAFs contribute to the suppression of anti-tumor immune responses by various mechanisms [100, 101]. These include inducing angiogenesis and metastasis, expressing specific molecules and receptors, and recruiting immunosuppressive cells into the TME, all facilitating tumor cell growth. Moreover, studies have highlighted that changes in the metabolic status of CAFs can influence the overall metabolic milieu of the TME. This alteration in metabolic conditions can "reshape" the intercellular communication within the TME, further affecting tumor progression [102]. Consequently, the presence and frequency of CAFs are increasingly recognized as biomarkers indicative of poor prognosis in OC patients [103]. Advancements in single-cell sequencing technology have revealed increasingly refined phenotypic states of CAFs. This detailed characterization is of significant importance for the development of precise treatment strategies in OC, offering insight into targeted therapies that could disrupt the supportive role of CAFs in cancer progression.

In a comprehensive study, Siel Olbrecht et al. conducted a systematic analysis of stromal cell subtypes in high-grade serous fallopian tube

and ovarian cancers [104]. They identified specific subtypes of stromal cells, including mesothelial cells (FB_CALB2), myofibroblasts (FB_MYH11), and cancer-associated fibroblasts driven by transforming growth factor ß (FB_COMP), and established a clear association between these cell types and the prognosis of OC patients. The study found that mesothelium-derived fibroblasts contribute significantly to the OC microenvironment subtype and are characterized by the high expression of profibrotic genes. On the other hand, the other two subtypes of CAFs, namely myofibroblasts and TGF-ß-driven CAFs, were shown to release interleukin-6 (IL-6). This release of IL-6 is instrumental in promoting cell growth, migration, neovascularization, and chemotherapy resistance in ovarian cancer, thereby negatively impacting patient prognosis [105].

Similarly, the study by Tongtong Kan et al. focused on the recurrence of OC [106]. Their study pointed out that a group of CAFs that highly express RGS5 are closely related to the distant metastasis and recurrence of OC. The research of Songwei Feng et al. pointed out the disease-specific gene expression characteristics in CAF. These genes are mainly related to nuclear factor kappa B (NF-κB), hypoxia and TNFA signaling of the Wnt β-catenin signaling pathway and can be induced under certain conditions [107]. To some extent, it affects the sensitivity of OC cells to anti-tumor drugs.

Recognizing the critical role of CAFs as a key matrix component and mediator between tumors and immune cells, spatial transcriptomics has emerged as a valuable tool to elucidate their characteristics in OC. Elaine Stur et al. [108] pioneered the application of this technology in OC research. Their study identified that Cluster 8, predominantly comprising cells indicative of epithelial-mesenchymal transition (EMT)-like cells, mesenchymal cells, endothelial cells, and myofibroblasts, was more prevalent in tissue samples from the poor treatment response (PR) group than those from the good treatment response (ER) group. The physical proximity and interaction of these cells suggest their role as a functional group worsening OC progression. However, this study did not provide detailed annotations of these cell types or protein-level validation of marker gene expression.

Building on this, Sammy Ferri-Borgogno et al. conducted a more in-depth study using spatial transcriptomics [109]. They discovered that the absence of specific CAF subtypes in advanced HGSC correlated with longer patient survival. In simpler terms, the CAF clusters in OC patients associated with longer survival were characterized by the expression of αSMA and VIM but lacked traditional CAF markers such as FAP, PDGFRα, and PDGFRβ. Crucially, their research indicated that in short-survival OC patients, CAFs are more likely to form a physical barrier, impeding the infiltration of individual immune cells into the tumor, thereby reducing the effectiveness of immune cell-mediated tumor cell killing. This observation helps explain why a higher density of IIC near the tumor mass correlates with a better prognosis in OC patients.

