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Original Article

Single-cell and spatial transcriptomics reveal cellular heterogeneity and immune niches in ovarian cancer drug resistance

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Abstract

Background: *Ovarian cancer mortality remains high because of late diagnosis, recurrence, and treatment resistance. Bulk transcriptomics cannot resolve cell-specific resistance states or their spatial organization. This review examines how single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) improve our understanding of the cellular and spatial mechanisms underlying drug resistance in ovarian cancer.*

Methods: *We narratively reviewed recent literature on ovarian cancer, platinum and PARP inhibitor resistance, immune low-response phenotypes, scRNA-seq, ST, and tumor microenvironment remodeling.*

Results: *Evidence supports a resistance ecosystem comprising resistant epithelial subclones, cancer-associated fibroblast/extracellular-matrix barriers, myeloid-rich suppressive niches, dysfunctional T cells, and immune-active tertiary lymphoid structure-like regions. scRNA-seq assigns resistance programs to specific cell states, whereas ST localizes these states to tumor-stromal interfaces, immune-excluded regions, and myeloid-rich foci.*

Conclusion: *These approaches complement one another in defining ovarian cancer resistance as a cellular and spatial ecosystem. Translation will require larger longitudinal cohorts, spatial multi-omics, and functional validation.*

Keywords: Ovarian Cancer; Drug Resistance; Single-Cell RNA Sequencing; Spatial Transcriptomics; Tumor Microenvironment

Introduction

Ovarian cancer is one of the most lethal gynecologic malignancies. The disease is frequently diagnosed after regional or distant spread, and prognosis is strongly associated with stage at diagnosis. According to the Surveillance, Epidemiology, and End Results (SEER) Program, the 5-year relative survival is 31.5% for distant-stage disease, compared with 91.9% for localized disease[1]. Recent population-based analyses also show substantial global variation in

ovarian cancer incidence and histological subtype distribution[2]. This gap reflects two deeply intertwined problems: late clinical presentation and the near-inevitability of recurrence after initial treatment response.

Standard management combines cytoreductive surgery with platinum-taxane chemotherapy. For appropriately selected patients, maintenance therapy with PARPi or anti-angiogenic agents has improved progression-free survival[3-6]. Nevertheless, primary refractory disease, acquired platinum resistance, and secondary PARPi resistance continue to undermine long-term control[7-9]. High-grade serous ovarian carcinoma (HGSOC) the most common and aggressive histotype is marked by chromosomal instability, extensive copy-number alterations and pronounced interpatient and intrapatient heterogeneity[10]. Yet genomic alterations alone do not fully explain why patients with histologically similar tumors respond differently to the same regimen.

Part of the answer lies outside the tumor cell. Resistant cancer cells evolve within a complex tumor microenvironment (TME) that includes immune cells, cancer-associated fibroblasts (CAFs), endothelial cells, extracellular matrix (ECM), and ascites-associated cells all spatially organized into distinct stromal compartments. Single-cell and genomic studies have demonstrated that malignant and immune clonal dynamics are closely interwoven in ovarian cancer, implying that therapy response is co-determined by tumor evolution and local immune pressure[11-14]. Drug resistance, in this view, is better understood as an ecological process: resistant epithelial states, stromal barriers, and immunosuppressive neighborhoods reinforce one another in ways that bulk profiling cannot resolve.

Bulk RNA sequencing captures population-level transcriptomic signatures. In doing so, it averages signals across all cell types present in a sample. A resistance-associated transcript could originate from epithelial tumor cells, macrophages, fibroblasts, or any other TME component bulk data alone cannot tell. Nor can bulk profiles reveal whether resistant states are scattered across a lesion or concentrated at tumor-stromal interfaces, perivascular niches, or immune-excluded margins. scRNA-seq and ST were developed precisely to solve these attribution and localization problems. The purpose of this review is to synthesize how these two technologies have been applied to ovarian cancer drug resistance, and to identify the gaps that must be closed before cell-state and niche-level findings can inform clinical decision-making.

2. Materials and methods

2.1. Literature search strategy

A narrative literature review was conducted to identify studies relevant to ovarian cancer drug resistance, scRNA-seq, ST, and the tumor immune microenvironment. We searched PubMed, Web of Science, Google Scholar, and publisher databases using combinations of the following terms: 'ovarian cancer,' 'high-grade serous ovarian cancer,' 'single-cell RNA sequencing,' 'spatial transcriptomics,' 'drug resistance,' 'platinum resistance,' 'PARP inhibitor resistance,' 'immune microenvironment,' 'immune niche,' 'cancer-associated fibroblast,' and 'tumor-associated macrophage.' The search covered publications from January 2018 through June 2026, with earlier methodological reports retained when they provided essential technical context for example, the original spatial transcriptomics proof-of-concept by Ståhl et al.[15].

