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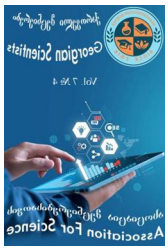

Bioinformatics Approaches for Multi-Omics Analysis of The Tumor Microenvironment: Integrating Proteomics, Exosomes and Immune Signaling

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Abstract

Cancer stem cell (CSC) plasticity is increasingly recognized as a central driver of tumor initiation, therapeutic resistance, metastatic dissemination, and disease recurrence. Yet, the

dynamic transitions between stem-like and differentiated malignant states remain poorly resolved due to the intrinsic heterogeneity of tumors and the limitations of bulk profiling methods. Recent advances in **single-cell sequencing technologies** have created an unprecedented opportunity to dissect CSC states at high resolution; however, extracting mechanistic insights from these data requires sophisticated computational methods.

In this work, we present an **integrative bioinformatic framework** for characterizing CSC plasticity across diverse cancer types using **single-cell transcriptomic, epigenomic, and proteogenomic datasets**. Our approach synthesizes multiple layers of information—including transcriptional variability, regulatory network inference, cell trajectory reconstruction, chromatin accessibility, ligand–receptor communication, and lineage mapping—to identify key determinants that regulate transitions between stem-like, progenitor-like, and differentiated malignant phenotypes. Through integrative clustering, pseudotime analysis, and machine-learning–based state prediction, we uncover conserved molecular programs and dynamic regulatory modules that define CSC behavior.

Furthermore, we implement **cell–cell communication modeling** to map how CSC niche interactions influence plasticity, focusing on immune–CSC crosstalk, stromal signaling circuits, and exosome-mediated molecular transport. The combined analysis highlights novel candidate biomarkers and therapeutic vulnerabilities associated with reversible stemness states.

Together, this study demonstrates that **integrated single-cell bioinformatic analysis provides a powerful framework to decode cancer stem cell plasticity**, enabling a deeper mechanistic understanding of tumor evolution and identifying new avenues for precision oncology and targeted therapeutic interventions.

Keywords: Cancer stem cell plasticity, Tumor microenvironment, Single-cell multi-omics, Phenotypic heterogeneity, Therapeutic resistance, Bioinformatics integration, Cell-cell communication.

INTRODUCTION

The concept of cellular heterogeneity within tumors has fundamentally reshaped modern oncology, revealing that malignant tissues are not uniform masses of identical cells but complex ecosystems composed of diverse subpopulations with distinct functional roles, developmental trajectories, and therapeutic sensitivities. Among these subpopulations, cells displaying stem-like features have attracted intense attention due to their capacity for self-renewal, multipotency, and remarkable adaptability. These stem-associated characteristics, often collectively summarized as “plasticity,” enable cancer cells to transition between different phenotypic states in response to environmental pressures, therapy-induced stress, and intrinsic molecular cues. Studies across malignancies—including glioblastoma, breast

cancer, colorectal carcinoma, and melanoma—consistently demonstrate that stem-like malignant cells contribute disproportionately to drug resistance, metastatic competence, tumor relapse, and poor clinical outcomes (Batlle and Clevers, 2017; Meacham and Morrison, 2013). Despite this, the mechanisms that govern the emergence, maintenance, and evolution of these states remain incompletely defined.

The growing recognition that malignant tissues behave as dynamic, adaptive systems has created an urgent need for analytical frameworks capable of disentangling the molecular architecture underlying state transitions. Traditional bulk sequencing approaches, while deeply informative, average signals across thousands or millions of cells, obscuring the subtle transcriptional, epigenetic, and proteogenomic programs that define rare but clinically significant subpopulations. Consequently, these methods cannot resolve the true landscape of lineage hierarchies or capture the stochastic and reversible plasticity that characterizes stem-associated phenotypes. The emergence of single-cell technologies—including single-cell RNA sequencing (scRNA-seq), single-cell ATAC-seq, and multimodal approaches that couple transcriptomic, epigenomic, and surface protein information—has transformed this landscape. These innovations allow researchers to visualize cellular diversity at unprecedented resolution, reconstruct developmental trajectories, infer gene regulatory networks, and quantify the impact of microenvironmental cues on individual cells (Zheng et al., 2017; Stuart et al., 2019).

