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Understanding the immune microenvironment of ovarian cancer

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Ovarian cancer remains the most lethal gynecological malignancy, yet immune checkpoint inhibitors (ICIs) demonstrate limited clinical efficacy due to profound $CD8^{+-}$ T cell exhaustion within a highly heterogeneous tumor immune microenvironment (TME). Single-cell RNA sequencing (scRNA-seq) has revolutionized our understanding of this complex ecosystem by resolving rare immune subsets, reconstructing lineage trajectories, and mapping dynamic intercellular signaling networks. Emerging single-cell atlases reveal that terminally exhausted T cells evolve from plastic progenitor populations, notably $GZMK^{+-}$ subsets, while ascitic fluid functions as a distinct immunomodulatory reservoir that continuously shapes intratumoral immune dynamics. Furthermore, multi-compartment transcriptomic profiling uncovers myeloid–lymphoid crosstalk that actively sustains localized immunosuppression and drives therapeutic resistance. These high-resolution molecular insights are increasingly leveraged to accurately predict ICI responsiveness, rationally design novel combinatorial regimens, and strategically guide next-generation adoptive cellular therapies. This review summarizes the scRNA-seq-driven advances in characterizing the ovarian cancer TME, emphasizing how single-cell resolution dismantles immunosuppressive barriers to T cell reinvigoration and establishes a robust translational framework for precision immunotherapy.

KEYWORDS
chimeric antigen receptor T-cell therapy, immune checkpoint inhibitors, immune microenvironment, ovarian cancer, single-cell RNA sequencing, T cell exhaustion

1 Introduction

Ovarian cancer constitutes the most lethal gynecological malignancy (1, 2). The tumor immune microenvironment (TME), composed of heterogeneous immune cell populations, critically governs anti-tumor immune responses. However, alterations in cellular composition and functional states can reprogram the TME toward an immunosuppressive niche, thereby accelerating tumor progression and metastasis (3, 4). The tumor immune microenvironment, composed of heterogeneous immune cell populations, critically governs anti-tumor immune responses; however, context-dependent alterations in cellular composition and functional states can reprogram the TME toward an immunosuppressive niche, thereby accelerating tumor progression and metastasis (3, 5). However, immune checkpoint

inhibitors (ICIs), despite their transformative impact in multiple malignancies, have yielded modest benefits in ovarian cancer, underscoring the imperative to decipher the complex immune dynamics conferring therapeutic resistance (6, 7). The emergence of single-cell RNA sequencing and integrative multi-omics platforms has empowered unprecedented resolution in mapping the heterogeneous immune architecture of ovarian cancer, revealing previously unappreciated cellular subsets and functional states (8). This review critically appraises contemporary advances in delineating the ovarian cancer immune microenvironment through single-cell technologies and evaluates their translational potential for refining immunotherapeutic paradigms, thereby establishing a conceptual foundation for precision oncology in ovarian cancer.

2 Single-cell sequencing in ovarian cancer immune microenvironment exploration

2.1 Overview of single-cell sequencing technology

Single-cell sequencing represents a paradigm shift in biomedical investigation, enabling genome-wide molecular profiling at individual cellular resolution (9). By isolating discrete cells for independent genomic or transcriptomic interrogation, this approach elucidates cellular heterogeneity, developmental dynamics, and pathogenic mechanisms with unprecedented precision (10, 11). scRNA-seq is currently the most widely used form of single-cell sequencing technology (12). It allows for high-resolution analysis of the transcriptome of individual cells, helping to identify new cell types, track cellular state transitions, and investigate functional differences between cells (13, 14). Following the seminal work of Tang et al. (2009) (15), technological advances—including Smart-seq2, Drop-seq, and 10x Genomics—have markedly improved sequencing throughput, sensitivity, and analytical robustness (16). Additionally, single-cell sequencing technology has transcended transcriptomics to encompass genomics, epigenomics, proteomics, and chromatin accessibility assays (scATAC-seq) (17, 18). In recent years, the advent of integrated multi-omics frameworks now enables concurrent interrogation of transcriptional, epigenetic, and spatial dimensions within individual cells, providing a systems-level understanding of cellular identity and function (19, 20). These capabilities have established single-cell sequencing as an indispensable tool for characterizing the complex immune microenvironments of malignancies, wherein cellular diversity and functional plasticity critically govern therapeutic efficacy and resistance patterns.

