



Single-Cell Technologies in Natural Product Pharmacology for Resolving Cellular Heterogeneity, Mechanism of Action, Resistance, and Context-Specific Therapeutic Response

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ABSTRACT

Natural products act within biologically heterogeneous systems in which pharmacological effects may differ among cell types, transient states, tissue niches, and resistant subpopulations. Population-averaged measurements can therefore obscure context-specific responses or incorrectly compress several cellular mechanisms into a single apparent effect. Single-cell and spatial technologies offer a route to resolve this heterogeneity, but their use in natural-product pharmacology remains methodologically uneven. We conducted a systematic mapping review of peer-reviewed literature published from 2017–2026 addressing single-cell technologies in natural-product pharmacology. Eligibility and charting distinguished treatment-exposed single-cell experiments from studies using pre-existing single-cell datasets to inform subsequent pharmacological investigation, and from methodological evidence required to interpret analytical validity. A verifiable candidate registry contained 54 records, of which 16 were excluded and 38 formed the mapped evidence base. Raw database-yield and full-text-stage denominators that could not be reconstructed reproducibly were not inferred. The map indicates a strong concentration of single-cell transcriptomic studies, particularly in cancer, inflammatory disease, traditional-medicine formulations, and immune-microenvironment research. Spatial, epigenomic, proteomic, and multimodal approaches remain less common. Cell-resolved studies frequently identify treatment-associated subpopulations or nominate mechanisms, whereas stronger mechanistic interpretation generally depends on orthogonal experimental validation. Direct evidence addressing resistance, persistence, temporal dynamics, dose dependence, and human treatment contexts is comparatively sparse. Single-cell technologies expand natural-product pharmacology from average response toward context-resolved pharmacological interpretation. Their principal value is analytical discrimination rather than automatic causal proof. Progress requires clearer separation of cell-state association, mechanistic inference, target validation, resistance biology, and translational evidence.

Key Words: Natural products, Single-cell RNA sequencing, Cellular heterogeneity, Pharmacological mechanism, Drug resistance, Spatial multi-omics

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INTRODUCTION

Natural products remain a major source of chemically distinctive pharmacological scaffolds and therapeutic

hypotheses, but their biological effects are frequently investigated in systems composed of multiple interacting cell populations rather than homogeneous targets [1]. This

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creates an interpretive problem. A bulk transcriptomic, proteomic, inflammatory, or phenotypic response can represent a uniform cellular effect, a large effect confined to one population, opposing responses in different populations, or a shift in the relative abundance of cellular states. These possibilities are pharmacologically different even when they produce similar population-averaged measurements.

Single-cell technologies provide a means to separate these possibilities. In natural-product research, single-cell and multiomic approaches have been proposed as complements to target identification because they can connect chemical intervention to cell identity, state, and molecular context [2]. More broadly, single-cell RNA sequencing has become relevant to drug discovery by resolving response heterogeneity, pathway activity, disease-associated populations, and treatment-linked state transitions that would otherwise remain aggregated [3]. Their contribution, however, should not be equated with direct target engagement or causal mechanism.

Recent syntheses of traditional-medicine applications show a rapidly expanding but heterogeneous literature in which single-cell profiling is increasingly used to interpret pharmacological response while modality coverage, validation depth, and analytical standardization remain uneven [4, 5]. This review therefore maps where single-cell evidence is presently concentrated, what kinds of pharmacological conclusions it can support, and which mechanistic, resistance, temporal, multimodal, and translational questions remain inadequately resolved.

Mapping questions and eligibility framework

The review was designed around four linked questions. First, which single-cell technologies have actually been applied to natural products, extracts, or multi-component traditional formulations? Second, what kinds of cellular heterogeneity are resolved after intervention? Third, how are cell-type-specific observations converted into mechanism-of-action, differential-sensitivity, resistance, immune, or tissue-context claims? Fourth, where do modality, experimental design, computational analysis, or validation limitations restrict interpretation?

Single-cell transcriptomics, epigenomics, protein-level profiling, spatially resolved measurements, and multimodal combinations were treated as analytically distinguishable evidence layers rather than interchangeable versions of the same assay [6]. Eligibility also distinguished intervention-exposed profiling from analyses in which existing single-cell or spatial datasets were used to identify a candidate cell state or target before separate pharmacological validation. This distinction is proposed here because the two designs answer different questions: the first observes cellular response after exposure, whereas the second uses single-cell information to inform a pharmacological hypothesis.

The operational boundaries used to convert these questions into an auditable search-and-selection process are summarized in **Table 1**.

Table 1. Operational search and selection framework for mapping single-cell evidence in natural-product pharmacology

Selection dimension	Operational treatment	Inclusion boundary	Interpretive consequence
Intervention	Purified natural product, defined extract, or multi-component natural-product formulation	Pharmacological relevance had to be explicit	Compound-level attribution was not assumed for complex formulations
Single-cell evidence	Transcriptomic, epigenomic, protein-level, spatial, or multimodal cellular profiling	Cell-resolved evidence had to contribute to a review question	Bulk-only studies were outside the core map
Exposure relationship	Treatment-exposed profiling or single-cell-informed pharmacology	Design type was charted separately	External atlases were not treated as evidence of treatment-induced change
Biological question	Heterogeneity, mechanism, sensitivity, resistance, persistence, immune response, or tissue context	At least one mapped domain had to be supported	Merely mentioning single-cell methods was insufficient
Validation	Orthogonal assays, perturbation, localization, functional testing, or comparative controls where reported	Validation was charted, not required uniformly	Mechanistic confidence remained graded
Evidence boundary	Preclinical, human tissue, computational, or methodological	Transferability was recorded explicitly	Cell-resolved association was not converted into clinical efficacy

Figure 1 makes the selection logic auditable while preserving the limits of the verified search ledger: only candidate-registry and final-inclusion counts are shown

numerically where those quantities were reproducibly established.