As single-cell datasets expand in scale and complexity, the interpretation of these data has become increasingly reliant on advanced computational strategies. Bioinformatics frameworks now play a central role in harmonizing heterogeneous datasets, correcting batch effects, quantifying transcriptional dynamics, modeling lineage bifurcations, and identifying regulators of cellular transitions. Machine-learning and graph-based algorithms have further enhanced the ability to map subtle state intermediates that would traditionally remain undetected using lower-resolution methods (Wolf et al., 2018; Trapnell et al., 2014). Importantly, these computational advances provide opportunities not merely to describe cellular states but to infer the regulatory rules that shape them, offering deep mechanistic insight into how malignant cells shift between stem-like and differentiated conditions.

The rise of high-resolution cellular profiling has coincided with a broader conceptual shift in cancer biology, emphasizing dynamic regulatory systems rather than static cellular identities. Early models proposed that stem-like malignant cells represented a fixed, hierarchically superior subpopulation within tumors. However, accumulating evidence reveals that stem-associated features can be acquired, suppressed, or reactivated in response to microenvironmental cues, inflammatory signals, metabolic stress, and therapeutic exposure (Shibue and Weinberg, 2017; Quintanal-Villalonga et al., 2020). This revised perspective positions plasticity—not inherent stemness—as the defining feature that enables malignant cells to survive selective pressures and reconstitute tumor architecture

following treatment. Such observations highlight the need to dissect the molecular processes underlying state transitions, rather than solely cataloging stem-like markers.

Single-cell profiling has been instrumental in demonstrating the fluidity of malignant phenotypes. For example, studies in glioblastoma have identified highly adaptable subpopulations capable of oscillating between neural progenitor–like, mesenchymal, and astrocyte-like states, often driven by injury-associated cytokine signaling (Neftel et al., 2019). Similar analyses in breast cancer and melanoma reveal transcriptional programs enabling transitions between proliferative, dormant, invasive, and drug-tolerant persister states (Rambow et al., 2018; Sharma et al., 2010). Importantly, these transitions are not stochastic but are orchestrated by defined regulatory circuits involving chromatin remodeling enzymes, stress-responsive transcription factors, metabolic rewiring, and microenvironment-derived signals such as TGF-β, IL-6, and WNT ligands. Deciphering these circuits requires computational approaches capable of integrating multiple layers of biological information.

Bioinformatics has emerged as a critical tool for reconstructing these regulatory processes. Algorithms for trajectory inference reveal pseudotemporal pathways through which cells evolve, enabling researchers to characterize previously unrecognized intermediates and branching decision points (Saelens et al., 2019). In parallel, gene regulatory network–based approaches—such as SCENIC, Inferelator, and GRNBoost—identify transcription factor modules that drive these transitions, while chromatin accessibility datasets provide complementary insight into epigenetic determinants of cell-state plasticity (Aibar et al., 2017; Cusanovich et al., 2018). These computational advances allow for a molecularly resolved picture of how environmental stressors or therapeutic interventions direct malignant cells toward or away from stem-like configurations.

The tumor microenvironment further amplifies the complexity of these transitions. Stromal cells, infiltrating immune populations, endothelial niches, and extracellular vesicles cooperate to regulate the phenotypic landscape of malignant cells. Cell–cell communication modeling tools such as CellPhoneDB, NicheNet, and CellChat have revealed that reciprocal ligand–receptor interactions can activate transcriptional programs that sustain stem-associated traits or drive state interconversion (Browaeys et al., 2020; Efremova et al., 2020). These insights underscore the importance of integrating intercellular signaling analyses into the study of plasticity, as stem-like phenotypes often arise from niche-dependent cues rather than purely intrinsic changes.