2.2 The immune microenvironment in ovarian cancer

The TME is a crucial component of the tumor microenvironment, primarily composed of immune cells, chemokines, and cytokines, forming a complex ecosystem (4, 21, 22). Reciprocal crosstalk between malignant and immune cells reprograms cellular phenotypes through cytokine secretion and microenvironmental remodeling, thereby facilitating immune evasion and disease progression (23, 24). In the ovarian cancer TME, the interactions between tumor cells and immune cells continuously impact tumor progression and therapeutic responses, highlighting the importance of characterizing the heterogeneity within the tumor immune microenvironment for developing effective personalized treatment strategies (8, 25). In the TME, the degree of infiltration of $CD8^{+-}$ tumor-infiltrating lymphocytes (TILs) is one of the key factors influencing the strength of the immune response (26, 27). High levels of $CD8^{+-}$ TIL infiltration are typically associated with better prognosis, as $CD8^{+-}$ TILs exert antitumor effects by secret ing granzyme and tumor necrosis factor, and by activating interferon-γ (IFN-γ) signaling pathways (28). However, prolonged exposure to the tumor immune microenvironment can lead to the functional exhaustion of these cytotoxic T cells. They may start to express inhibitory receptors such as programmed death-1 (PD-1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), eventually transforming into exhausted T cells (Tex) that lose their antitumor activity (29).

Regulatory T cells (Tregs) further modulate immune suppression and promote immune evasion, which is typically associated with poor prognosis in ovarian cancer patients (30, 31). Notably, a large meta-analysis found no significant association between Treg levels and prognosis in ovarian cancer patients (32). This discrepancy may be due to the lack of detailed immune cell subtype classification or differences in the histological localization of immune cells. In addition to lymphocytes, myeloid cells, such as tumor-associated macrophages (TAMs), also play an important role in the tumor immune microenvironment (33, 34). TAMs are the most abundant immune cells in ovarian cancer tissues and ascites, typically exhibiting an immunosuppressive M2 phenotype, which contributes to the establishment of pre-metastatic niches (35, 36). Although M1 macrophages are generally associated with better prognosis, evidence suggests that they may promote tumor cell metastasis by activating the nuclear factor-κB (NF-κB) signaling pathway (37). This indicates that even homologous immune cells can exhibit different phenotypes and functions in different tumor immune microenvironments, with these characteristics changing in response to cell interactions and tumor progression. Single-cell sequencing technologies facilitate a more detailed understanding of immune cell subpopulations, differentiation origins, and intercellular interactions within the tumor immune microenvironment, which is crucial for developing targeted therapeutic strategies and predicting disease prognosis (38, 39).

3 Roles of scRNA-seq in the immune microenvironment of ovarian cancer

scRNA-seq is a powerful tool that reveals gene expression profiles at the single-cell level. It is crucial for uncovering the transcriptional heterogeneity and functional states of various cell

types within the immune microenvironment of ovarian cancer (40, 41). This technology plays a significant role in identifying immune cell subpopulations, tracking the differentiation pathways of these subpopulations, and studying intercellular communication (42, 43).