2.2. Evidence synthesis

We did not attempt a systematic review or meta-analysis. The studies in this field differ too widely in platform (10x Chromium, Smart-seq2, Visium, Slide-seq, MERFISH, Xenium),

sample type (primary tumor, ascites, omental metastasis, organoid), treatment exposure (treatment-naïve, post-platinum, post-PARPi), and computational pipeline to permit quantitative synthesis. Instead, evidence was synthesized narratively and interpreted according to three tiers: (i) direct ovarian cancer single-cell or spatial evidence; (ii) indirect evidence from broader TME studies; and (iii) technology-driven hypotheses awaiting functional validation. This tiered approach is intended to distinguish well-supported findings from plausible-but-unproven conjecture.

3. Biological basis of drug-resistance heterogeneity in ovarian cancer

Ovarian cancer resistance operates at multiple levels simultaneously genetic, transcriptional, phenotypic, and spatial. In HGSOC, chromosomal instability and near-universal TP53 mutations coexist with variable homologous recombination deficiency (HRD), diverse copy-number alteration landscapes, and multiple coexisting tumor subclones. Chemotherapy and targeted therapy act on this heterogeneous population as selective pressures, enriching rare pre-existing resistant states or driving stress-adaptive transcriptional programs. The critical point is that resistance is dynamic. A tumor sampled before and after treatment is not the same disease.

At the tumor-cell level, platinum resistance and PARPi resistance share considerable mechanistic overlap. Both involve altered DNA damage response: impaired drug uptake or increased efflux, restored homologous recombination capacity (through BRCA reversion mutations or RAD51 upregulation), replication-fork protection, and rewired cell-cycle checkpoint signaling via ATR-CHK1-WEE1[7-9]. These pathways are well characterized individually. What is less appreciated is that they do not operate in isolation from the microenvironment.

CAFs, tumor-associated macrophages (TAMs), myeloid-derived suppressor cell (MDSC)-like populations, regulatory T cells, and endothelial cells each contribute to a microenvironmental context that shapes treatment outcome. CAF-derived ECM deposition can physically impede drug penetration. TAMs and MDSCs suppress cytotoxic T-cell function[16]. Regulatory T cells dampen antitumor immunity[17]. Hypoxia and metabolic rewiring further tilt the balance toward tolerance[18]. These extrinsic influences help explain a long-standing clinical puzzle: why tumors with similar genomic profiles can respond quite differently to the same therapy.

Spatial organization adds yet another dimension. Within a single ovarian tumor, immune-infiltrated regions often coexist with immune-excluded zones, myeloid-rich foci, and dense stromal barriers. A lesion classified as "immune-active" by bulk analysis might still harbor localized compartments where T cells are physically separated from malignant cells or trapped within suppressive myeloid neighborhoods. Interpreting resistance-associated gene programs without reference to cell identity and spatial context is therefore incomplete at best, and misleading at worst.

4. Single-cell transcriptomics reveals resistance-associated cellular states

4.1. Resistant epithelial tumor-cell states

The fundamental contribution of scRNA-seq to resistance research is not marker discovery per se it is the assignment of markers to biologically interpretable cell states. By separating malignant epithelial cells from immune and stromal compartments, scRNA-seq makes it possible to ask which tumor-cell states are enriched in resistant versus sensitive samples, and whether those states pre-exist treatment or emerge under selective pressure.

Several resistance-associated epithelial phenotypes have been recurrently identified across ovarian cancer scRNA-seq studies. These include proliferative clusters with elevated cell-cycle gene expression, stress-response clusters enriched for heat-shock and oxidative-stress pathways, epithelial-mesenchymal transition (EMT)-like cells, DNA repair-high populations, and stem-like subclones. Some appear to be present at low frequency before treatment, consistent with a pre-existing resistance model; others expand following chemotherapy or PARPi exposure, suggesting therapy-induced adaptation. A key challenge is distinguishing genuine resistant states from transient stress responses that would regress if treatment pressure were removed.

One of the most concrete examples comes from Han et al.[19], who performed integrated scRNA-seq and ST on platinum-resistant HGSOC. They identified a TACSTD2-overexpressing epithelial cluster enriched in platinum-resistant tumors and linked it to Rap1/PI3K/AKT signaling. This finding is notable because it moves beyond descriptive cell-type annotation toward a candidate resistance pathway although functional confirmation through perturbation experiments is still needed. Studies like this illustrate both the promise and the current limits of single-cell approaches: they can nominate resistant subclones and pathways, but they cannot prove causality alone. Figure 1 illustrates the interconnected cellular and molecular network driving heterogeneous drug resistance in ovarian cancer.