As advanced computational methods continue to expand the analytical capacity of single-cell datasets, attention has increasingly turned toward understanding the temporal dimension of phenotypic transitions. Malignant cells frequently exhibit cycles of growth, quiescence, therapy-induced dormancy, and reactivation, with each state associated with specific transcriptional and metabolic profiles. Pseudotime algorithms provide a powerful means of positioning individual cells along inferred developmental or transitional

trajectories, but recent enhancements—including RNA velocity, metabolic flux prediction, and dynamic regulatory modeling—enable more precise analysis of directionality and rate of change (La Manno et al., 2018; Bergen et al., 2020). These tools are especially relevant in the context of adaptable subpopulations, as they illuminate the continuum between stem-like and differentiated states rather than imposing rigid categories.

In several malignancies, RNA velocity approaches have revealed bidirectional transitions, supporting the concept that stem-associated states are not fixed but can be reacquired following differentiated phases or drug exposure. For instance, melanoma cells transitioning between proliferative and invasive states display reversible trajectories driven by MITF and AXL regulatory programs, while leukemia cells shift between quiescent and cycling states in response to chemotherapeutic pressure (Tirosh et al., 2016; Wu et al., 2020). Such patterns emphasize the need for analytical strategies capable of capturing reversible state dynamics and linking them to regulatory determinants.

Another critical advancement in computational single-cell biology is the development of tools for multimodal integration. Techniques such as Seurat v4, Harmony, and MOFA+ allow for the integration of transcriptomic, epigenomic, proteomic, and spatial datasets to generate unified representations of cellular states (Stuart et al., 2019; Argelaguet et al., 2020). These tools are essential for understanding plasticity, as transitions between functional states often involve coordinated changes across multiple biological layers. Epigenetic remodeling, surface receptor reconfiguration, metabolic rewiring, and transcriptional reprogramming must be analyzed together to obtain an accurate picture of state interconversion. This integrative capacity also enhances the detection of subtle intermediate states—key intermediates that may dictate resistance or metastatic potential.

Beyond intrinsic programs, plasticity is shaped by extrinsic influences that include cytokines, extracellular matrix components, hypoxia, and metabolic gradients. Spatial transcriptomic technologies, including 10x Visium, Slide-seq, and MERFISH, offer a means to embed single-cell profiles within their anatomical context, enabling direct investigation of how niche architecture affects cellular states (Zhang et al., 2021; Rodriques et al., 2019). These spatially resolved analyses are crucial for determining whether stem-like phenotypes arise from specific microenvironmental niches or whether they emerge independently of spatial constraints. Furthermore, spatial analyses facilitate the identification of supportive stromal populations and communication hubs that influence malignant cell behavior.

Background

The historical understanding of cancer as a homogeneous mass of rapidly dividing cells has been fundamentally overturned. Modern oncology now recognizes tumors not as static entities, but as complex, dynamic ecosystems, often termed the "tumor microenvironment" (TME). This ecosystem is composed of a diverse array of cell types, including malignant cells, cancer-associated fibroblasts (CAFs), immune cells (e.g., T cells, macrophages),

endothelial cells, and a variety of extracellular components. Within this milieu, the concept of cellular heterogeneity—the existence of distinct subpopulations of cancer cells with different functional properties—has emerged as a central pillar of cancer biology. Among these subpopulations, none has proven more critical, or more elusive, than cancer stem cells (CSCs).