3.1 Identification and characterization of immune cell subpopulations

Ovarian cancer exhibits multifaceted heterogeneity across molecular, anatomical, and cellular axes, with dynamic spatiotemporal evolution during disease progression and therapeutic intervention. Single-cell RNA sequencing (scRNA-seq) enables high-resolution deconvolution of immune microenvironment architectures, facilitating the systematic identification of functionally distinct immune subpopulations with translational potential (8, 44). Such subsets may serve as precision therapeutic targets or predictive biomarkers for treatment stratification. For instance, BRCA-mutant ovarian tumors demonstrate enhanced $CD8^{+-}$ T cell infiltration relative to wild-type counterparts, concomitant with expansion of $IBA1^{+-}$ $CD163^{+-}$ macrophages—a population implicated in hepatic pathogenesis yet requiring further mechanistic characterization in BRCA-deficient ovarian malignancies (45). A single-cell atlas of multiple tissues in ovarian cancer using scRNA-seq revealed the critical role of ascitic fluid in shaping the tumor immune microenvironment. The study showed that in chemotherapy-responsive patients, mucosal-associated invariant T (MAIT) cells were highly enriched in ascitic fluid, whereas in non-responsive patients, these cells exhibited dysfunction. This suggests that activated MAIT cells in ascitic fluid may serve as an important biological marker for predicting chemotherapy response (46). It was found that effector regulatory T cells (eTregs) were significantly enriched in ovarian cancer patients with homologous recombination deficiency (HRD) (47),. Their numbers were influenced by the HRD status and the use of poly (ADP-ribose) polymerase (PARP) inhibitors. Hence, scRNA-seq-driven subpopulation characterization not only refines our understanding of ovarian cancer immunobiology but also provides a rational foundation for biomarker development and personalized immunotherapy design.

3.2 Differentiation trajectories of immune cells

scRNA-seq coupled with pseudotime reconstruction elucidates the dynamic differentiation trajectories of immune cells within the ovarian cancer TME, enabling precise mapping of rare transitional states and lineage commitment mechanisms (48, 49). For T lymphocytes, trajectory inference based on continuous transcriptional cascades delineates functional state transitions with high temporal resolution (50, 51). Integration of T cell receptor (TCR) clonotyping further traces clonal evolution across developmental stages, revealing that exhaustion-associated genes are predominantly upregulated during terminal differentiation, whereas proliferation-related transcripts exhibit distinct temporal dynamics (40). This indicates that the functional state of T cells changes during the differentiation process, and blocking the differentiation of immune cells towards an immunosuppressive phenotype in crucial differentiation pathways may be an important direction for future immunotherapy. The TCR-based STARTRAC algorithm identified that $GZMK^{+}$ $CD8^{+}$ effector memory T cells derived from ascites shared TCR sequences with Tex cells from the primary lesions and omental metastatic lesions. This suggests that the former may be a potential important source of differentiation for the latter (46). Clinically, elevated $GZMK^{+-}$ T cell abundance correlates with favorable immunotherapy responses in other solid malignancies (52), suggesting these cells retain substantial functional plasticity. Consequently, therapeutic strategies aimed at enhancing the tumor-homing capacity of ascitic $GZMK^{+-}$ $CD8^{+-}$ T cells may circumvent terminal differentiation and restore cytotoxic efficacy.

3.3 Intercellular communication in the TME

Cellular crosstalk within the tumor immune microenvironment critically orchestrates tumor progression and therapeutic resistance, predominantly mediated through ligand–receptor signaling networks (53, 54). Leveraging curated interaction databases, computational frameworks now enable systematic inference of previously uncharacterized intercellular circuits. For instance, ovarian cancer tumor cells recruit $CXCR6^{+-}$ T cells via CXCL16 overexpression, an axis essential for sustaining $CD8^{+-}$ cytotoxic functionality (55). During tumor progression, such tumor-driven recruitment exemplifies how malignant cells exploit chemokine signaling to shape immune infiltration patterns, yielding both pro-inflammatory and immunosuppressive outcomes. Importantly, ligand–receptor interactions extend beyond tumor–immune interfaces to encompass immune–immune coordination. Researchers constructed a pan-cancer single-cell atlas of tumor-infiltrating natural killer (NK) cells, revealing that $LAMP3^{+-}$ dendritic cells engage NK cells through IL-15/IL-15R and Nectin2/TIGIT axes, resulting in suppressed NK cytotoxicity proximal to these dendritic subsets (56). Similarly, Yang et al. (57) mapped tumor-infiltrating B cell (TIB) heterogeneity across malignancies, identifying CXCL12/CXCR4-mediated chemotaxis as a key mechanism governing B cell localization within ovarian cancer tissues (Figure 1). By decoding these spatially resolved signaling architectures, scRNA-seq provides a mechanistic foundation for disrupting immunosuppressive circuits and enhancing antitumor immunity through targeted intervention of critical ligand–receptor pairs.