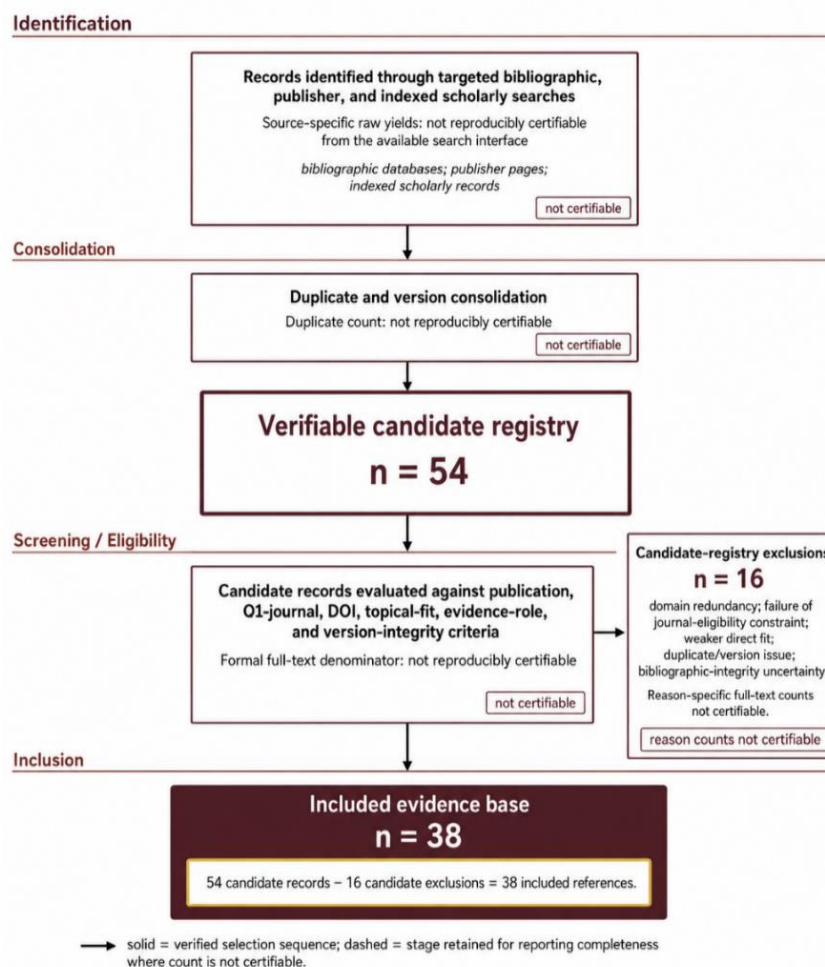


Figure 1. Auditable evidence-selection flow for the systematic mapping of single-cell natural-product pharmacology

Protocol and reporting framework

The review followed systematic mapping logic: the objective was to characterize the distribution, design, modality, evidential role, and interpretive maturity of a heterogeneous literature rather than to estimate a pooled therapeutic effect. Reporting was aligned with PRISMA-ScR principles because those principles require transparent specification of eligibility, selection, charting, and synthesis processes for evidence maps and related scoping designs [7]. The review was not designed as a meta-analysis, systematic effectiveness comparison, bibliometric study, or quantitative ranking of natural products.

The unit of mapping was the eligible journal article, but extraction operated at several levels within each article: intervention, biological system, single-cell platform, cell population or state, pharmacological question, mechanistic evidence, computational strategy, validation design, and transferability boundary. Where a study contained several modalities, they were charted separately rather than compressed into a single generic “single-cell” category.

A protocol-level distinction was also maintained between what the underlying source reported and what this review synthesized across sources. Reported cell-type shifts, pathway changes, target observations, or experimental validations were mapped as evidence-derived findings. Cross-study categories such as “treatment-exposed single-cell evidence” and “single-cell-informed pharmacology” were used as proposed organizing constructs and were not presented as previously validated taxonomies.

Eligibility criteria

Eligible records were peer-reviewed journal articles published from 2017 through 2026 with a verifiable DOI and Q1 journal status in a relevant subject category. Each source had to support at least one predefined domain: single-cell platform use, cellular heterogeneity, mechanism inference, differential sensitivity, resistance or persistence, immune or microenvironmental response, temporal or dose-dependent analysis, computational strategy, reproducibility, or translational boundary. Natural products included purified compounds, chemically defined natural-product preparations, extracts, and multi-

component traditional formulations. Their evidential interpretation remained chemically specific: an effect observed after a complex formulation was not reassigned to an individual constituent without direct evidence. Conference papers, preprints without a retained final journal version, theses, websites, patents, non-peer-reviewed reports, duplicate versions, DOI duplicates, and adjacent studies lacking a direct mapping role were excluded.

