Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2026, Volume 6, Issue 1, Page No: 131-141

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Single-Cell and Spatial Technologies Enter Natural-Product Pharmacology: A State-of-the-Art Review of Heterogeneous Response, Cell-State Selectivity, and Mechanistic Resolution

Charlotte Dupuis[1]*, Hugo Perrin[1], Elise Morel[2], Thomas Martin[3]

[1] Department of Single-Cell Pharmacology and Natural Products, Faculty of Pharmacy, University of Lille, Lille, France.
[2] Department of Spatial Technologies and Cell-State Selectivity, Faculty of Pharmacy, University of Montpellier, Montpellier, France.
[3] Department of Mechanistic Resolution in Natural-Product Pharmacology, Faculty of Pharmacy, University of Nantes, Nantes, France.

***E-mail** ✉ charlotte.dupuis@univ-lille.fr
Received: 09 February 2026; **Revised:** 24 May 2026; **Accepted:** 26 May 2026

ABSTRACT

Natural-product pharmacology has conventionally inferred activity from averaged biochemical, cellular, tissue, or organism-level measurements. These approaches remain indispensable, but averaging can obscure which cellular states respond, which remain refractory, where pharmacologically relevant species accumulate, and whether apparent pathway normalization reflects direct action or changing cellular composition. Single-cell and spatial technologies create a complementary analytical layer by resolving treatment-associated states, intercellular relationships, tissue neighborhoods, and local molecular distributions. This state-of-the-art review examines how these technologies are entering natural-product research, with particular attention to heterogeneous response, cell-state selectivity, tissue context, and the evidential steps required for stronger mechanistic interpretation. Current applications span defined phytochemicals, complex herbal interventions, perturbational transcriptomics, spatial transcriptomics, and spatial metabolomics. Collectively, the literature shows that cell-resolved measurements can reveal pharmacological responses concealed by bulk assays and can prioritize cellular compartments or interactions for subsequent validation. However, cell-state association does not itself establish causal mechanism, formula-level effects cannot automatically be assigned to individual constituents, and spatial colocalization does not prove target engagement. We therefore propose an evidence-bounded interpretation architecture in which cellular response, local exposure, pathway perturbation, and orthogonal mechanistic validation remain distinct until experimentally connected. The near-term opportunity is not simply to add single-cell or spatial assays to natural-product studies, but to design perturbation experiments in which chemical identity, dose, time, cellular state, tissue location, and validation are jointly interpretable.

Keywords: Natural products, Single-cell transcriptomics, Spatial omics, Pharmacology, Cell-state selectivity, Heterogeneous drug response

How to Cite This Article: Dupuis C, Perrin H, Morel E, Martin T. Single-Cell and Spatial Technologies Enter Natural-Product Pharmacology: A State-of-the-Art Review of Heterogeneous Response, Cell-State Selectivity, and Mechanistic Resolution. Spec J Pharmacogn Phytochem Biotechnol. 2026;6(1):131-41. https://doi.org/10.51847/9vTnALJMXq

Introduction

Natural products occupy an unusual position in contemporary pharmacology. Their chemical diversity provides valuable starting points for drug discovery, yet their biological effects are frequently studied through assays that compress multicellular systems into one concentration, one pathway score, or one average transcriptional response. Single-cell RNA sequencing (scRNA-seq) has expanded drug discovery by allowing target biology, pharmacological response, resistance, and biomarker hypotheses to be examined at the level of heterogeneous cellular populations rather than only bulk samples [1]. This distinction is especially important when a treatment

changes the abundance of a cell population while simultaneously changing transcription within that population: the same bulk signal can arise from biologically different processes.

Natural-product research is beginning to exploit this resolution. Recent synthesis of traditional Chinese medicine studies documents increasing use of scRNA-seq to interrogate cell-type-specific responses, disease-associated subpopulations, immune remodeling, and candidate mechanisms after treatment with herbal medicines or their constituents [2]. The technological premise is broader than descriptive cell atlasing. Perturbational platforms such as sci-Plex demonstrated that large numbers of chemical perturbations can be multiplexed and read transcriptionally at single-cell resolution, making heterogeneous chemical response itself an experimentally tractable object [3].

Spatial information adds a second missing coordinate. Integrating single-cell and spatial transcriptomics can distinguish cellular identity from tissue position and relate inferred cellular states to neighboring populations and local microenvironments [4]. For natural-product pharmacology, the conceptual opportunity is therefore not merely higher-dimensional profiling. It is a shift from asking whether a compound, extract, or formula changes a tissue-average endpoint toward asking which cells change, in what state, at what location, after what exposure, and with what evidence that the observed state participates in the pharmacological mechanism. This review evaluates that shift while maintaining a strict distinction between response localization, mechanistic nomination, causal validation, and translational inference.