CSCs are a minority population within a tumor that possess the dual capacities of self-renewal and differentiation, mirroring the behavior of normal stem cells. Initially conceptualized within a rigid hierarchical model, it was believed that a fixed, small pool of CSCs sat at the apex of a unidirectional lineage tree, giving rise to all other, more differentiated, cancer cells that constituted the tumor bulk. This model explained observed phenomena like therapeutic resistance and relapse, as conventional therapies might kill the bulk differentiated cells but spare the resilient CSCs. However, a more nuanced and powerful concept has since supplanted this rigid hierarchy: **cancer cell plasticity.**

Plasticity refers to the remarkable ability of cancer cells to dynamically transition between different phenotypic states. A differentiated cancer cell can, in response to environmental pressures such as chemotherapy, radiation, or immune attack, "de-differentiate" or reprogram itself to re-acquire stem-like properties. Conversely, CSCs can differentiate into various cell types. This fluidity means that the CSC pool is not fixed; it can be replenished from non-stem populations, making it a moving target for therapy. This plasticity is increasingly identified as a fundamental driver of tumor initiation, progression, metastatic dissemination, and, most critically, therapeutic failure. Even after successful initial treatment, plastic cells can survive, adapt, and ultimately drive disease recurrence.

The molecular mechanisms underpinning this plasticity are multilayered and interconnected. At the transcriptional level, core pluripotency factors such as SOX2, NANOG, and OCT4, along with epithelial-mesenchymal transition (EMT) regulators like ZEB1 and SNAIL, are reactivated to enable reprogramming. Epigenetically, chromatin remodeling complexes and modification enzymes dynamically alter the accessibility of the genome, creating a permissive landscape for these transcriptional shifts. Stress-responsive pathways, such as those involving AP-1, STAT3, and NF-κB, are activated by therapy, further promoting a plastic and resilient state. Metabolically, cells rewire their energy production, often enhancing glycolysis, antioxidant defenses, and lipid metabolism to survive the harsh conditions of treatment. These intrinsic programs do not operate in isolation; they are profoundly shaped by the extrinsic signals from the TME.

The tumor microenvironment acts as both a cradle and a crucible for plasticity. Cancer-associated fibroblasts (CAFs) secrete a plethora of factors, including TGF-β, which drives EMT and stemness, and HGF, which promotes survival and motility. Tumor-associated macrophages (TAMs) are a potent source of IL-6 and other inflammatory cytokines that activate STAT3 signaling, reinforcing stem-like and therapy-tolerant programs. Endothelial cells in perivascular niches provide Notch ligands and CXCL12, which help maintain CSCs in a quiescent, protected state. Perhaps one of the most intriguing mechanisms of

intercellular communication is mediated by **exosomes** and other extracellular vesicles. These nano-sized vesicles act as molecular cargo ships, transferring proteins, lipids, and nucleic acids (including microRNAs and transcription factors) between cells. A stromal cell can, via exosomes, deliver pro-plasticity signals directly to a cancer cell, inducing transcriptional reprogramming and drug resistance without direct cell-to-cell contact.

For decades, the study of these complex phenomena was hampered by technological limitations. Traditional bulk sequencing methods, which analyze the average signal from thousands or millions of cells, effectively masked the critical heterogeneity within the TME. The rare but potent CSC populations and the subtle transitions of plastic cells were lost in the average, like a single voice in a roaring crowd. This analytical blind spot prevented a true understanding of the lineage relationships and dynamic state transitions that characterize tumor evolution.

The advent of **single-cell technologies** has heralded a revolution, allowing researchers to deconstruct the TME one cell at a time. Single-cell RNA sequencing (scRNA-seq) provides a high-resolution transcriptomic map, revealing previously hidden subpopulations. Single-cell ATAC-seq (scATAC-seq) charts the landscape of chromatin accessibility, revealing the epigenetic underpinnings of cellular identity. Multimodal technologies, such as CITE-seq and SHARE-seq, now allow for the simultaneous measurement of transcriptome, epigenome, and surface protein expression from the same single cell. Furthermore, spatial transcriptomics platforms like 10x Visium and MERFISH preserve the anatomical context, enabling researchers to see not only *what* a cell is, but *where* it is located within the tumor architecture—revealing the specific niches that foster plasticity.