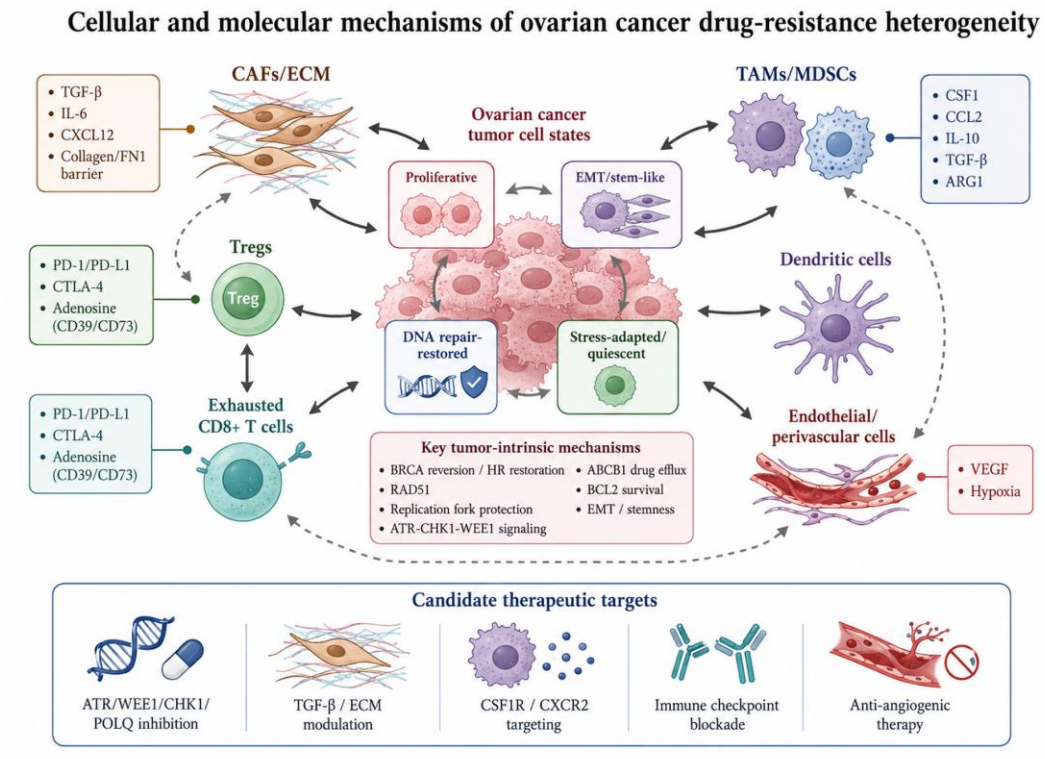


Figure 1. Schematic overview of cellular and molecular mechanisms underlying heterogeneous drug resistance in ovarian cancer.

Central malignant epithelial cells adopt diverse resistant phenotypes: proliferative, EMT/stem-like, DNA repair-restored, and stress-quiescent states. Bidirectional crosstalk between tumor cells and surrounding stromal and immune populations, including CAFs, TAMs/MDSCs, dendritic cells, endothelial cells, Tregs and exhausted $CD8^+$ T cells, shapes therapy-refractory microenvironmental niches. Core tumor-intrinsic resistance pathways and promising therapeutic intervention strategies targeting key nodes are illustrated.

4.2. Fibroblast and extracellular matrix programs

CAFs are among the most consequential non-malignant cells in the ovarian cancer TME, yet their heterogeneity is only beginning to be appreciated. Single-cell profiling has distinguished at least three broad CAF programs: inflammatory CAFs (characterized by cytokine and chemokine expression), myofibroblast-like CAFs (enriched for contractile and ECM-remodeling genes), and a less consistently defined population sometimes called antigen-presenting CAFs[20]. The nomenclature across studies is not yet standardized, which complicates cross-study comparison.

What makes CAFs relevant to drug resistance is their functional output. CAF-derived ECM components, including collagens, fibronectin (FN1), and periostin (POSTN), may alter tissue stiffness, restrict drug penetration and contribute to T-cell exclusion in selected spatial contexts. TGF-β signaling from CAFs can further suppress immune activity and promote EMT-like programs in adjacent tumor cells. In resistance research, CAFs should not be dismissed as passive background stroma. Their proximity to resistant tumor subclones, CD8+ T cells and TAMs likely determines whether they function primarily as physical barriers, survival niches or immune-modulating hubs. Integrated scRNA-seq and ST can help distinguish CAF programs that are broadly distributed across a tumor from those concentrated in treatment-resistant regions a distinction with translational implications that still requires functional validation.