Information sources and search strategy

Searches combined single-cell terminology with natural-product, pharmacological, mechanistic, resistance, heterogeneity, immune, spatial, multimodal, temporal, computational, and translational terms. Variants included “single-cell RNA sequencing,” “scRNA-seq,” “single-cell transcriptomics,” “single-cell ATAC,” “scATAC,” “CyTOF,” “spatial transcriptomics,” “spatial multi-omics,” “cell state,” “subpopulation,” “differential sensitivity,” “resistance,” “persistence,” “mechanism of action,” and “tumor microenvironment.”

Searches were intentionally broader than individual final table categories so that selection was not driven toward a predetermined exhibit structure. Candidate records were checked through bibliographic, indexed, publisher, and DOI-linked scholarly records. Because the available search environment did not retain a reproducible export of every raw database hit, source-specific yield counts and raw duplicate-removal totals were not reconstructed retrospectively. The auditable review denominator therefore begins at the verified candidate registry rather than at an invented identification count.

Evidence selection

Screening proceeded from the verified candidate registry using publication eligibility, Q1 status, DOI integrity, direct article-domain relevance, evidence-role specificity, and version integrity. A record was retained only when it could support an identifiable manuscript claim, methodological decision, limitation, or mapping category rather than merely mentioning single-cell technology or natural products.

The verified registry contained 54 candidate records. Sixteen were not retained because of domain redundancy, journal-eligibility failure, weaker direct relevance, duplicate or version issues, or unresolved bibliographic-integrity concerns, leaving 38 included references. These 16 exclusions are registry-level decisions and are not recast as reason-specific full-text exclusions because a reproducible full-text denominator was unavailable. This distinction prevents the review flow from appearing more granular than the underlying audit trail permits.

Data charting and taxonomy development

A structured charting framework captured bibliographic identity, publication year, natural-product or formulation class, biological system, data source, intervention design, single-cell or spatial modality, computational analysis, focal pharmacological question, cell type or state, mechanism-related evidence, comparator, orthogonal validation, reported limitation, uncertainty source, and transferability boundary. Missing information was treated as unreported rather than inferred.

For synthesis, four evidence classes were proposed. Treatment-exposed single-cell evidence comprised experiments in which the intervention preceded cell-resolved profiling. Single-cell-informed pharmacology comprised studies using existing single-cell or spatial datasets to nominate a biological context, target, or response signature followed by separate pharmacological investigation. Methodological or benchmark evidence supported interpretation of platform, integration, statistical, or reproducibility constraints. Field synthesis evidence supported terminology, scope, and maturity assessment.

These categories were designed to protect a central evidential boundary. An atlas can identify a plausible responsive cell population without demonstrating that a natural product caused that state. Conversely, treatment-associated transcriptional change can establish a cell-resolved response without independently establishing direct molecular target engagement. Both observations are informative, but they occupy different levels of pharmacological inference.

Mapping and synthesis approach

The synthesis was descriptive and configurational rather than effect-size based. Studies were mapped first by evidence class, then by single-cell modality, natural-product class, biological setting, and pharmacological question. Within each stratum, interpretation focused on what the data resolved that a population-average assay would not: differential cell-state response, localization of a candidate mechanism, detection of rare or persistent subpopulations, immune or stromal remodeling, tissue-context dependence, temporal or dose-conditioned changes, or computational nomination of pharmacologically relevant states.

Mechanistic claims were subsequently graded conceptually according to the evidential transition they represented. Cell-type enrichment or differential expression was treated as association within a resolved cellular context. Concordant pathway activity strengthened biological plausibility but did not by itself establish target engagement. Orthogonal perturbation, protein-level measurement, localization, functional testing, or matched

mechanistic experimentation increased interpretive confidence when reported. This hierarchy is proposed as an organizing principle for the present map rather than as a validated scoring instrument.

Cross-study comparison also preserved difficult differences instead of normalizing them away. Purified compounds were separated from complex formulations; preclinical intervention studies from human tissue studies; treatment-exposed single-cell data from public-atlas analyses; and ordinary heterogeneous response from resistance or persistence. No pooled estimate was calculated because modalities, interventions, disease systems, sampling schemes, biological replicates, and validation strategies were too heterogeneous for a meaningful common effect metric. The resulting map therefore addresses where evidence exists, what inferential level it reaches, and which questions remain structurally underrepresented, rather than asking which natural product is “best.”

Evidence-selection results

The mapped corpus showed a field that is methodologically broader than its dominant experimental practice. Contemporary single-cell research encompasses transcriptomic, epigenomic, proteomic, spatial, and integrated multiomic approaches, but the natural-product evidence base was concentrated most strongly in transcriptome-resolved intervention studies [8]. The included literature ranged from purified compounds to extracts and multi-component traditional formulations and from cancer models to inflammatory, metabolic, cardiovascular, skeletal, infectious, and wound-healing systems.

The map was therefore interpreted by evidential function rather than publication count alone. Treatment-exposed studies were separated from single-cell-informed pharmacology using pre-existing atlases, while general methodological papers were retained only where they established analytical boundaries. Resistance-specific, human treatment-associated, temporally resolved, spatial, and genuinely multimodal studies constituted smaller evidence strata. Consequently, absence of a large mapped stratum was treated as an evidence-coverage gap rather than evidence that the corresponding pharmacological phenomenon is unimportant.