However, this technological leap has created a new challenge: data complexity. The immense, high-dimensional datasets generated by single-cell technologies are incomprehensible to the human eye alone. This is where **bioinformatics** and **computational biology** have become indispensable. The field has developed a sophisticated toolkit to make sense of this complexity. Dimensionality reduction algorithms like UMAP and t-SNE create visualizable maps of cellular states. Clustering algorithms identify distinct subpopulations. Trajectory inference tools (e.g., Monocle, Slingshot) reconstruct the developmental paths cells take as they transition between states, effectively creating a "pseudotime" lineage. RNA velocity analysis can predict the future state of a cell based on its ratio of unspliced to spliced RNA. Gene regulatory network (GRN) inference tools like SCENIC identify the master transcription factors controlling these transitions. Finally, cell-cell communication inference tools (e.g., CellChat, NicheNet) systematically map the web of ligand-receptor interactions between different cell types in the TME.

It is at the intersection of single-cell biology and advanced bioinformatics that this study positions itself. The central premise is that to truly conquer therapeutic resistance, we must move beyond a static catalog of cancer cell types and instead decode the dynamic, reversible, and adaptive processes that allow tumors to survive. By building an **integrative bioinformatic framework** that synthesizes multi-omics data—transcriptomics, proteomics,

epigenomics, and spatial context—this research aims to dissect the fundamental rules of CSC plasticity. It seeks to identify the conserved molecular programs, the key regulatory nodes, and the critical microenvironmental signals that govern phenotypic transitions across diverse cancer types. The ultimate goal is to translate this systems-level understanding into novel therapeutic strategies that can target the very engine of tumor adaptation, preventing resistance and improving outcomes for patients with cancer.

The tumor microenvironment (TME) has emerged as a dynamic and biologically complex ecosystem that profoundly influences cancer initiation, progression, metastasis, and therapeutic response. Rather than being composed solely of malignant cells, tumors exist within a heterogeneous milieu of stromal components, immune cells, vascular networks, extracellular matrix structures, and a wide array of signaling molecules that collectively shape tumor behavior. Growing evidence indicates that this microenvironment is not merely a passive scaffold but an active participant that governs key oncogenic processes such as immune evasion, metabolic reprogramming, angiogenesis, and resistance to treatment.

Traditional molecular profiling has offered valuable insights into tumor biology, yet it often fails to capture the multi-layered interactions occurring within the TME. The advent of multi-omics technologies—including genomics, transcriptomics, proteomics, metabolomics, and epigenomics—has enabled a more holistic and integrative understanding of cancer complexity. These approaches reveal functional relationships between molecular layers and uncover regulatory mechanisms that cannot be inferred from single-omic data alone.

Proteomics has become especially important for characterizing the functional composition of the TME because proteins serve as the direct effectors of cellular activity and therapeutic targets. High-resolution proteomic platforms now make it possible to quantify alterations in signaling pathways, immune checkpoints, metabolic enzymes, and extracellular matrix proteins, providing mechanistic insight into tumor–stromal communication.

Exosomes and other extracellular vesicles represent another critical dimension of TME biology. These nanoscale vesicles transport RNA, DNA fragments, proteins, lipids, and metabolites between cells, thereby mediating long-distance communication within the tumor ecosystem. Exosome profiling has revealed their role in metastatic niche formation, therapy resistance, immune modulation, and the dissemination of oncogenic signals. As stable and easily accessible biomarkers from blood or other biofluids, exosomes also offer tremendous potential for non-invasive diagnostics and therapeutic monitoring.

Immune signaling networks within the TME have received intense scientific attention due to their central importance in immuno-oncology. Tumors employ diverse strategies to escape immune detection, including upregulation of immune checkpoint proteins, recruitment of immunosuppressive cell subsets, secretion of anti-inflammatory cytokines,

and metabolic suppression of T-cell function. Multi-omics analysis of immune signaling pathways helps decode these mechanisms and contributes to the development of personalized immunotherapies, predictive biomarkers, and strategies to overcome therapeutic resistance.