What bulk natural-product pharmacology cannot resolve

Bulk measurements remain appropriate when the relevant biological unit is genuinely homogeneous or when the objective is an integrated tissue-level endpoint. Their limitation appears when pharmacological response is distributed unevenly across cell types or states. Contemporary single-cell and spatial analyses of drug response and resistance show that treatment-sensitive, adaptive, resistant, stromal, and immune programs can coexist within the same specimen, meaning that a tissue-average expression change can conceal both responding and nonresponding compartments [5]. A bulk decrease in an inflammatory transcript, for example, cannot by itself distinguish transcriptional suppression within macrophages from depletion, redistribution, or phenotypic replacement of the macrophage population.

Natural-product experiments already illustrate this problem. In diabetic nephropathy, rosmarinic-acid treatment was examined by scRNA-seq alongside additional molecular measurements, revealing nonuniform responses across renal epithelial and immune populations, including proximal tubular, podocyte, macrophage, and natural-killer-cell compartments [6]. The value of the single-cell layer is not that every differential gene becomes mechanistic evidence; rather, it identifies where a whole-kidney treatment effect is concentrated and which cellular hypotheses warrant orthogonal testing.

Three-dimensional organization introduces another source of hidden variation. Spatially engineered spheroid experiments have shown that cells occupying different positions can exhibit different transcriptomic responses to the same drug exposure, plausibly reflecting gradients in penetration, metabolism, nutrients, oxygenation, or stress [7]. Although this example is not natural-product-specific, the experimental principle is directly relevant to compounds whose solubility, binding, metabolism, or tissue access may be heterogeneous.

Finally, apparent cellular heterogeneity can itself be technically produced. Cell recovery, ambient RNA, doublets, normalization, batch structure, and integration choices can change the observed single-cell landscape [8]. Thus, replacing a bulk assay with scRNA-seq does not remove uncertainty; it redistributes uncertainty from averaging toward sampling, measurement, annotation, and inference. The appropriate comparison is therefore not “bulk versus single cell” as competing technologies, but whether the biological question requires information that averaging necessarily discards.

Single-cell technologies entering the field

The most direct entry point is treatment-linked scRNA-seq in which the administered intervention and sampled biological system are experimentally connected. In sepsis-induced acute lung injury, a study of Yantiao Formula combined chemical characterization with scRNA-seq and experimental validation, using cell-resolved data to examine changes in the pulmonary immune environment and macrophage polarization [9]. Such designs can localize formula-associated responses, but constituent–target attribution remains a separate problem: predicted or docked formula constituents do not become validated causal ligands merely because their candidate pathways overlap a responding cell state.

Defined natural products permit tighter attribution. Syringin has been investigated in experimental rheumatoid arthritis using single-cell analysis to resolve macrophage–fibroblast-like-synoviocyte interactions in the treated inflammatory environment [10]. Cryptotanshinone studies likewise illustrate how scRNA-derived immune-state information can be combined with experimental pharmacology to investigate treatment-associated remodeling rather than relying only on bulk inflammatory markers [11]. These studies shift the evidential unit from "the tissue responded" to "specific cellular compartments and communication programs changed after a defined intervention."

The strongest mechanistic use of single-cell data occurs when the technology is embedded inside a larger validation chain. Dihydrotanshinone I was studied using biochemical target-engagement approaches, metabolomics, co-culture experiments, and scRNA-seq, allowing cell-resolved tumor-microenvironment changes to be interpreted alongside independent evidence concerning PHGDH inhibition and serine metabolism [12]. Similarly, multi-omic investigation of tubeimoside II combined single-cell analysis with other molecular measurements while examining its interaction with anti-PD-1 treatment [13]. Neither design implies that scRNA-seq alone proves mechanism; rather, single-cell data become more informative when molecular perturbation, cell-state change, and functional outcome can be triangulated.

The resulting evidence configurations differ sufficiently that they should not be treated as interchangeable. **Table 1** summarizes what each configuration can legitimately resolve and where its mechanistic interpretation should stop.

Table 1. Evidence configurations emerging from single-cell natural-product pharmacology

Evidence configuration	Primary information gained	Mechanistic value	Principal interpretation boundary
Defined compound + scRNA-seq	Cell-type and state-specific treatment response	Localizes responsive compartments	Differential expression alone does not identify the causal target
Formula/extract + scRNA-seq	Multicellular response to a complex intervention	Resolves responder populations and communication changes	Effects cannot automatically be assigned to one constituent
Single-cell + orthogonal molecular assays	Cell-state change linked to independent biochemical evidence	Supports mechanistic triangulation	Concordance still requires perturbational testing of the proposed link
Multiplex chemical perturbation	Response distributions across compounds, doses, or states	Reveals heterogeneous perturbation structure	Transcriptomic similarity is not functional equivalence
Re-analysis/target nomination	Candidate vulnerable states or pathways	Generates prioritization hypotheses	Prediction or colocalization is not pharmacological validation

Note: The categories synthesize distinct evidential roles represented in the reviewed literature. They are analytical groupings, not validated evidence grades.

Spatial technologies and tissue context

Single-cell dissociation resolves cellular identity at the cost of much native positional information. Spatial technologies address a complementary question: whether treatment-associated cells, transcripts, proteins, metabolites, or chemical species occupy biologically meaningful tissue locations. Current spatial multi-omics methods differ in molecular coverage, resolution, tissue compatibility, and whether cellular identity is measured directly or computationally inferred [14]. For pharmacology, this means that "spatially resolved" is not a single evidential category; the relevant resolution must match the proposed biological claim.