Single-cell platforms used in natural product pharmacology

Platform choice affects what constitutes an observable pharmacological response. Multicenter benchmarking has demonstrated that scRNA-seq technologies can differ in capture characteristics and measurement performance [9], while atlas-level integration benchmarks show that

computational harmonization itself can materially alter cross-dataset comparability [10]. Multimodal integration adds another layer because transcriptomic, chromatin, protein, and related measurements require modality-aware alignment rather than simple concatenation [11]. Accordingly, cross-study consistency cannot be inferred solely from the shared label “single-cell.”

Within the mapped natural-product literature, scRNA-seq was the principal platform for resolving treatment-associated cell identities, proportions, transcriptional states, and inferred communication. Epigenomic, spatial, protein-level, and combined designs were less frequent but provided additional information that transcript abundance alone cannot supply. Emerging computational models can learn representations across large single-cell multiomic collections [12], but their availability should not be mistaken for validated natural-product-specific pharmacological prediction.

The resulting platform landscape therefore suggests increasing technical capacity but uneven application. Transcriptomics currently provides the broadest empirical bridge between natural-product exposure and cellular heterogeneity, whereas multimodal designs offer deeper contextual resolution at substantially lower evidential density.

Cellular heterogeneity and state resolution

Treatment-exposed single-cell studies repeatedly showed why aggregate response is insufficient. In breast cancer, compound Kushen injection combined with chemotherapy was associated with cell-resolved changes in the tumor environment rather than a single uniform tumor response [13]. Shenmai injection combined with PD-1 inhibition similarly revealed altered antitumor immune states in a non-small-cell lung-cancer model [14]. These studies demonstrate heterogeneity of treatment-associated response, but combination designs limit attribution to the natural-product component alone.

Cannabidiol provides a stronger multimodal example because transcriptomic and chromatin-accessibility measurements jointly resolved macrophage and tumor-microenvironment changes in experimental colon cancer [15]. Outside oncology, artesunate treatment in experimental sepsis exposed heterogeneous liver-cell responses [16], while *Sanguisorba officinalis* extract altered inflammatory cell states and pathway-associated signals in experimental colitis [17].

Across these examples, cellular heterogeneity is pharmacologically meaningful when it changes interpretation of who responds, in what state, and within which microenvironment. The map does not support a universal cellular-response taxonomy. Rather, it indicates that similar aggregate outcomes may arise through distinct

cell populations and that treatment-associated state resolution should precede broad mechanistic generalization.

Mechanism-of-action inference

Single-cell studies were frequently used to move from a gross therapeutic phenotype toward a more localized mechanistic hypothesis. Cordycepin treatment, for example, altered tumor-immune states relevant to TIGIT blockade in a colon-cancer model [18]. Shi-Bi-Man treatment in psoriasis was associated with changes involving the IL-23/Th17 axis and endothelial activation [19], whereas rosmarinic acid produced cell-resolved changes related to oxidative stress and inflammation in experimental diabetic nephropathy [20]. Du-Zhong-Wan localized fracture-healing effects to bone-marrow stromal-cell biology and YAP/ β -catenin/VEGF-associated signaling [21], and Lapagyl was linked to Foxp3-positive regulatory T-cell and chemokine-associated responses after ultraviolet injury [22].

Some studies moved further by joining cell localization to orthogonal mechanistic tests. Specnuezhenide evidence linked protection against osteoporosis to METTL3 within LEPR-positive bone-marrow stromal cells [23]. Dihydroartemisinin studies in rheumatoid arthritis connected single-cell changes to a matrix-metalloproteinase-associated cellular environment [24]. Shen-Bai-Jie-Du decoction localized colorectal-tumor effects to immunosuppressive dendritic-cell differentiation and TMEM131-TNF-associated signaling [25]. Spatial multiomic analysis of Shenhua Tablet extended this logic by retaining renal anatomical context alongside molecular reprogramming [26], while eriodictyol experiments supported a ZBP1-related inflammatory and necrotic mechanism in acute pancreatitis [27].

The map nevertheless identifies a critical inferential boundary. Differential expression, pathway enrichment, or altered cell abundance identifies a cell-conditioned mechanistic hypothesis, not necessarily direct molecular engagement. This is especially important in single-cell-informed pharmacology: indirubin studies used disease-context single-cell and proteomic evidence to nominate

MAC1/RAGE-related biology before subsequent pharmacological testing [28], and ginsenoside Rb1 analysis used an existing single-cell dataset to help identify HO-1-associated context before separate ferroptosis-focused validation [29]. These studies can strengthen mechanistic reasoning, but the pre-existing atlas cannot itself demonstrate that the natural product produced the mapped cellular state.

Resistance and differential sensitivity

Resistance requires stricter evidence than the simple observation that different cells respond differently. Persistent or senescent states may survive an intervention without representing genetically or mechanistically defined drug resistance. In post-sepsis liver, single-cell analysis identified a senescence landscape relevant to intervention with dasatinib plus quercetin [30]. This establishes persistence-related pharmacological heterogeneity, but it does not make senescence synonymous with resistance.

Other recent studies illustrate differential sensitivity without directly testing resistant clones. Cycloviobuxine D altered immune populations across CyTOF and scRNA-seq measurements in hepatocellular carcinoma [31], while combined single-cell and spatial transcriptomics localized treatment-associated responses to *Tripterygium wilfordii* Polyglycosidium in ulcerative colitis [32]. Human granulation-tissue analysis after *Calvatia lilacina* treatment resolved wound-associated cellular programs with extensive orthogonal validation [33], and Shenfu Qiangxin pill studies decomposed HFpEF-associated responses by cardiac cell population [34].