4.3. Myeloid populations and immunosuppressive niches

Myeloid cells are arguably the most plastic component of the ovarian cancer TME. scRNA-seq has identified multiple macrophage states in ovarian tumors, though their functional significance remains debated. Among the best-characterized are APOE-high macrophages, TREM2+ macrophages (associated with lipid metabolism and tissue remodeling), SPP1+ macrophages (linked to ECM organization and angiogenesis), and MRC1+ cells with features traditionally labeled "M2-like." Marker-defined populations such as C1QA-C-expressing macrophages have also been described, along with cells enriched for antigen-presentation genes.

We emphasize that marker expression is not function. A macrophage expressing TREM2 and SPP1 in the tumor core might behave quite differently from one with the same markers at the invasive edge or in a perivascular niche. This is precisely why scRNA-seq alone is insufficient for understanding myeloid contributions to resistance. Spatial analyses by Mateiou et al.[21] and others have shown that macrophage-rich immune niches are focal rather than diffuse, and that their distribution within tumors is non-random[21-22]. These observations support the concept of myeloid-rich neighborhoods as targetable resistance compartments but also highlight the need for spatial context in interpreting marker data.

4.4. T-cell dysfunction and immune low-response states

The limited efficacy of immune checkpoint inhibitors in ovarian cancer is well documented, and it cannot be explained by checkpoint molecule expression alone. scRNA-seq has revealed considerable heterogeneity within the T-cell compartment: cytotoxic effector cells, tissue-resident memory-like T cells, exhausted T cells (characterized by PD-1, TIM-3, and LAG-3 co-expression), proliferating T-cell subsets, and FOXP3+ regulatory T cells can all coexist within the same tumor[23]. The functional significance of each population depends on factors beyond abundance including antigen presentation capacity, the density and activation state of

suppressive myeloid cells, the presence of stromal barriers, and spatial proximity to malignant cells.

A critical translational question is whether T-cell dysfunction in ovarian cancer is reversible. Tumors with preserved CD8+ T-cell and dendritic-cell neighborhoods might respond to immunotherapy combinations aimed at reinvigorating exhausted effectors. Tumors dominated by CAF-ECM barriers or myeloid-rich suppression, by contrast, are unlikely to benefit from checkpoint blockade alone; they would require niche-remodeling strategies to physically and functionally restore T-cell access to tumor cells. This distinction is directly relevant to the immune low-response phenotype described by Yan et al.[24], in which T-cell dysfunction, TAM enrichment, and stromal exclusion converge[23-25]. Candidate cell populations, characteristic transcriptional programs and their inferred roles in ovarian cancer chemoresistance and PARPi resistance are outlined in Table 1.

Table 1. Cell Populations And Candidate Programs Implicated In Ovarian Cancer Drug Resistance.

Cell population	Representative states or markers	Potential role in resistance	Evidence interpretation
Malignant epithelial cells	DNA repair programs, EMT-like states, stress-response genes, TACSTD2	Platinum and PARPi resistance; relapse-seeding states	Direct scRNA-seq/ST evidence emerging; functional validation limited
CAFs and ECM-rich stroma	TGF-β, FN1, COL1A1/COL3A1, POSTN, myofibroblast-like programs	Drug penetration barrier; immune exclusion; survival niche formation	Likely context-dependent; spatial localization is critical
TAMs and MDSC-like myeloid cells	APOE, TREM2, SPP1, MRC1, C1QA-C	T-cell suppression; angiogenesis; matrix remodeling; therapy tolerance	Strong TME plausibility; functions require spatial and experimental validation
T cells and Tregs	CD8+ cytotoxic cells, exhausted T cells (PD-1/TIM-3/LAG-3+), tissue-resident memory-like cells, FOXP3+ Tregs	Immune surveillance or immune escape depending on state and location	Abundance alone is insufficient; neighborhood context required
B cells and TLS-like regions	B cells, plasma cells, dendritic-cell proximity, lymphoid aggregates	Potential immune-active niches; treatment-response modifiers	Promising but not yet established as routine biomarkers

Note: CAF, cancer-associated fibroblast; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; MDSC, myeloid-derived suppressor cell; PARPi, poly(ADP-ribose) polymerase inhibitor; ST, spatial transcriptomics; TAM, tumor-associated macrophage; TLS, tertiary lymphoid structure.