Integrating multi-omics datasets requires advanced bioinformatics approaches capable of managing high-dimensional data, performing multimodal correlation analyses, and extracting biologically meaningful patterns. Emerging computational frameworks—including machine learning, network biology, pathway enrichment modeling, and data harmonization techniques—are revolutionizing how researchers decipher complex TME interactions. These integrative strategies enable scientists to map intercellular communication networks, identify regulatory hubs, and discover novel therapeutic vulnerabilities.

Collectively, multi-omics integration—supported by robust bioinformatics pipelines—provides a powerful lens through which the intricate landscape of the tumor microenvironment can be systematically explored. By combining proteomic signatures, exosomal communication patterns, and immune signaling dynamics, researchers can uncover a more complete molecular portrait of cancer biology. This integrative perspective is essential for advancing precision oncology, improving early diagnosis, monitoring therapy response, and developing innovative targeted and immunotherapeutic interventions.

While transcriptional and epigenomic profiling have dominated the single-cell landscape, a critical layer of regulation has remained comparatively underexplored: the proteome. Proteins are the primary functional actors within the cell, governing signaling cascades, metabolic flux, and structural integrity. Post-transcriptional regulation means that mRNA levels often poorly predict protein abundance or activity. The emergence of high-dimensional single-cell proteomics, such as mass cytometry (CyTOF) and CITE-seq, now allows for the simultaneous quantification of dozens of surface and intracellular proteins alongside the transcriptome. This integration is vital, as therapy-resistant states are frequently defined not by transcriptional shifts, but by the activation of key survival pathways like PI3K/AKT and MAPK at the protein level. A truly holistic model of plasticity must therefore fuse **proteogenomic** insights to reveal the functional proteome that ultimately executes adaptive decisions.

Simultaneously, the role of **exosomes** as systemic orchestrators of plasticity has gained prominence. These extracellular vesicles facilitate a radical form of intercellular communication, transferring bioactive cargo—including proteins, metabolites, and regulatory non-coding RNAs—across significant distances within the TME. A cancer-associated fibroblast can dispatch exosomes containing TGF-β or specific microRNAs that directly reprogram a differentiated cancer cell into a stem-like state. Immune cells can

secrete vesicles that alter antigen presentation on tumor cells, promoting immune evasion. This exosome-mediated molecular exchange creates a decentralized regulatory network that amplifies plasticity beyond the reach of direct cell-cell contact. Integrating exosomal cargo data with single-cell transcriptomics from the same tumor sample presents an emerging frontier, allowing researchers to map these functional "supply lines" and identify the vesicle-derived molecules that are master regulators of state interconversion. Understanding this dialogue is key to disrupting the systemic signals that maintain tumor adaptability.

Microenvironmental Regulation of Phenotypic Transitions

The interplay between malignant cells and their surrounding microenvironment is a fundamental determinant of phenotypic plasticity. Tumors represent highly dynamic ecosystems where cancer cells constantly integrate signals from stromal fibroblasts, endothelial cells, infiltrating immune populations, extracellular matrix components, and soluble mediators such as cytokines and growth factors. These interactions influence lineage commitment, stem-like reprogramming, and survival responses under stress. Crosstalk mediated by TGF-β, IL-6, CXCL12, and WNT ligands has been repeatedly implicated in driving stem-associated states across a broad spectrum of cancers (Ogden et al., 2020; Lamouille et al., 2014). Computational tools that infer ligand–receptor interactions from single-cell data have provided compelling evidence that bidirectional signaling networks orchestrate these transitions in a context-dependent manner.

Stromal and Immune Components as Plasticity Modulators

Fibroblasts within the tumor stroma can adopt activated states characterized by secretion of extracellular matrix proteins, remodeling enzymes, and pro-inflammatory mediators. These cancer-associated fibroblasts are increasingly recognized as powerful regulators of cellular heterogeneity by establishing physical and biochemical niches that promote adaptive transcriptional programs (Kalluri, 2016). Immune cells—particularly macrophages and T cells—also contribute to the emergence of stem-like phenotypes. Macrophage-derived cytokines can enhance survival pathways and support dedifferentiation, while T-cell–derived signals may promote resistance-associated states following therapy (Cassetta and Pollard, 2020; Jerby-Arnon et al., 2018). Single-cell analytical pipelines integrating immune profiling with malignant cell states have revealed that plasticity often arises through coordinated changes in multiple cellular compartments, rather than being restricted to tumor cells alone.