Natural-product research offers an instructive example through spatial metabolomics. In a zebrafish model, wedelolactone and demethylwedelolactone were examined using mass-spectrometry imaging integrated with liver transcriptomics, enabling investigators to map distributions of parent compounds and metabolites while relating treatment to endogenous metabolic changes [15]. This contributes a pharmacologically important layer that transcriptomics alone cannot supply: where chemical species are detected. Yet the transcriptomic component was liver-level rather than single-cell-resolved, so spatial chemical localization should not be misrepresented as proof that a particular cell type experienced a defined intracellular exposure.

Spatial transcriptomics can instead locate multicellular mechanisms. In thyroid cancer, spatial transcriptomic analysis contributed to identifying a cancer-associated-fibroblast program linked to tumor dedifferentiation;

subsequent co-culture and functional work examined the proposed metabolic interaction and resveratrol as an intervention [16]. Here, spatial evidence gains mechanistic value because tissue localization is connected to experiments that interrogate the proposed interaction, although clinical transfer remains unresolved.

A different use is discovery rather than post-treatment mechanism. Single-cell and spatial profiling of inflammatory breast cancer identified heterogeneous immune and tumor compartments and was followed by screening of a natural-product collection, nominating sanguinarine and α-mangostin as candidate immunomodulatory agents [17]. Such work illustrates a reverse direction of inference: spatial biology defines a vulnerability, after which natural products are screened against it. The nominated compounds still require exposure, target-engagement, and context-specific validation.

Likewise, studies combining single-cell and spatial target mapping with virtual screening can connect tissue-contextualized target hypotheses to natural-product chemical space [18]. Their evidential endpoint is candidate prioritization rather than demonstrated pharmacology. The distinction matters because spatial proximity, target expression, chemical docking, and treatment response occupy different layers of evidence.

Figure 1 integrates the review's proposed interpretation architecture: cell-state and spatial resolution reduce specific ambiguities of bulk pharmacology, but stronger natural-product mechanism claims require explicit passage through exposure and validation boundaries rather than simple accumulation of omics layers.

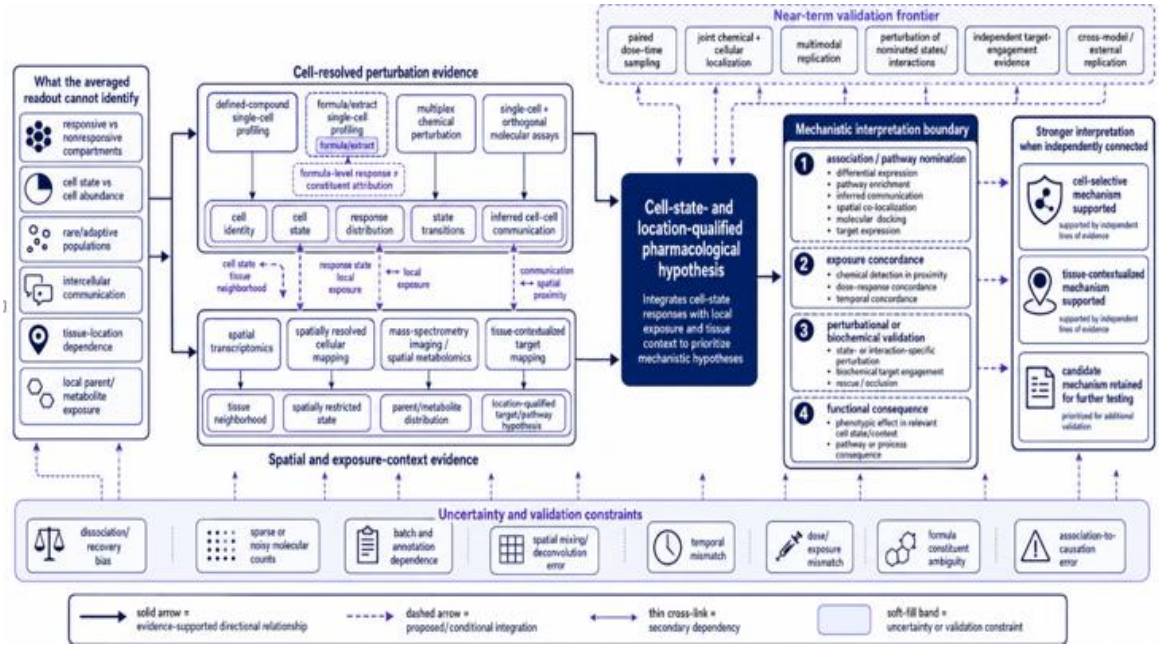

Figure 1. From averaged activity to cell-state- and location-qualified natural-product pharmacology

Cell-state selectivity and heterogeneous drug response

Cell-state selectivity is more specific than cell-type selectivity. Two cells assigned to the same lineage can occupy transcriptionally, metabolic, proliferative, stressed, differentiated, or treatment-adapted states that differ in pharmacological susceptibility. This distinction is increasingly consequential for drug-response modeling. PERCEPTION demonstrated that single-cell tumor transcriptomes can be used to model treatment response and resistance while explicitly retaining intratumoral cellular heterogeneity [19]. Cellular adaptation under treatment can also progress along structured continua rather than dividing cleanly into sensitive and resistant populations [20]. These observations argue against interpreting a natural product as having one invariant "effect on macrophages," "effect on epithelial cells," or "effect on cancer cells"; the relevant unit may instead be a particular state within that lineage.