3.4 Immune microenvironment characteristics of ovarian cancer

Ovarian cancer is a highly heterogeneous malignancy, and its complex tumor microenvironment (TME) plays a critical role in disease progression and treatment response (58, 59). Using 10x Genomics single-cell RNA sequencing and T-cell receptor (TCR) analysis, a study investigated 39 samples derived from five anatomical sites—primary tumors, omental metastases, ascites, peripheral blood, and pelvic lymph nodes—collected from 14 patients with ovarian cancer (60). This study revealed the global immune landscape of ovarian cancer and demonstrated that the immune

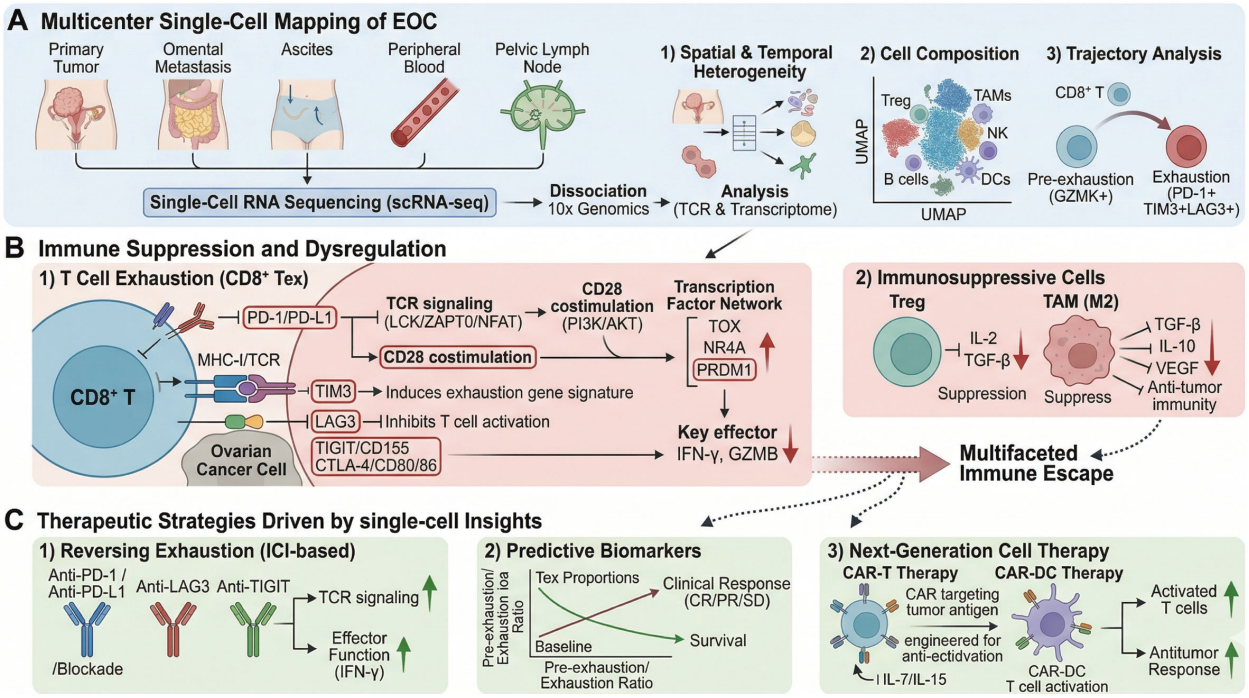

FIGURE 1
Multicenter single-cell RNA sequencing applied to epithelial ovarian cancer to analyze spatial and temporal heterogeneity, cell composition, and trajectory analysis.