The clearest resistance-focused example in the map was dihydroartemisinin exposure of isogenic *Plasmodium falciparum* carrying sensitive or resistant K13 states, which revealed stage- and genotype-conditioned transcriptional responses [35]. This supports a distinction between ordinary heterogeneous response, persistent state, differential sensitivity, and experimentally defined resistance.

Table 2 compares these evidence forms without treating them as interchangeable.

Table 2. Study characteristics distinguishing heterogeneous response, persistence, differential sensitivity, and direct resistance evidence

Evidence form	Representative system	Cell-resolved design	Primary pharmacological information	Principal boundary
Heterogeneous response	Cancer and inflammatory models	Treatment-exposed scRNA-seq	Unequal response among cell types or states	Does not by itself establish resistance
Persistent state	Post-sepsis liver	Single-cell state mapping with senolytic intervention	Persistence-associated vulnerable populations	Senescence is not equivalent to drug resistance
Differential sensitivity	HCC, ulcerative colitis, HFpEF, wound repair	scRNA, CyTOF, spatial or human-tissue profiling	Context-specific variation in intervention response	Population differences may remain mechanistically unresolved



Direct resistance comparison	Artemisinin-sensitive versus resistant malaria parasites	Isogenic, treatment-exposed single-cell transcriptomics	Genotype- and stage-conditioned response under drug pressure	Disease- and genotype-specific; not universally transferable
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Context-specific therapeutic response

Context specificity emerged across biological scale. A treatment-associated signal could depend on tissue architecture, immune composition, disease stage, cell differentiation state, combination therapy, or whether the cellular map was generated before or after exposure. A 2026 baicalein study illustrates the distinction particularly well: public single-cell and spatial datasets were integrated to derive a candidate non-small-cell lung-cancer signature, which was then interrogated experimentally [36]. The resulting evidence supports pharmacologically informed target prioritization, but the public atlas does not become an intervention-response experiment merely because baicalein is subsequently tested.

The same principle applies to immune and microenvironmental studies. Treatment-associated macrophage, dendritic-cell, T-cell, stromal, epithelial, or endothelial responses should be interpreted within the disease and sampling context that generated them. Human tissue evidence strengthens biological relevance but does not automatically establish comparative clinical effectiveness, while animal models can provide mechanistic resolution without guaranteeing human transferability.

Temporal and dose context are similarly important. Endpoint-only profiling can conflate transient adaptation, durable reprogramming, selective depletion, and population replacement. The mapped literature contains examples with dose or stage information, but comparatively few designs were structured specifically to distinguish these temporal alternatives.

Coverage gaps across tissues, diseases, and compound classes

The evidence base is concentrated in cancer, inflammatory disease, traditional Chinese medicine formulations, and immune-microenvironment questions. Purified compounds such as cannabidiol, artesunate, cordycepin, rosmarinic acid, specnuezhenide, dihydroartemisinin, eriodictyol, indirubin, ginsenoside Rb1, and baicalein provide chemically narrower interventions, whereas decoctions, extracts, pills, injections, and polyglycoside preparations introduce compositional complexity that limits constituent-level attribution.

The review map therefore reveals several neglected dimensions. Direct single-cell resistance studies are uncommon. Human intervention-associated data remain limited relative to preclinical models. Proteomic and epigenomic single-cell approaches are markedly less

represented than transcriptomics, and spatially resolved designs are only beginning to connect cellular state with tissue position. Longitudinal, repeated-dose, recovery, and post-treatment persistence designs are also sparse.

These gaps matter because different questions require different evidence. A transcriptomic endpoint can identify a responsive population; it cannot by itself determine whether that population was transiently perturbed, selectively eliminated, rendered resistant, displaced spatially, or causally responsible for the therapeutic phenotype.

RESULTS AND DISCUSSION

This mapping review indicates that single-cell technologies are changing the resolution of pharmacological inference more convincingly than they are changing the evidential rules for mechanism. Their immediate contribution is to reveal heterogeneity hidden by bulk measurements: which population changes, which state persists, which immune compartment is remodeled, and where a candidate mechanism appears most biologically plausible. The evidence is strongest when such localization is followed by orthogonal molecular or functional experiments.

A proposed interpretive hierarchy follows from the map. Cell-resolved association should be treated as the first evidential layer; pathway coherence as mechanistic plausibility; orthogonal molecular or functional testing as strengthened mechanism evidence; and direct perturbational or engagement evidence as a higher mechanistic standard. None of these layers alone establishes clinical effectiveness. This hierarchy is deliberately not a numerical score because the reviewed designs are too heterogeneous to justify a universal weighting scheme.

The map also suggests that the field should resist two symmetrical errors: treating single-cell complexity as mechanism simply because it is high dimensional, and dismissing cell-state associations because they are not yet causal. Properly bounded, such associations can identify the experiments needed to discriminate competing mechanistic explanations.

Methodological maturity and reproducibility

Single-cell pharmacology inherits the statistical and technical vulnerabilities of the underlying platforms. Differential-expression analyses can yield false discoveries when large numbers of cells are treated as independent biological replicates rather than preserving the



experimental unit [37]. Spatial technologies add their own reproducibility requirements, including explicit quality metrics and dataset-level assessment [38].