This review therefore proposes four analytically distinct forms of cell-state selectivity: response enrichment, in which a pre-existing state responds more strongly than neighboring states; state displacement, in which treatment changes the abundance of a state; state transition, in which cells move along an adaptive or differentiation trajectory; and state persistence, in which a subpopulation remains comparatively refractory. These categories are interpretations to be tested, not properties that can be inferred solely from cluster-wise differential expression. Computational models add another qualification: benchmarking of single-cell drug-response models shows

substantial dependence on dataset, task, representation, and evaluation design, so model-derived sensitivity should not be treated as an experimentally established cellular phenotype [21].

Natural-product experiments illustrate why these distinctions matter. Single-cell analysis of the multicomponent natural product Shi-Bi-Man localized treatment-associated changes to hair-follicle stem and dermal-papilla-cell compartments and implicated altered intercellular signaling [22]. Yet a change in population size, pathway activity, or inferred communication can have several explanations, including selective survival, proliferation, differentiation, migration, or transcriptional reprogramming. Resolving heterogeneous natural-product response therefore requires both a cellular denominator and a mechanistic comparator: which state existed before treatment, what became of it after treatment, and whether the inferred transition survives independent experimental interrogation.

Table 2 compares the principal technologies by the pharmacological question they can answer and, equally importantly, by the inference they cannot establish on their own.

Table 2. Analytical capabilities and interpretation boundaries of cell-resolved technologies in natural-product pharmacology

Technology or analysis	Pharmacological information resolved	Particularly useful for	Interpretation boundary
scRNA-seq	Cell identities and transcriptional states	Locating heterogeneous treatment responses	Expression change is not target engagement
Multiplex perturbational scRNA-seq	Compound-, dose-, or condition-linked state responses	Comparing response distributions and adaptation	Transcriptomic similarity is not pharmacological equivalence
Spatial transcriptomics	Transcript-defined states with tissue position	Mapping neighborhoods and spatial response programs	Spatial association is not causal interaction
Spatial metabolomics / mass-spectrometry imaging	Local parent, metabolite, or endogenous molecular distributions	Relating chemical exposure to tissue regions	Detection does not identify intracellular target or responding cell
Integrated single-cell–spatial analysis	Cell states interpreted within tissue architecture	Connecting heterogeneity, neighborhood, and treatment response	Computational integration inherits modality-specific uncertainty

Note: The distinctions are an analytical synthesis proposed for interpretation; they are not a validated ranking of technologies.

Mechanistic opportunities created by cell-resolved analysis

The principal mechanistic opportunity is localization of the causal question. Instead of testing whether an intervention changes an entire tissue, investigators can nominate a responsive cell state, its interacting partners, and a restricted pathway for subsequent perturbation. Single-cell spatial multi-omics has demonstrated that treatment resistance can be associated with multicellular microenvironmental configurations rather than only tumor-cell-intrinsic programs [23]. Such evidence narrows where mechanism should be sought, but the spatially associated state remains a candidate mediator until it is experimentally perturbed.

Perturbational single-cell methods offer a second advance because chemical treatment becomes part of the experimental design rather than a retrospective label. Multiplex chemical-genomics approaches can resolve heterogeneous cellular responses to targeted perturbations and identify state-dependent pathway dependencies [24]. Genome-scale Perturb-seq extends the same logic to genetic perturbation, providing a route for testing whether genes or regulatory programs nominated from natural-product response maps are functionally necessary for a phenotype [25]. In a natural-product workflow, these methods are most valuable after a compound-associated cellular state has been observed: genetic perturbation can distinguish a pathway that merely changes alongside treatment from one that modifies the response.

Spatial response modeling adds a third layer. SpaRx was developed to infer spatial heterogeneity of drug response from spatial and single-cell transcriptomic information [26]. The relevant opportunity for natural-product pharmacology is not to convert such predictions directly into efficacy claims, but to use them to prioritize tissue regions or cellular neighborhoods for measurement and validation. This is particularly important when local exposure may differ from nominal dose.

The proposed mechanistic chain is therefore deliberately discontinuous: chemical intervention → observed cellular response → nominated state/pathway → independent perturbation or target-engagement evidence →

functional consequence. No single omics layer spans that chain automatically. Ligand–receptor inference can prioritize intercellular communication; trajectory analysis can nominate transitions; spatial analysis can nominate neighborhoods; and metabolite imaging can qualify local exposure. Their mechanistic value increases when these independent measurements address competing explanations rather than simply reproduce the same association in different feature spaces.