5. Spatial transcriptomics reconstructs resistance-associated immune niches

5.1. Spatial tumor subclones and local microenvironments

ST assigns transcriptional states to physical tissue coordinates. In HGSOC, this technology has revealed that tumor microenvironments are spatially discrete rather than continuous. Denisenko et al. [22], using ST on ovarian cancer subclones, demonstrated distinct microenvironments and autocrine signaling loops that would have been invisible to dissociated

single-cell methods. For drug resistance research, this finding has a direct implication: resistant subclones are not uniformly distributed across a tumor. They occupy selected regions tumor-stromal interfaces, hypoxic cores, perivascular niches, and immune-excluded margins each with a distinct local microenvironment.

Spatial subclone mapping also bridges two levels of heterogeneity that are usually studied separately: genomic (clonal architecture) and microenvironmental (cellular composition). Subclone-specific ligand-receptor patterns, visible only when tumor genotypes are overlaid with spatial coordinates, can reveal how genetically distinct tumor subclones communicate with adjacent stromal and immune cells. This capability is unique to ST and cannot be replicated by dissociated scRNA-seq. Indeed, a recent multi-modal study of 160 tumor sites from 42 HGSOC patients demonstrated that anatomical location and mutational processes jointly shape immune evasion mechanisms, underscoring the necessity of spatial context for interpreting resistance[14].

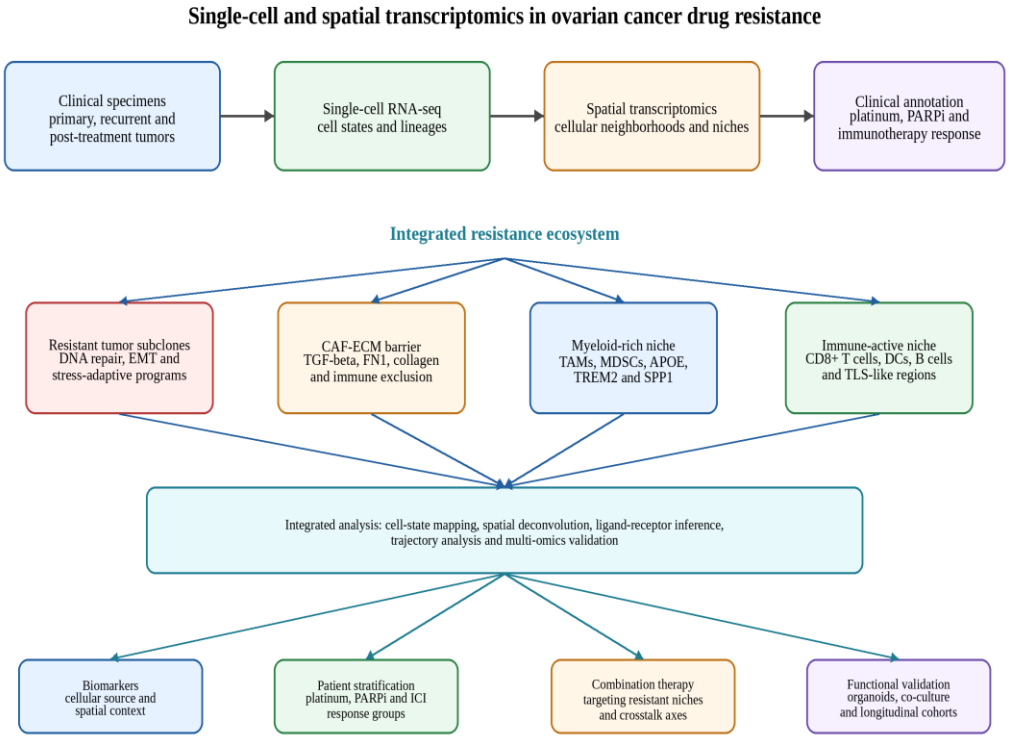


Figure 2 summarizes the multi-step research pipeline applying single-cell and spatial transcriptomics to unravel spatially structured drug-resistance ecosystems in ovarian cancer, together with key translational outputs.

Figure 2. Integrated framework for single-cell and spatial transcriptomic analysis of ovarian cancer drug-resistance heterogeneity. Clinical specimens (primary, recurrent, post-treatment) are profiled by scRNA-seq and ST, then integrated with treatment annotation. The resulting resistance ecosystem comprises resistant epithelial subclones, CAF-ECM barriers, myeloid-rich niches and immune-active regions. Downstream applications include biomarker discovery, patient stratification, combination-therapy hypothesis generation and functional validation. CAF, cancer-associated fibroblast; DC, dendritic cell; ECM, extracellular matrix; ICI, immune checkpoint inhibitor; MDSC, myeloid-derived suppressor cell; PARPi, poly(ADP-ribose) polymerase inhibitor; scRNA-seq, single-cell RNA sequencing; ST, spatial transcriptomics; TAM, tumor-associated macrophage; TLS, tertiary lymphoid structure.