DCs

DCs are pivotal antigen-presenting cells within the immune system, playing a vital role in modulating immune responses and sustaining immune tolerance. In the TME of OC, DCs exhibit multifaceted functions. They can elicit antitumor immune responses; however, they can also be co-opted by tumors to facilitate tumor progression and immune escape. In a typical immune setting, mature DCs initiate and sustain T cell-mediated anti-tumor immunity [110, 111]. It is important to note the presence of diverse DC subpopulations within the OC TME. Studies have identified an abundance of cytokines such as TGF-β, IL-10, and CXCL-12 secreted by tumor cells in this environment. These cytokines, particularly under the influence of CXCL-12, attract plasmacytoid DC precursors [112-114]. Upon entering the TME, these DCs secrete IL-10, inhibiting T cell-mediated tumor destruction. Furthermore, research indicates that certain bone marrow-derived DCs in the TME express high levels of immune checkpoint-related genes, notably PD-L1, and Fgl2 [115] (as identified using single-cell RNA sequencing). These DCs contribute to the TME's suppressive nature by increasing Treg cell proportions and hindering T cell proliferation. Similar DC phenotypes have been observed in the ascites of OC patients with peritoneal metastasis. These predominantly plasmacytoid DCs, often immature, are implicated in promoting metastatic OC progression through angiogenesis and are termed tumor-associated dendritic

cells (tDCs). However, recent studies by Tsing-Lee Tang-Huau et al. using single-cell RNA sequencing have identified a subset of monocyte-derived DCs (mo-DCs) in the ascites of OC patients [116]. These mo-DCs can induce cytotoxic CD8+ T cell differentiation, highlighting their potential use in anti-tumor strategies, particularly those aimed at enhancing the cross-presenting capabilities of DCs.

Future clinical directions

For current immunotherapy therapies, the immunosuppressive properties of the TME are a major challenge to achieving therapeutic applications [117]. This property is mainly manifested by the presence of Tregs, TAMs, CAFs, and the exhaustion or functional decline of killer T cells [118, 119]. This problem applies to OC. Therefore, the therapeutic strategy of enhancing the function of immune killer cells by inhibiting immunosuppressive cells is the current focus of tumor immunotherapy.

Single-cell sequencing technologies provide unparalleled insight into the heterogeneity of stromal cells within the TME, a crucial aspect in cancer therapeutics. This advanced technology highlights the therapeutic potential of various immune cell subsets and CAFs in the stroma, paving the way for targeted therapeutic strategies [120]. For example, research has used this approach to explore therapeutic avenues such as reversing immunosuppression in the TME by targeting TAMs. Strategies include blocking TAM recruitment, inducing their apoptosis, or altering their immune functions to favor anti-tumor responses [121, 122]. Additionally, the development of DC-based vaccines has been enhanced by single-cell insights, which facilitate the reprogramming of patients' own DCs to elicit specific immune responses against cancer antigens [123, 124].

Moreover, single-cell technologies have also shifted the landscape of tumor prognosis evaluation. Traditionally, bulk RNA sequencing was used to identify genomic signatures predictive of outcomes [125]; however, single-cell analyses have revealed that the proportion and diversity of specific cell types within the TME can provide more precise prognostic indicators [126, 127]. This includes evaluating the tumor ecosystem diversity index (EDI), the Shannon diversity index for cell types, and the overall cellular diversity through unsupervised clustering techniques. Such detailed assessments have been applied to breast cancer and are beginning to inform OC prognosis as well. The initial OC classification based on immune molecular subtypes is being supplemented by deeper insight into the TME [128]. Advanced single-cell and microdissection techniques are critical in identifying key cellular populations that influence OC patient outcome [129], further underscored by research linking the EMT process [130] and specific prognostic genes within the NOTCH1 signaling pathway to progression and survival rates [131].

The future of single-cell sequencing in oncology holds transformative potential, especially when it integrates with technologies like artificial intelligence (AI) and machine learning (ML) to enhance the precision and predictive power of cancer treatment. These advancements are expected to allow for more personalized therapeutic approaches by providing deeper insight into the tumor microenvironment and its complex cellular interactions. Such progress promises to not only refine existing therapies but also facilitate the development of novel, cell-based interventions that could lead to more effective treatments and possibly cures for challenging conditions such as OC. As these technologies evolve, they are likely to play a crucial role in both advancing treatment efficacy and exploring preventative strategies to combat cancer at its onset.