Current applications in natural-product research

Natural-product research entered the cell-resolved domain before the present surge in transcriptomic studies. High-resolution phenotypic profiling demonstrated that natural products can generate heterogeneous single-cell phenotypes that are obscured by population averages, establishing an early experimental precedent for treating within-sample variation as pharmacological information [27]. More recent reviews of traditional Chinese medicine and single-cell omics document a widening application space that includes immune remodeling, disease-associated cellular subpopulations, intercellular communication, target nomination, and treatment-response characterization [28]. A complementary synthesis emphasizes that single-cell measurements are increasingly being combined with transcriptomic, metabolomic, network, and pharmacological evidence rather than used as isolated descriptive atlases [29].

The field nevertheless remains heterogeneous in evidential maturity. Some studies administer a chemically defined phytochemical and profile the responding tissue; others investigate an extract or formula whose pharmacologically active species remain only partly resolved. Still others begin with a disease atlas and subsequently search natural-product space for compounds predicted or experimentally screened to perturb a selected state. These designs answer different questions. The broader natural-product discovery literature already emphasizes that chemical identification, biological activity, mechanism, and translational development are separate stages of evidence [30]; single-cell and spatial technologies increase resolution within those stages but do not erase the distinctions between them.

Table 3 organizes the current application landscape according to what has been achieved and which evidential link most commonly remains open.

Table 3. Evidence synthesis and unresolved links in current cell-resolved natural-product applications

Application pattern	Principal contribution	Evidence that can be strengthened	Characteristic unresolved link
Defined phytochemical treatment	Locates compound-associated responder states	Orthogonal biochemical or genetic validation	Responsive state → causal molecular target
Formula or extract treatment	Resolves multicellular response to the administered material	Exposure-resolved constituent analysis	Formula response → responsible chemical species
Disease atlas followed by natural-product screening	Prioritizes state-specific candidate interventions	Prospective perturbational testing	Target/state nomination → demonstrated pharmacology
Spatial chemical mapping	Qualifies regional exposure and metabolism	Co-registered cell-state measurements	Chemical localization → intracellular response
Multimodal mechanistic study	Triangulates cellular, molecular, and functional evidence	Independent replication and perturbation	Concordant association → causal mechanism

Note: "Unresolved link" denotes the next evidential connection required; it does not imply that every study must use every modality.

The evidence accumulated across these applications supports a second synthesis complementary to **Figure 1**. Rather than organizing technologies by modality, **Figure 2** maps natural-product studies by the evidential distance between an administered material and a mechanistically qualified cell-state response.

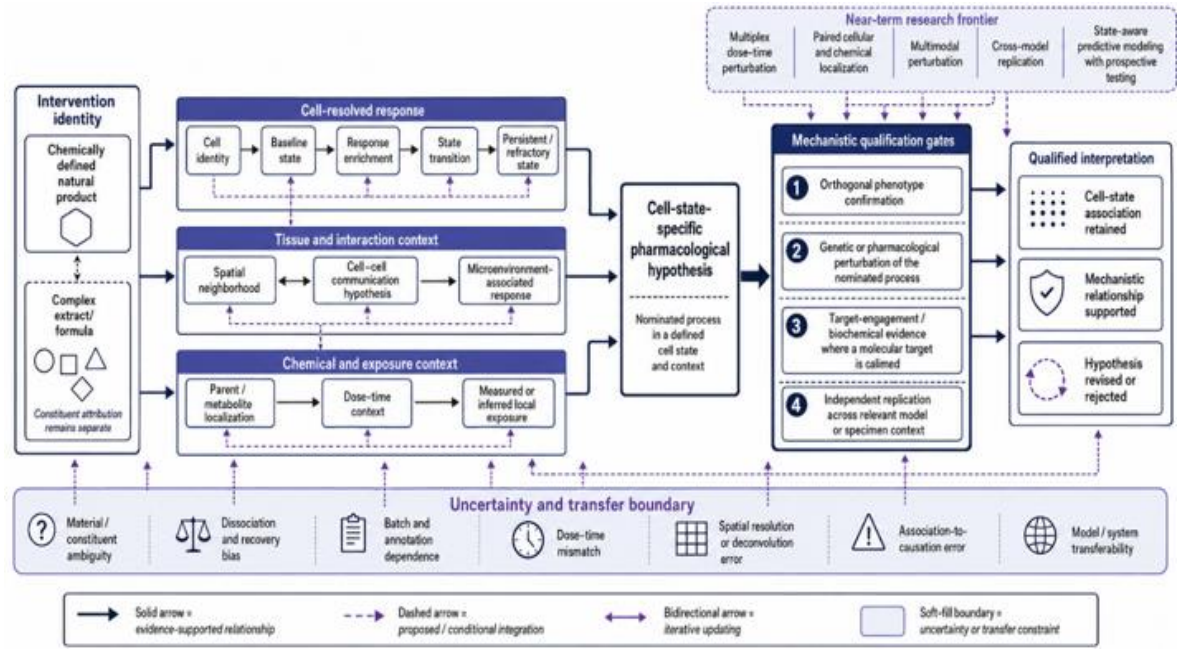

Figure 2. Evidence maturation from administered natural product to mechanistically qualified cell-state response

Technical and interpretive barriers

Greater biological resolution also creates additional routes to error. Tissue dissociation can induce transcriptional programs and alter the apparent representation of cellular subpopulations [31]. Storage, dissociation strategy, and the choice between single-cell and single-nucleus workflows can likewise introduce systematic differences in recovered cell composition and gene expression [32]. Consequently, a treatment-associated "missing" state can reflect biological depletion, poor recovery, or sample handling, while an induced stress program can be mistaken for pharmacological reprogramming. These risks are especially important when treated and control tissues differ in fragility, fibrosis, necrosis, or extracellular-matrix composition.