Accordingly, methodological maturity should be judged not by cell number alone but by biological replication, intervention controls, sampling design, batch handling,

transparent preprocessing, appropriate statistical units, independent validation, and fidelity between the claimed mechanism and the measurement actually performed.

Table 3 maps the methodological maturity domains that most strongly determine how far a single-cell natural-product study can be interpreted.

Table 3. Evidence-maturity map for interpreting single-cell natural-product pharmacology

Maturity domain	Stronger evidence pattern	Common failure mode	Interpretation protected
Experimental unit	Independent biological replication with matched controls	Treating cells as independent subjects	Statistical validity
Exposure design	Defined treatment, dose, timing, and comparator	Unclear intervention context	Pharmacological attribution
Cellular measurement	Modality appropriate to the biological question	Equating transcription with protein or function	Assay interpretation
Mechanistic validation	Orthogonal perturbation, protein, localization, or functional testing	Pathway enrichment presented as mechanism	Causal restraint
Data integration	Transparent batch handling and modality-aware analysis	Over-harmonization or dataset-driven clusters	Cross-study comparability
Spatial analysis	Explicit quality assessment and anatomical validation	Colocalization presented as causal signaling	Tissue-context inference
Translation	Independent models or relevant human material	Preclinical response presented as clinical efficacy	Transferability

Priority gaps for translational pharmacology

The highest-priority advance is not simply more single-cell sequencing. Future studies should link chemically characterized interventions to replicated biological designs, dose and time structure, cell-resolved profiling, and orthogonal mechanism testing. Complex natural-product formulations require especially careful attribution because a cell-specific response to the mixture cannot automatically be assigned to an individual constituent.

Resistance studies should deliberately sample pre-treatment, early response, residual, and recurrent states where biologically feasible. Multimodal designs should be chosen when the additional modality addresses a specific uncertainty—for example, chromatin accessibility for regulatory state, CyTOF for protein-defined immune phenotypes, or spatial measurements for tissue localization—rather than added solely for analytical sophistication.

Translational progress also requires more human material, independent cohorts, and explicit separation of predictive signatures from measured treatment responses. Public single-cell atlases are valuable hypothesis-generating resources, but prospective treatment-exposed validation remains necessary before a candidate cellular signature can be regarded as a pharmacodynamic or response marker.

Review limitations

This map is constrained by the heterogeneity of both natural-product chemistry and single-cell study design. Purified molecules, botanical extracts, injections, decoctions, and multi-component formulations cannot be assumed to represent equivalent intervention classes.

Disease models, sampling times, platforms, preprocessing strategies, validation experiments, and biological replication also vary substantially.

The review further distinguishes a verified candidate registry from unavailable raw-search denominators. Because complete database-level yields and reason-specific full-text counts could not be reconstructed reproducibly, they were not estimated. The selection figure therefore reports only auditable counts.

The literature is also weighted toward preclinical and transcriptomic studies. Consequently, the map may characterize current publication concentration more strongly than the underlying biological opportunity space. Finally, single-cell association, computational target nomination, spatial colocalization, and pathway enrichment were not interpreted as causal mechanism unless supported by the corresponding experimental evidence.

CONCLUSION

Single-cell technologies have begun to reposition natural-product pharmacology from population-averaged response toward cell-, state-, and context-resolved interpretation. The mapped evidence shows particular value for identifying heterogeneous therapeutic responses, localizing candidate mechanisms, resolving immune and microenvironmental remodeling, and distinguishing persistent or resistant populations.

The field remains methodologically asymmetric. Transcriptomics is substantially more developed than proteomic, epigenomic, spatial, and multimodal



applications, while human, longitudinal, dose-resolved, and resistance-focused studies remain comparatively limited. The principal opportunity is therefore not to treat single-cell resolution as mechanistic proof, but to use it to formulate more discriminating pharmacological hypotheses and to connect those hypotheses to appropriate orthogonal validation. Progress toward translational use will depend on preserving the distinction between cellular association, mechanistic evidence, predictive interpretation, and demonstrated therapeutic response.