5.2. Immune-excluded, myeloid-rich, and immune-active niches

Ovarian cancer is often labeled "immunologically cold." This characterization, while not entirely wrong, oversimplifies a more nuanced spatial reality. Mateiou et al.[21], using digital spatial profiling, identified distinct ovarian tumor immune phenotypes with markedly different spatial distributions of checkpoint markers, macrophages, and immune-cell neighborhoods. Their work underscores that the same tumor can contain immune-active regions alongside zones of T-cell exclusion, macrophage-rich foci, and dense stromal barriers.

This spatial heterogeneity has functional consequences. A tumor with abundant CD8+ T cells in bulk analysis might still resist immunotherapy if those T cells are sequestered behind CAF-ECM barriers or surrounded by suppressive TAMs. Conversely, the presence of TLS-like immune-active regions characterized by B-cell aggregates, plasma cells, and dendritic-cell proximity might identify tumors in which antitumor immunity exists but is locally suppressed[25]. Ascites, a hallmark of advanced ovarian cancer, further remodels the immune ecosystem: single-cell analyses have shown that the ascitic microenvironment harbors distinct immune and malignant cell states compared to solid tumor deposits, potentially creating an additional compartment of drug-tolerant cells[26]. These distinctions matter clinically: they suggest that "immune-cold" is not a binary state but a spatially graded phenomenon, and that immunotherapy response might be improved by targeting the specific barrier stromal, myeloid, or checkpoint-mediated that dominates in a given region.

5.3. Spatial features of platinum and PARPi resistance

Platinum and PARPi resistance are typically discussed in terms of tumor-cell-intrinsic mechanisms: restored DNA repair, BRCA reversion, drug efflux. But spatial studies suggest that intercellular crosstalk contributes as well. Suzuki et al.[27], using Visium-based ST to compare PARPi-sensitive and PARPi-resistant HGSOC, found that resistant tumors exhibited not only cell-intrinsic transcriptional changes but also spatially constrained shifts in cell composition and ligand-receptor crosstalk. In other words, PARPi resistance was associated with niche-level remodeling, not just with restored DNA repair capacity.

Drawing on these studies, we propose that spatially informed analysis can distinguish at least four resistance patterns. The first is tumor-autonomous resistance, in which malignant subclones enriched for DNA damage-response or survival programs drive resistance independently of their microenvironment. The second is CAF-ECM immune-exclusion resistance, in which stromal barriers physically exclude both drugs and immune cells. The third is myeloid-rich suppressive resistance, dominated by TAM- and MDSC-mediated immunosuppression. The fourth is immune-active but functionally suppressed resistance, in which T-cell and dendritic-cell neighborhoods are preserved but locally inhibited. These patterns are not mutually exclusive; in fact, they frequently coexist within the same patient, which partly explains why responses vary across metastatic sites and treatment lines.

6. Integration of single-cell and spatial transcriptomics

6.1. Cell-state mapping and spatial deconvolution

A widely adopted integration strategy uses scRNA-seq as a reference atlas and deconvolves ST data to estimate cell-type composition at each spatial location. This approach is particularly necessary for Visium-type platforms, where each capture spot spans multiple cells and the resulting transcriptomic profile is a mixture. Computational tools such as Cell2location[28] and Robust Cell Type Decomposition (RCTD)[29] have been developed to perform this deconvolution, each with different assumptions about gene expression variability and

reference quality. By transferring cell-type signatures from scRNA-seq to spatial coordinates, investigators can infer where resistant epithelial states, TAMs, CAFs, and T-cell subsets are enriched and how these distributions relate to histological architecture.

It is worth noting that deconvolution accuracy depends heavily on the quality of the scRNA-seq reference and the degree of concordance between the single-cell and spatial platforms. Reference atlases built from treatment-naïve tumors may fail to capture resistant states present in post-treatment samples. Batch effects between datasets, differences in gene capture efficiency, and dissociation-induced artifacts in scRNA-seq all introduce noise into the deconvolution output. Computational integration tools such as Harmony[30] can partially mitigate batch effects when combining datasets from different platforms or laboratories, but they cannot fully compensate for biological differences in sample preparation. These limitations argue for a practical strategy of combining broad discovery with targeted validation: scRNA-seq and Visium-like ST can identify candidate niches, while multiplex immunofluorescence, imaging mass cytometry, or digital pathology can validate selected markers and cell-cell relationships in larger, clinically annotated cohorts.