Multimodal integration does not eliminate these limitations. Single-cell multi-omics platforms differ in molecular coverage, capture efficiency, throughput, and compatibility with native tissue architecture [33]. Computational integration can align datasets and improve shared-state representation, but it necessarily depends on assumptions about correspondence among modalities and samples; modern dictionary-learning approaches expand scalable integration without making biological equivalence automatic [34]. Batch correction that forces two conditions into a common representation can also suppress real treatment biology if nuisance variation and pharmacological signal are confounded.

Natural-product studies add two domain-specific complications. First, chemical exposure must remain explicit. Nominal administration of an extract, formula, or poorly characterized preparation does not identify which constituent, metabolite, or transformed species reached the responding cell. Second, time is part of cell state: early stress, adaptive signaling, cell death, repopulation, and tissue repair may occupy different trajectories. A single terminal sampling point can therefore make sequential processes appear simultaneous.

Interpretive errors frequently arise after analysis rather than during measurement. Cluster labels can be treated as fixed biological entities; ligand–receptor predictions can be written as demonstrated communication; enriched pathways can be called mechanisms; and pseudoreplicated cells can be mistaken for independent biological specimens. Spatial spots may contain multiple cells, while computational deconvolution introduces model dependence. These issues do not invalidate single-cell or spatial pharmacology. They define the evidential discipline required to prevent increased measurement resolution from becoming increased interpretive confidence without corresponding validation.

Near-term research frontiers

The near-term frontier is perturbation-first cell-resolved pharmacology. Multiplex pharmacotranscriptomic pipelines now permit many drug conditions to be linked directly to single-cell transcriptional responses, creating

a practical route to comparing doses, combinations, and heterogeneous state transitions within one experimental architecture [35]. The Dini and colleagues platform, for example, couples multiplex drug screening to single-cell RNA sequencing rather than profiling one treatment condition as an isolated endpoint. Natural-product studies could use this design principle to distinguish reproducible state-selective effects from terminal snapshots, provided compound identity and exposure are controlled.

A second frontier is computational transfer across increasingly large cell-state spaces. Foundation and transfer-learning approaches can learn biological representations that generalize across some perturbations or cellular contexts, but predictive transfer remains conditional on training coverage and task definition [36]. For natural products, chemical-space novelty adds another source of distribution shift. Models trained predominantly on conventional synthetic compounds or a narrow set of cancer drugs should therefore not be presumed to transfer to structurally diverse natural products, metabolites, or complex botanical exposures without explicit benchmarking.

Third, cell-resolved pharmacology must be connected to multiscale pharmacological measurement. Contemporary multiscale modeling emphasizes that adding biological detail is useful only when parameters, interfaces, calibration targets, and validation data remain identifiable [37]. A spatial cell state without local exposure information can explain where a response occurs but not necessarily why; conversely, tissue drug localization without cellular response can establish distribution without pharmacodynamic attribution.

Finally, single-cell chemical genomics is expanding from descriptive profiling toward experimentally testable response maps [38]. We propose that natural-product studies prioritize designs that jointly record verified intervention identity, biologically replicated dose–time perturbation, baseline cellular state, post-treatment state, relevant local exposure where measurable, and an independent test of the nominated mechanism. This is not proposed as a mandatory assay checklist. It is an evidence architecture: each added layer should eliminate a specific competing explanation. The strongest future studies will therefore be those in which single-cell and spatial technologies are chosen because they resolve a defined pharmacological ambiguity, not simply because they increase dimensionality.

Conclusion

Single-cell and spatial technologies change the unit of observation in natural-product pharmacology. A response that once appeared as one tissue-average change can now be decomposed into responding and persistent states, altered population structure, spatial neighborhoods, intercellular relationships, and regionally distributed chemical species. This resolution is particularly valuable for natural products because biological heterogeneity intersects with chemical complexity, metabolism, tissue access, and, for extracts and formulas, uncertain attribution of activity to individual constituents.

The central contribution of this review is an evidence-bounded interpretation architecture in which cellular response, spatial context, chemical exposure, mechanistic nomination, and causal validation remain separate until experimentally connected. Cell-resolved data can identify where an effect occurs and which states warrant testing; spatial measurements can qualify where those states and chemical species occur; perturbational and biochemical experiments can then determine whether nominated pathways or targets participate in the response. None of these layers substitutes automatically for the others.