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REFERENCES

- [1] Atanasov AG, Zotchev SB, Dirsch VM, International Natural Product Sciences Taskforce, Supuran CT. Natural products in drug discovery: Advances and opportunities. *Nat Rev Drug Discov.* 2021;20(3):200-16. doi:10.1038/s41573-020-00114-z
- [2] Zhu Y, Ouyang Z, Du H, Wang M, Wang J, Sun H, et al. New opportunities and challenges of natural products research: When target identification meets single-cell multiomics. *Acta Pharm Sin B.* 2022;12(11):4011-39. doi:10.1016/j.apsb.2022.08.022
- [3] Van de Sande B, Lee JS, Mutasa-Gottgens E, Naughton B, Bacon W, Manning J, et al. Applications of single-cell RNA sequencing in drug discovery and development. *Nat Rev Drug Discov.* 2023;22(6):496-520. doi:10.1038/s41573-023-00688-4
- [4] Jiang B, Si J, Luo H, Le L. Single-cell omics reveal the mechanisms of traditional Chinese medicines. *Phytomedicine.* 2025;147:157204. doi:10.1016/j.phymed.2025.157204
- [5] Chen X, Liu J, Lu P, Zhou J, Jiang L, Zhai Y, et al. Unraveling traditional Chinese medicine with single-cell RNA sequencing: Current applications and future frontiers. *Phytomedicine.* 2025;149:157556. doi:10.1016/j.phymed.2025.157556
- [6] Vandereyken K, Sifrim A, Thienpont B, Voet T. Methods and applications for single-cell and spatial multi-omics. *Nat Rev Genet.* 2023;24(8):494-515. doi:10.1038/s41576-023-00580-2
- [7] Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation. *Ann Intern Med.* 2018;169(7):467-73. doi:10.7326/M18-0850
- [8] Lim J, Park C, Kim M, Kim H, Kim J, Lee DS. Advances in single-cell omics and multiomics for high-resolution molecular profiling. *Exp Mol Med.* 2024;56(3):515-26. doi:10.1038/s12276-024-01186-2
- [9] Chen W, Zhao Y, Chen X, Yang Z, Xu X, Bi Y, et al. A multicenter study benchmarking single-cell RNA sequencing technologies using reference samples. *Nat Biotechnol.* 2021;39(9):1103-14. doi:10.1038/s41587-020-00748-9
- [10] Luecken MD, Büttner M, Chaichoompu K, Danese A, Interlandi M, Mueller MF, et al. Benchmarking atlas-level data integration in single-cell genomics. *Nat Methods.* 2022;19(1):41-50. doi:10.1038/s41592-021-01336-8
- [11] Hao Y, Hao S, Andersen-Nissen E, Mauck WM 3rd, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. *Cell.* 2021;184(13):3573-87.e29. doi:10.1016/j.cell.2021.04.048
- [12] Cui H, Wang C, Maan H, Pang K, Luo F, Duan N, et al. scGPT: Toward building a foundation model for single-cell multi-omics using generative AI. *Nat Methods.* 2024;21(8):1470-80. doi:10.1038/s41592-024-02201-0
- [13] Liu X, Bai M, Li H, Ye P, Duan X, Wu C, et al. Single-cell RNA-sequencing uncovers compound kushen injection synergistically improves the efficacy of chemotherapy by modulating the tumor environment of breast cancer. *Front Immunol.* 2022;13:965342. doi:10.3389/fimmu.2022.965342
- [14] Yu D, Yang P, Lu X, Huang S, Liu L, Fan X. Single-cell RNA sequencing reveals enhanced antitumor immunity after combined application of PD-1 inhibitor and Shenmai injection in non-small cell lung cancer. *Cell Commun Signal.* 2023;21(1):169. doi:10.1186/s12964-023-01184-3
- [15] Sun X, Zhou L, Wang Y, Deng G, Cao X, Ke B, et al. Single-cell analyses reveal cannabidiol rewires tumor microenvironment via inhibiting alternative activation of macrophage and synergizes with anti-PD-1 in colon cancer. *J Pharm Anal.* 2023;13(7):726-44. doi:10.1016/j.jpha.2023.04.013
- [16] He XL, Chen JY, Feng YL, Song P, Wong YK, Xie LL, et al. Single-cell RNA sequencing deciphers the mechanism of sepsis-induced liver injury and the therapeutic effects of artesunate. *Acta Pharmacol Sin.* 2023;44(9):1801-14. doi:10.1038/s41401-023-01065-y
- [17] Li C, Gong L, Jiang Y, Huo X, Huang L, Lei H, et al. Sanguisorba officinalis ethyl acetate extract attenuates ulcerative colitis through inhibiting PI3K-AKT/NF-κB/STAT3 pathway uncovered by single-cell RNA