6.2. Ligand-receptor analysis and inferred cellular communication

Ligand-receptor analysis has become a standard tool for inferring intercellular communication from transcriptomic data. Tools such as CellChat[31] and NicheNet[32] take different approaches: CellChat aggregates ligand-receptor expression across cell groups to infer signaling pathway activity, while NicheNet links ligand expression to downstream target gene regulation in receiver cells. In spatial contexts, both approaches gain biological interpretability, because potential sender and receiver cells can be constrained by physical proximity. A tumor-CAF interaction axis inferred from dissociated scRNA-seq data is a hypothesis; the same axis inferred from adjacent spots in ST data is a spatially grounded hypothesis.

We caution, however, that ligand-receptor inference remains fundamentally correlative. Expression of a ligand and its receptor does not prove functional signaling. Spatial adjacency does not prove causal interaction. Co-expression of a signaling pair might reflect shared transcriptional programs rather than genuine communication. Functional validation using organoid co-culture systems, ex vivo tumor slice cultures, patient-derived xenografts, or CRISPR-based perturbation is essential before any inferred communication axis can be considered a therapeutic target.

6.3. Trajectory, clonal evolution, and multi-omics integration

Trajectory inference algorithms attempt to reconstruct the developmental or transitional relationships between cell states. Tools evaluated in benchmarking studies by Saelens et al.[33] and methods such as Palantir[34] can suggest transitions from treatment-sensitive to resistant states, though such inferences are most reliable when longitudinal samples are available. In practice, most ovarian cancer scRNA-seq datasets are cross-sectional they capture a single time point per patient and trajectory reconstructions from cross-sectional data should be interpreted with considerable caution.

Integration with complementary data types substantially strengthens biological inference. Copy-number variation inference from scRNA-seq data, whole-exome sequencing, HRD scoring, TCR/BCR repertoire sequencing, and spatial proteomics each add a layer of information that pure transcriptomics cannot provide. The Seurat framework[35-36] and Scanpy[37] have become standard environments for such multi-modal integration. For clinical translation, the most informative datasets will combine pretreatment, on-treatment, and recurrent samples from the same patient, annotated with detailed treatment history and outcomes. Without

temporal information, it is extraordinarily difficult to determine whether a spatial niche causes resistance, is selected by therapy, or merely represents a consequence of prior treatment. The complementary utilities, main analytical outputs, contributions to resistance research and inherent limitations of distinct single-cell and spatial transcriptomic analytical strategies are systematically contrasted in Table 2.

Table 2. Complementary roles of single-cell and spatial transcriptomics in ovarian cancer resistance research.

Analytical layer	Main output	Contribution to resistance research	Key limitation
scRNA-seq cell atlas	Cell types, malignant states, immune/stromal programs	Identifies rare resistant states; attributes resistance transcripts to cellular sources	Loses spatial information; vulnerable to dissociation bias
ST mapping	Gene expression linked to tissue coordinates	Defines immune-excluded, myeloid-rich, CAF-rich, and immune-active regions	Resolution and sensitivity vary by platform
Spatial deconvolution (Cell2location, RCTD)	Estimated cell composition per spot	Maps scRNA-seq-defined states into tissue architecture	Depends on reference quality and computational assumptions
Ligand-receptor inference (CellChat, NicheNet)	Putative cell-cell communication axes	Prioritizes crosstalk pathways in resistant neighborhoods	Correlative; requires functional validation
Longitudinal multi-omics	Temporal changes in clones, cell states, niches	Distinguishes pre-existing resistance from therapy-selected adaptation	Requires matched samples and detailed clinical annotation

Note: scRNA-seq, single-cell RNA sequencing; ST, spatial transcriptomics; RCTD, Robust Cell Type Decomposition.

7. Discussion: translational implications and clinical challenges

The core translational promise of scRNA-seq and ST is to move biomarker development beyond decontextualized gene lists toward signatures that are aware of both cellular source and anatomical location. The same gene expressed by malignant epithelial cells and by myeloid cells can have entirely different implications. The same gene program distributed uniformly across a tumor versus concentrated at a tumor-stromal interface can carry different functional weight. In our view, cellular source and spatial context are not optional metadata they are essential components of biomarker interpretation.

For platinum therapy, integrated single-cell and spatial profiling could identify patients whose tumors harbor pre-existing resistant epithelial states, stromal barriers, or myeloid-rich niches before treatment begins. These patients might benefit from upfront combination strategies rather than sequential monotherapy. For PARPi therapy, spatial profiles could complement BRCA mutation testing and HRD scoring by revealing niche-level features such as immune activation or stromal remodeling that influence response beyond what genomic assays capture. For immunotherapy, spatial analysis could clarify why a given tumor is not responding: is it due to absence of effector cells? T-cell dysfunction? Treg enrichment? Myeloid suppression? Or physical exclusion by CAF-ECM barriers? Each of these failure modes implies a different therapeutic intervention.