Accordingly, the important transition is not from “bulk” to “single cell” as a simple technological upgrade. It is from averaged pharmacology to state-, location-, exposure-, and validation-qualified inference. Current natural-product applications demonstrate substantial analytical promise but uneven mechanistic maturity. The most productive next phase will couple chemically rigorous intervention design with longitudinal or multiplex cell-resolved perturbation, spatially relevant exposure measurement, orthogonal mechanism testing, and explicit treatment of uncertainty. Under those conditions, single-cell and spatial technologies can sharpen natural-product pharmacology without converting molecular resolution into unwarranted mechanistic certainty.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. 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
2. 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
3. Srivatsan SR, McFaline-Figueroa JL, Ramani V, Saunders L, Cao J, Packer J, et al. Massively multiplex chemical transcriptomics at single-cell resolution. Science. 2020;367(6473):45-51. doi:10.1126/science.aax6234
4. Longo SK, Guo MG, Ji AL, Khavari PA. Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. Nat Rev Genet. 2021;22(10):627-44. doi:10.1038/s41576-021-00370-8
5. Cheng X, Peng T, Chu T, Yang Y, Liu J, Gao Q, et al. Application of single-cell and spatial omics in deciphering cellular hallmarks of cancer drug response and resistance. J Hematol Oncol. 2025;18(1):70. doi:10.1186/s13045-025-01722-1
6. 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
7. King JJ, Mowla A, Kretzmann JA, Bhalla N, Sugianto G, Norret M, et al. Single-cell RNA sequencing profiles drug activity within spatially engineered 3D cultures. Nat Commun. 2026;17(1):7735. doi:10.1038/s41467-026-74587-8
8. Heumos L, Schaar AC, Lance C, Litinetskaya A, Drost F, Zappia L, et al. Best practices for single-cell analysis across modalities. Nat Rev Genet. 2023;24(8):550-72. doi:10.1038/s41576-023-00586-w
9. Liu D, Zhang Y, Bai B, Xiong X, Zhou Q, Shi R. Integration of single-cell RNA sequencing and network pharmacology to elucidate the effect of Yantiao Formula on alleviating ALI by regulating the polarization of alveolar macrophages. J Ethnopharmacol. 2025;343:119436. doi:10.1016/j.jep.2025.119436
10. Cong S, Wang N, Pei H, Li Z, Meng Y, Maimaitituersun S, et al. Syringin inhibits the crosstalk between macrophages and fibroblast-like synoviocytes to treat rheumatoid arthritis via PDE4. Phytomedicine. 2025;138:156401. doi:10.1016/j.phymed.2025.156401
11. Xie L, Zhong Y, Chen Y, Wang Y, Xian P, Liu S, et al. Cryptotanshinone alleviates immunosuppression in endometriosis by targeting MDSCs through JAK2/STAT3 pathway. Phytomedicine. 2025;136:156227. doi:10.1016/j.phymed.2024.156227
12. Qian C, Han H, Wan L, Huang Y, Luo Y, Wang Z, et al. Dihydrotanshinone I inhibits PHGDH to suppress serine synthesis and remodel the tumor microenvironment in non-small cell lung cancer. Phytomedicine. 2026;158:158366. doi:10.1016/j.phymed.2026.158366
13. Xu T, Liu Q, Ma H, Xu M, Zhang J, Wang Y, et al. Tubeimoside II potentiates anti-PD-1 therapy in non-small cell lung cancer by increasing ferroptosis sensitivity and remodeling the immune landscape. Phytomedicine. 2026;150:157660. doi:10.1016/j.phymed.2025.157660
14. 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
15. Chen P, Zhu Z, Geng H, Cui X, Han Y, Wang L, et al. Integrated spatial metabolomics and transcriptomics decipher the hepatoprotection mechanisms of wedelolactone and demethylwedelolactone on non-alcoholic fatty liver disease. J Pharm Anal. 2024;14(4):100910. doi:10.1016/j.jpha.2023.11.017
16. Ji X, Lv C, Wu X, Wu H, Chang Y, Liu Q, et al. Mechanism of cancer-associated fibroblast-driven thyroid cancer dedifferentiation via the ZFP57-PKM2 axis-mediated lactate secretion and therapeutic intervention with resveratrol. J Exp Clin Cancer Res. 2026;45(1):88. doi:10.1186/s13046-026-03675-w
17. Sun X, Xia J, Jiang H, Duan T, Zhang C, Li Q, et al. Single-cell and spatial transcriptome profiling identifies cellular heterogeneity and immunosuppressive tumor microenvironment in inflammatory breast cancer. J Adv Res. 2026;81:637-56. doi:10.1016/j.jare.2025.05.061