microenvironments of primary tumors and metastatic sites share common features, notably the enrichment of exhausted $CD8^+$ T cells and regulatory T cells (61). In contrast, immune cells enriched in ascites were predominantly effector and memory T cells, suggesting a pivotal role for memory T cells in reshaping the TME. Furthermore, macrophages exhibited site-specific differences in function and origin, deepening our understanding of how ascites contributes to tumor progression and offering new avenues for developing targeted immunotherapeutic strategies (62).

As a critical component of ovarian cancer progression, ascites presents a unique immune microenvironment (63, 64). Although tumor cells can be detected in ascitic fluid, the typical immunosuppressive features of solid tumor tissues are absent (65, 66). Instead, immune cells such as effector T cells, memory T cells, macrophages, and dendritic cells dominate the ascitic immune cell composition, reflecting an inflammatory environment (46, 67). Notably, ascitic fluid is rich in $GZMK^+CD8^+$ effector memory T cells, which may serve as a significant source of tumor-infiltrating exhausted T (Tex) cells (68). Subsequent research further highlights the anatomical localization of these transitional cells in ovarian cancer, suggesting that the ascitic microenvironment plays a key role in shaping the tumor immune landscape. Regarding macrophage origin, TAMs in primary and metastatic tumors primarily arise from monocytes, whereas macrophages in ascites are predominantly derived from embryonic tissue-resident macrophages (46, 69). These macrophage populations exhibit differences in gene expression and function, underscoring the necessity of single-cell sequencing to unravel their heterogeneity, a critical factor closely linked to tumor progression and immune microenvironment composition (70, 71).

In lymph nodes, B cells and $CD4^+$ T cells are the dominant populations, mirroring the immune characteristics observed in lymph nodes from other solid tumors and reinforcing their role as immune organs (72, 73). Compared with primary tumors and metastases, lymph nodes contain a lower proportion of $CD8^+$ T cells (74, 75). B cells in lymph nodes can be classified into naïve B cells, memory B cells, and plasma cells, with high expression levels of chemokines that mediate the homing of T cells and dendritic cells (76, 77). Although no studies to date have analyzed the heterogeneity of B cells and tumor-infiltrating lymphocytes (TILs) in lymph nodes, understanding the cross-tissue characteristics of B cells in ovarian cancer is essential for exploring their spatial heterogeneity. Thus, the immune microenvironment of ovarian cancer across multiple sites exhibits complex cellular composition and dynamic characteristics, with ascites potentially serving as a unique immune cell reservoir that influences tumor progression. These findings not only deepen our understanding of ovarian cancer biology but also provide important insights for the development of novel immunotherapeutic strategies.

4 Single-cell sequencing predicts immune response in ovarian cancer immunotherapy

Based on the observed strong antitumor immune responses and T cell exhaustion in ovarian cancer, immune checkpoint inhibitor (ICI) therapy has emerged as a promising strategy (78). Although many clinical trials involving PD-1/programmed death-ligand 1