These distinctions can help generate rational combination-therapy hypotheses. Tumor-autonomous DNA repair-associated resistance may support investigation of DDR combinations including ATR, WEE1, CHK1, or POLQ-directed approaches with platinum or PARPi [9]. CAF-ECM barrier-dominant tumors may require TGF-β or matrix-modulating strategies to reduce physical exclusion. Myeloid-rich tumors may benefit from exploration of TAM-targeting agents, such as CSF1R inhibitors, or MDSC-directed strategies [16]. Immune-active but checkpoint-high niches may be candidates for PARPi, anti-angiogenic therapy and immune checkpoint blockade combinations. These proposals remain conceptual. Prospective clinical trials stratifying patients by spatial niche type have not yet been conducted, and these recommendations should be considered hypothesis-generating rather than practice-changing.

8. Future directions

Several barriers stand between the current state of the field and clinically useful spatial biomarkers.

8.1. Sample size and study design

Most published ovarian cancer scRNA-seq and ST datasets include fewer than 10 patients per study, often combining different histological subtypes, anatomical sites, and treatment histories. This heterogeneity dilutes disease-specific signals and limits statistical power. Future studies should prioritize larger, more homogeneous cohorts ideally focused on HGSOC with matched primary, post-treatment, and recurrent samples from the same patients.

8.2. Platform limitations

Tissue dissociation for scRNA-seq can selectively damage or lose certain cell types, particularly macrophages and fragile stromal cells. Conventional spot-based platforms such as Visium v1/v2 capture multicellular regions, whereas higher-resolution approaches such as MERFISH [38] and Stereo-seq [39] provide subcellular mapping through targeted imaging or sequencing-based whole-transcriptome strategies. These platforms involve trade-offs among resolution, sensitivity, transcriptome breadth, field of view, tissue compatibility, and analytical complexity. Cross-platform integration is therefore often advantageous.

8.3. Temporal dynamics

The overwhelming majority of existing datasets are cross-sectional. Without longitudinal sampling, it is impossible to distinguish niches that cause resistance from those that are selected by therapy or arise as a consequence of treatment. Matched pre- and post-treatment biopsies, though logistically challenging in ovarian cancer, are the only way to address this question definitively.

8.4. Computational standardization

Results are sensitive to the choice of reference atlas, deconvolution method, batch-correction algorithm, and annotation strategy. The field lacks standardized definitions of spatial resistance niches, making cross-study comparison difficult. Community efforts to establish common analytical frameworks analogous to what the TCGA achieved for bulk genomics would substantially accelerate progress.

8.5. Functional validation

Ultimately, omics-defined niches must be tested for causal involvement in resistance. Organoid models [40], ex vivo tumor slice cultures, co-culture systems, and patient-derived xenografts each offer different advantages and limitations. Perturbation approaches genetic (CRISPR),

pharmacological, or antibody-based are needed to determine whether disrupting a candidate niche (e.g., depleting TREM2+ macrophages or breaking down CAF-ECM barriers) can sensitize resistant tumors to therapy. Without such experiments, niche-level observations remain correlational.

9. Conclusion

Ovarian cancer drug resistance is shaped by malignant cell plasticity, stromal remodeling, immune suppression, and the spatial organization of cellular neighborhoods. scRNA-seq resolves the first dimension identifying resistant cell states and attributing resistance-associated signals to specific cell types. ST restores the second mapping these states onto tissue architecture and revealing resistance-associated niches. Together, these technologies provide a framework for understanding why tumors with similar genomic features respond differently to platinum agents, PARPi, and immunotherapy.

The evidence reviewed here supports an ecological model of resistance: resistant epithelial subclones, CAF-ECM barriers, myeloid-rich immunosuppressive niches, and immune-active TLS-like regions interact as components of a broader system rather than acting in isolation. This perspective has direct implications for combination-therapy design, because different resistance patterns call for different therapeutic strategies.

At the same time, we must be candid about the limitations. Current datasets are small, cross-sectional, and platform-dependent. Functional validation has not kept pace with omics discovery. Spatial niche biomarkers have not been prospectively validated in clinical trials. Single-cell and spatial transcriptomics are not yet stand-alone clinical tools. What they provide and what no previous technology has is a framework for interpreting drug resistance as a cellular and spatial ecosystem. The challenge now is to convert that framework into actionable clinical intelligence.

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