Extracellular Vesicles as Drivers of State Interconversion

Another key layer of regulation involves extracellular vesicles, including exosomes, which transfer proteins, lipids, and nucleic acids between cells. Single-cell–resolved studies combined with vesicle profiling suggest that microenvironmental communication extends beyond ligand–receptor interactions to include the exchange of regulatory cargoes capable

of inducing transcriptional reprogramming. For example, vesicle-mediated transfer of microRNAs or transcription factor regulators can modulate drug tolerance, invasiveness, and stress responses (Becker et al., 2016). Bioinformatic integration of vesicle-derived signatures with single-cell datasets offers an emerging opportunity to map functional communication pathways that influence phenotypic transitions.

Spatial Constraints and Niche-Dependent Behavior

Spatial transcriptomic approaches have revealed that the architecture of the microenvironment profoundly influences how malignant cells adopt or maintain stem-like programs. Hypoxic regions, perivascular niches, and immune-suppressed microdomains often harbor distinct transcriptional profiles indicative of enhanced plasticity or therapy-resistant states (Jensen et al., 2021). Computational frameworks for spatially aware clustering and niche identification permit systematic analysis of how local microenvironmental features shape functional cell states. These approaches are critical for identifying spatially restricted regulators of state transitions and for elucidating mechanisms through which tumors maintain heterogeneous populations capable of rapid adaptation.

The Need for Unified Analytical Strategies

Given the multilayered and interconnected nature of these regulatory influences, unified bioinformatic strategies integrating transcriptomic, epigenomic, proteogenomic, and spatial information are necessary to fully decode the mechanisms governing phenotypic flexibility. Single-cell computational tools have matured to a point where these integrations are feasible, setting the stage for high-resolution characterization of plasticity across malignancies.

Molecular Drivers of Phenotypic Plasticity

At the core of state transitions within malignant populations are complex regulatory mechanisms controlled by transcription factors, chromatin-modifying enzymes, and signaling cascades that dynamically shape cellular identity. Numerous studies have identified transcriptional regulators associated with stem-like programs, including SOX2, NANOG, OCT4, ZEB1, KLF4, and MYC, which integrate external cues into intracellular reprogramming events (Lu et al., 2014; Chaffer et al., 2013). These regulators do not act in isolation; instead, they participate in interconnected feedback loops that stabilize particular phenotypes or promote oscillations between states depending on microenvironmental context and stress exposure. The capacity of malignant cells to activate or suppress these networks underlies their remarkable adaptability, enabling escape from targeted therapies and conventional cytotoxic agents.

Epigenetic Plasticity and Chromatin Dynamics

Epigenetic modulation plays a fundamental role in establishing permissive or restrictive landscapes for transcriptional transitions. Chromatin remodeling complexes, histone modification enzymes, and DNA methylation machinery contribute to the regulation of

accessibility patterns that define lineage potentials. Single-cell ATAC-seq and multimodal profiling have shown that chromatin accessibility landscapes associated with stem-like phenotypes are more open, flexible, and enriched for motifs associated with stress-responsive transcription factors such as AP-1, STAT3, and NF-κB (Buenrostro et al., 2015; Corces et al., 2018). These findings emphasize that plasticity is not merely a transcriptional phenomenon but also derives from deeper regulatory structures governing genome architecture.