(PD-L1) inhibitors for ovarian cancer have shown limited patient benefits, single-cell-level studies investigating the mechanisms underlying inadequate T cell responses within tumor tissues may provide a new theoretical foundation for optimizing ICI strategies and predicting treatment responses (7, 79). In optimizing ICI therapy, the primary focus is on reversing T cell exhaustion. One approach involves targeting rare cell populations identified through scRNA-seq, which could serve as potential therapeutic targets (80, 81). As previously noted, effector T cells and exhausted T (Tex) cells are not two entirely independent cell states. Precursor T cell subpopulations, such as $GZMK^+$ cells, precede the exhaustion state, and preventing their transition into Tex cells represents a potential therapeutic direction (52, 82). Moreover, given that $GZMK^+$ T cells are predominantly enriched in the ascitic environment, targeting exhausted precursor T cells in ascites may be a key entry point for overcoming the poor efficacy of ICIs in ovarian cancer. Another strategy is to target transcription factor networks associated with T cell exhaustion, as well as a series of proteins significantly linked to exhaustion, such as TIGIT, lymphocyte activation gene 3 (LAG3), and insulin-like growth factor-like family receptor 1 (IGFLR1) (83, 84). However, clinical trials of ICIs targeting these molecules in ovarian cancer patients have not yet been conducted, highlighting the need to explore the heterogeneity of Tex cells and to discover novel characteristic immune checkpoints and regulatory transcription factors. Furthermore, scRNA-seq data can be used to predict ICI treatment responses by analyzing dynamic changes in cell types and composition (85, 86).

By tracking the entire treatment course of colorectal cancer patients receiving systemic anti-PD-1 therapy, it's found that patients achieving complete response (CR) had a higher proportion of Tex cells and elevated expression of exhaustion-related genes in $CD8^+$ T cells at baseline (87). This suggests that the composition and status of Tex cells could serve as reliable indicators for tumor efficacy assessment. Similarly, C5-ZNF683 pre-exhaustion subpopulation within $CD8^+$ T cells has been found in non-small-cell lung cancer patients, with further validation through multi omics data showing that a higher ratio of pre-exhausted to exhausted cells correlates with better patient prognosis (88, 89). Although preliminary attempts at ICI treatment in ovarian cancer have not yielded satisfactory results, beyond the development of ICIs, the mechanisms of T cell exhaustion elucidated by scRNA-seq can also facilitate the development of novel immune cell therapies, such as vaccines, chimeric antigen receptor T-cell immunotherapy (CAR-T), and chimeric antigen receptor dendritic cell immunotherapy (CAR-DC). These therapies may reverse T cell exhaustion and the immunosuppressive microenvironment, thereby enhancing effector T cell activity.

5 Conclusion

Single-cell RNA sequencing has substantially advanced our understanding of the immune microenvironment in ovarian cancer, revealing the cellular complexity and functional heterogeneity that underlie immune escape and therapeutic resistance. In particular, scRNA-seq has identified diverse immune cell subsets, spatially and transcriptionally distinct T cell states, and exhaustion-associated programs that may limit the efficacy of immune checkpoint inhibitors (ICIs). These findings indicate that ovarian cancer is not simply an immunologically "cold" tumor, but rather a highly heterogeneous immune ecosystem in which dysfunctional cytotoxic T cells, immunosuppressive myeloid populations, regulatory T cells, and stromal components interact to restrain effective anti-tumor immunity. Therefore, immune microenvironmental features identified by scRNA-seq may provide valuable diagnostic and prognostic information, including immune infiltration patterns, exhaustion signatures, and cell–cell communication networks associated with disease progression, recurrence, and treatment failure.

Clinically, integrating scRNA-seq-derived immune signatures into biomarker development may help refine patient stratification and guide individualized therapeutic strategies for ovarian cancer. Targeting key exhaustion-related molecules and pathways, reversing immunosuppressive cellular interactions, and combining ICIs with therapies that remodel the tumor microenvironment may enhance anti-tumor immune responses. Moreover, scRNA-seq can support the development of innovative immune cell-based therapies by identifying functionally relevant T cell subsets, macrophage states, and ligand–receptor interactions that influence treatment sensitivity. Although challenges remain in standardization, validation, cost, and clinical translation, single-cell technologies offer a powerful framework for connecting immune microenvironmental biology with precision diagnosis and therapy. Future studies integrating scRNA-seq with spatial transcriptomics, proteomics, and clinical outcome data may ultimately transform ovarian cancer immunotherapy from broadly applied treatment into a biomarker-driven, patient-specific strategy.

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