18. Wu KJ, Li JC, Liu LM, Huang XJ, Mo LH, Ling JW, et al. Nitidine chloride inhibits colorectal cancer by targeting BUB1: Mechanistic insights from molecular dynamics simulation, spatial transcriptomics, and single-cell RNA sequencing. BMC Gastroenterol. 2025;25(1):818. doi:10.1186/s12876-025-04423-8
19. Sinha S, Vegesna R, Mukherjee S, Kammula AV, Dhruba SR, Wu W, et al. PERCEPTION predicts patient response and resistance to treatment using single-cell transcriptomics of their tumors. Nat Cancer. 2024;5(6):938-52. doi:10.1038/s43018-024-00756-7
20. França GS, Baron M, King BR, Bossowski JP, Bjornberg A, Pour M, et al. Cellular adaptation to cancer therapy along a resistance continuum. Nature. 2024;631(8022):876-83. doi:10.1038/s41586-024-07690-9
21. Wang Q, Pan Y, Zhou M, Tang Z, Wang Y, Wang G, et al. scDrugMap: Benchmarking large foundation models for drug response prediction. Nat Commun. 2026;17(1):730. doi:10.1038/s41467-025-67481-2
22. Han M, Li C, Zhang C, Song C, Xu Q, Liu Q, et al. Single-cell transcriptomics reveals the natural product Shi-Bi-Man promotes hair regeneration by activating the FGF pathway in dermal papilla cells. Phytomedicine. 2022;104:154260. doi:10.1016/j.phymed.2022.154260
23. Quek C, Pratapa A, Bai X, Al-Eryani G, Pires da Silva I, Mayer A, et al. Single-cell spatial multiomics reveals tumor microenvironment vulnerabilities in cancer resistance to immunotherapy. Cell Rep. 2024;43(7):114392. doi:10.1016/j.celrep.2024.114392
24. McFaline-Figueroa JL, Srivatsan S, Hill AJ, Gasperini M, Jackson DL, Saunders L, et al. Multiplex single-cell chemical genomics reveals the kinase dependence of the response to targeted therapy. Cell Genom. 2024;4(2):100487. doi:10.1016/j.xgen.2023.100487
25. Replogle JM, Saunders RA, Pogson AN, Hussmann JA, Lenail A, Guna A, et al. Mapping information-rich genotype-phenotype landscapes with genome-scale Perturb-seq. Cell. 2022;185(14):2559-75.e28. doi:10.1016/j.cell.2022.05.013
26. Tang Z, Liu X, Li Z, Zhang T, Yang B, Su J, et al. SpaRx: Elucidate single-cell spatial heterogeneity of drug responses for personalized treatment. Brief Bioinform. 2023;24(6):bbad338. doi:10.1093/bib/bbad338
27. Kremb S, Voolstra CR. High-resolution phenotypic profiling of natural products-induced effects on the single-cell level. Sci Rep. 2017;7:44472. doi:10.1038/srep44472
28. Wu L, Wu Q, Du X, Ling M, Tong H. Revolutionizing pharmacological research of traditional Chinese medicine with single-cell omics technologies. Fitoterapia. 2025;186:106846. doi:10.1016/j.fitote.2025.106846
29. 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
30. Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, et al. Natural products in drug discovery: Advances and opportunities. Nat Rev Drug Discov. 2021;20(3):200-16. doi:10.1038/s41573-020-00114-z
31. van den Brink SC, Sage F, Vértesy Á, Spanjaard B, Peterson-Maduro J, Baron CS, et al. Single-cell sequencing reveals dissociation-induced gene expression in tissue subpopulations. Nat Methods. 2017;14(10):935-6. doi:10.1038/nmeth.4437
32. Denisenko E, Guo BB, Jones M, Hou R, de Kock L, Lassmann T, et al. Systematic assessment of tissue dissociation and storage biases in single-cell and single-nucleus RNA-seq workflows. Genome Biol. 2020;21(1):130. doi:10.1186/s13059-020-02048-6
33. Baysoy A, Bai Z, Satija R, Fan R. The technological landscape and applications of single-cell multi-omics. Nat Rev Mol Cell Biol. 2023;24(10):695-713. doi:10.1038/s41580-023-00615-w
34. Hao Y, Stuart T, Kowalski MH, Choudhary S, Hoffman P, Hartman A, et al. Dictionary learning for integrative, multimodal and scalable single-cell analysis. Nat Biotechnol. 2024;42(2):293-304. doi:10.1038/s41587-023-01767-y
35. Dini A, Barker H, Piki E, Sharma S, Raivola J, Murumägi A, et al. A multiplex single-cell RNA-Seq pharmacotranscriptomics pipeline for drug discovery. Nat Chem Biol. 2025;21(3):432-42. doi:10.1038/s41589-024-01761-8
36. Theodoris CV, Xiao L, Chopra A, Chaffin MD, Al Sayed ZR, Hill MC, et al. Transfer learning enables predictions in network biology. Nature. 2023;618(7965):616-24. doi:10.1038/s41586-023-06139-9
37. Munn LL, Jain RK. Challenges and opportunities for the next generation of computational tumor models. PLoS Biol. 2025;23(7):e3003269. doi:10.1371/journal.pbio.3003269

38. Wyatt A, Hoffer-Hawlik K, Giglio RM, Azizi E, McFaline-Figueroa JL. An expanded role for single-cell chemical genomics profiling in drug discovery. Biochem J. 2026;483(2):211-28. doi:10.1042/BCJ20253273