Conclusion

Perceptions of cancer research have significantly evolved in recent decades, particularly in understanding the immune microenvironment's role [132]. Initially, tumors were primarily viewed as cellular-level genetic alterations. Research predominantly focused on the uncontrolled cell cycle and aberrant proliferation capabilities of tumor cells [133]. The introduction of the immune surveillance hypothesis marked a pivotal shift in tumor studies. In the 21st century, advancements in molecular biology and immunology have highlighted the TME concept [134]. Traditional biological research strategies, when applied to the complex and heterogeneous TME, have revealed certain limitations. However, the advent of single-cell technology has enabled high-precision and

high-resolution studies of the TME, significantly enhancing our understanding and offering new insights for treatment strategies. This review described the current understanding of the OC TME as revealed by single-cell technology studies up to January 2024, with a focus on stromal cells. The TME matrix comprises various cell types, predominantly T cells, macrophages, B cells, NK cells, fibroblasts, and DCs [132, 135]. Therefore, this article specifically discussed these cell types, summarizing and examining their phenotypes, expression profiles, functional heterogeneity, and roles in immunosuppression.

Despite the groundbreaking insights offered by single-cell sequencing technology in mapping the OC landscape, its application remains underutilized within this context. A critical observation is that the technology's coverage of stromal cells in the tumor TME does not encompass the full spectrum of cellular diversity, notably omitting significant cell types such as neutrophils, whose role in OC merits special attention [136]. Although neutrophils have been a focal point in single-cell research across other diseases [137], their mention in OC studies hs been sparse. This oversight may stem from intrinsic limitations of single-cell technologies. According to the official instructions of the 10x platform (https://www.10xgenomics.com/support/software/cell-ranger/latest/tutorials/cr-tutorial-neutrophils), scRNA-Seq faces challenges that include cell viability loss and RNA degradation during sample preparation, which restrict sequencing depth and lead to the omission of certain cell types. Moreover, the cost implications of single-cell methodologies, especially when compared to spatial transcriptomics, pose significant barriers to their widespread adoption in OC research. Financial and technical constraints have thus far hindered the large-scale deployment of these technologies in the study of OC.

Furthermore, the analytical processes currently available for single-cell data are somewhat limited, necessitating future advancements in multi-omics data integration and algorithm development. Presently, the most prevalent approach involves a synergistic combination of batch sequencing, scRNA-Seq, and spatial transcriptomics, each compensating for the other's limitations to better capture tumor heterogeneity. Emerging techniques such as single-cell assay for transposase-accessible chromatin sequencing (scATAC-Seq) [138], cellular indexing of transcriptomes and epitopes by sequencing (CITE-Seq) [139], and antigen-specific analysis by sequencing (ASAP-Seq) [140] promise further analytical depth. In terms of computational advancements, preliminary efforts to amalgamate single-cell sequencing with AI, particularly ML and deep learning (DL) models, have demonstrated substantial potential [141]. These AI-driven methodologies are adept at navigating the complex multidimensional data characteristic of single-cell analyses, offering a promising avenue for future research.

Taken together, single-cell technology has emerged as a pivotal tool in elucidating the intricacies and therapeutic challenges of the TME in OC, offering unparalleled cellular and spatial resolution. This innovative approach unveils the TME's cellular heterogeneity, uncovers novel therapeutic targets, and enhances the precision of disease prognosis assessments. As these technologies advance, we anticipate gaining profound insight into the TME's complex mechanisms in OC, leading to the development of effective treatment for OC. Consequently, the incorporation of single-cell technology in OC research facilitates progress toward achieving more accurate and individualized cancer treatment.

Disclosure of conflict of interest

None.

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