- sequencing. *Phytomedicine*. 2023;120:155052. doi:10.1016/j.phymed.2023.155052
- [18] Chen R, Feng C, Chen L, Zheng X, Fang W, Wu S, et al. Single-cell RNA sequencing indicates cordycepin remodels the tumor immune microenvironment to enhance TIGIT blockade's anti-tumor effect in colon cancer. *Int Immunopharmacol*. 2024;126:111268. doi:10.1016/j.intimp.2023.111268
- [19] Zhang C, Cao X, Zhao L, Ni Z, Du H, Qu J, et al. Traditional Chinese medicine Shi-Bi-Man ameliorates psoriasis via inhibiting IL-23/Th17 axis and CXCL16-mediated endothelial activation. *Chin Med*. 2024;19(1):38. doi:10.1186/s13020-024-00907-z
- [20] Chen J, Zhang Q, Guo J, Gu D, Liu J, Luo P, et al. Single-cell transcriptomics reveals the ameliorative effect of rosmarinic acid on diabetic nephropathy-induced kidney injury by modulating oxidative stress and inflammation. *Acta Pharm Sin B*. 2024;14(4):1661-76. doi:10.1016/j.apsb.2024.01.003
- [21] Dong R, Wei J, Tian S, Wang J, Ma Y, Li Y, et al. Single-cell RNA transcriptomics reveals Du-Zhong-Wan promotes osteoporotic fracture healing via YAP/ β -catenin/VEGF axis in BMSCs. *Phytomedicine*. 2024;135:155572. doi:10.1016/j.phymed.2024.155572
- [22] Xie Y, Yang A, Li N, Zheng H, Zhong Y, Jin Y, et al. Lapagyl mitigates UV-induced inflammation and immunosuppression via Foxp3⁺ Tregs and CCL pathway: A single-cell transcriptomics study. *Phytomedicine*. 2024;129:155679. doi:10.1016/j.phymed.2024.155679
- [23] Wei J, Dong R, Ma Y, Wang J, Tian S, Tu X, et al. Single-cell sequencing reveals that specnuezhenide protects against osteoporosis via activation of METTL3 in LEPR⁺ BMSCs. *Eur J Pharmacol*. 2024;981:176908. doi:10.1016/j.ejphar.2024.176908
- [24] Guo Q, Wang Q, Chen J, Zhao M, Lu T, Guo Z, et al. Dihydroartemisinin regulated the MMP-mediated cellular microenvironment to alleviate rheumatoid arthritis. *Research (Wash D C)*. 2024;7:0459. doi:10.34133/research.0459
- [25] Tao Y, Ma Y, Gu L, Zhang Y, Zhang Q, Zhou L, et al. Single-cell RNA sequencing reveals Shen-Bai-Jie-Du decoction retards colorectal tumorigenesis by regulating the TMEM131-TNF signaling pathway-mediated differentiation of immunosuppressive dendritic cells. *Acta Pharm Sin B*. 2025;15(7):3545-60. doi:10.1016/j.apsb.2025.05.013
- [26] Yin F, Li P, Liu C, Zheng Y, Yan G, Wang M, et al. Spatially resolved multi-omics reveals the renal cortex-metabolic reprogramming of Shenhua Tablet for intervention on IgA nephropathy. *Phytomedicine*. 2025;141:156742. doi:10.1016/j.phymed.2025.156742
- [27] Tian S, Xin G, Zhang K, Wen A, Yu X, Ni C, et al. Eriodictyol from *Scutellariae Barbata* alleviates inflammation and necrosis via ZBP1-dependent signaling in acute pancreatitis. *Phytomedicine*. 2025;145:156926. doi:10.1016/j.phymed.2025.156926
- [28] Lin J, Wang C, Yang G, Liu Y, Da Y, Zhou W, et al. Targeting the cell adhesion related ligands MAC1 with indirubin to inhibit AGE-RAGE signaling and mitigate colitis in an mouse model. *Phytomedicine*. 2025;149:157504. doi:10.1016/j.phymed.2025.157504
- [29] He S, Ye H, Wang Q, He Y, Liu X, Song J, et al. Ginsenoside Rb1 targets to HO-1 to improve sepsis by inhibiting ferroptosis. *Free Radic Biol Med*. 2025;226:13-28. doi:10.1016/j.freeradbiomed.2024.11.007
- [30] Lavarti R, Cai L, Alvarez-Diaz T, Medina-Rodriguez T, Bombin S, Raju RP. Senescence landscape in the liver following sepsis and senolytics as potential therapeutics. *Aging Cell*. 2025;24(1). doi:10.1111/ace.14354
- [31] Ma F, Zhang L, Fan Y, Xu Z, He Y, Sun J, et al. Integrating CyTOF with scRNA-seq reveals that Cyclovirobuxine D inhibits HCC progression through hepatic immune microenvironment remodeling. *Phytomedicine*. 2026;150:157751. doi:10.1016/j.phymed.2025.157751
- [32] Shu J, Qin D, Tu W, Zhao D, Yang Q, Shao S, et al. Single-cell and spatially resolved transcriptomics elucidate therapeutic mechanism of *Tripterygium wilfordii* Polyglycosidum in ulcerative colitis. *Phytomedicine*. 2026;150:157569. doi:10.1016/j.phymed.2025.157569
- [33] He T, Wang K, Mo R, Guo J, Jiang B, Mu R, et al. Single-cell RNA sequencing reveals the therapeutic mechanism of *Calvatia lilacina* in promoting wound healing of anal fistula. *Chin Med*. 2026;21(1):14. doi:10.1186/s13020-025-01293-w
- [34] Yang Y, Gao H, Fu Z, Hu W, Li Y, Li H, et al. Single-cell transcriptomics reveals how Shenfu Qiangxin pill ameliorates HFpEF by modulating cardiac cellular heterogeneity. *Chin Med*. 2026;21:199. doi:10.1186/s13020-026-01456-3
- [35] Connelly SV, Cunningham C, Petrow I, Rustamzade N, Zuromski J, Sipolski DM, et al. Single-cell transcriptional changes of artemisinin-sensitive K13C580 and artemisinin-resistant K13580Y *Plasmodium falciparum* upon dihydroartemisinin exposure. *Front Cell Infect Microbiol*. 2026;16:1816105. doi:10.3389/fcimb.2026.1816105

- [36] Wu D, Liu C, Zhan L, Liang M, Li Q, Lian Q. Integrative single-cell and spatial transcriptomics analysis reveals a baicalein-responsive 10-gene signature for non-small cell lung cancer. *Transl Oncol.* 2026;70:102853. doi:10.1016/j.tranon.2026.102853
- [37] Squair JW, Gautier M, Kathe C, Anderson MA, James ND, Hutson TH, et al. Confronting false discoveries in single-cell differential expression. *Nat Commun.* 2021;12(1):5692. doi:10.1038/s41467-021-25960-2
- [38] Plummer JT, Dezem FS, Cook DP, Park J, Zhang L, Liu Y, et al. Standardized metrics for assessment and reproducibility of imaging-based spatial transcriptomics datasets. *Nat Biotechnol.* 2026;44(7):1213-25. doi:10.1038/s41587-025-02811-9