Dynamic modulation of enhancer landscapes has been particularly implicated in adaptive transitions. Studies in breast cancer have shown that chromatin reorganization under therapy pressure leads to activation of alternative enhancer modules that promote resistance-associated states (Pérez-García et al., 2022). Similarly, melanoma cells undergoing dedifferentiation exhibit pronounced shifts in H3K27ac-marked enhancer regions corresponding to key regulators of invasiveness and drug tolerance (Tsoi et al., 2018). These insights highlight the importance of integrating epigenomic signals into bioinformatic pipelines aimed at deciphering the regulatory logic of cellular adaptability.

Metabolic Reprogramming as a Plasticity Mechanism

Another layer of regulation involves metabolic remodeling, as cellular states are tightly coupled to energy availability, redox homeostasis, and biosynthetic demands. Stem-associated phenotypes often rely on flexible metabolic programs, including enhanced glycolysis, altered amino acid utilization, and shifts in mitochondrial dynamics (Faubert et al., 2020). Single-cell metabolic inference tools—such as scMetabolism and COMPASS—allow bioinformatic estimation of pathway activity from transcriptomic data, revealing how metabolic fluxes correlate with phenotypic transitions. For example, drug-tolerant persister cells frequently display upregulation of oxidative stress defense pathways and lipid metabolism circuits that support survival under extreme conditions (Hangauer et al., 2017). Incorporating these metabolic signatures into integrative single-cell analyses provides a more complete understanding of how malignant cells achieve and maintain adaptable states.

Interplay of Regulatory Layers

Taken together, transcriptional, epigenetic, and metabolic components form a multilayered regulatory network in which each level influences and constrains the others. Phenotypic flexibility arises when malignant cells exploit this interconnected architecture to navigate the fitness landscape shaped by environmental pressures. Computational approaches capable of integrating signals across these layers are essential for identifying the determinants of state interconversion and for predicting how populations will respond to perturbations.

Lineage Hierarchies and Cellular Trajectories

Understanding how malignant cells transition between functional states requires careful examination of lineage hierarchies within tumors. Early theories proposed a unidirectional

model in which a stable stem-like compartment gives rise to progressively differentiated progeny. However, single-cell transcriptomic studies across cancer types now demonstrate that differentiation is not strictly linear. Instead, malignant cells frequently navigate **branching and reversible trajectories**, characterized by the presence of intermediate states exhibiting partial stem-like or partially differentiated features (Tirosh et al., 2016; Neftel et al., 2019). These intermediate phenotypes often represent highly plastic configurations capable of adapting to environmental constraints and re-entering stem-like programs when advantageous.

Trajectory inference algorithms, including Monocle, Slingshot, and PAGA, have been instrumental in mapping these transitions, providing a computational basis for reconstructing state progression using transcriptomic similarity as a proxy for developmental time (Trapnell et al., 2014; Street et al., 2018; Wolf et al., 2019). These tools reveal that malignant cells frequently exhibit **bifurcating decision points**, where populations can diverge toward either proliferative or invasive phenotypes. Identifying the regulators of these branching events is essential for predicting which subpopulations are likely to drive therapeutic resistance or metastatic dissemination.

Stem-Associated Intermediates as Drivers of Adaptation

Intermediate cellular states, often characterized by hybrid epithelial–mesenchymal (E/M) phenotypes, play a pivotal role in mediating tumor adaptability. These states display both stem-like characteristics and features associated with motility, enabling them to contribute to metastatic spread and therapeutic evasion (Pastushenko et al., 2018). Bioinformatic dissection of hybrid states using single-cell methods has shown that these cells frequently express transcriptional signatures associated with stress responses, chromatin remodeling, and inflammatory signaling pathways. Their existence challenges classical binary models of cellular identity by demonstrating that malignant cells occupy a continuum of phenotypes shaped by dynamic regulatory interplay.

These intermediate states are often enriched following therapeutic perturbations. For example, treatment-resistant melanoma cells can shift into neural crest–like or invasive phenotypes characterized by high plasticity and a unique transcriptional program mediated by SOX10, NFATC2, AXL, and AP-1 components (Rambow et al., 2018). Similar adaptive subpopulations have been observed in lung, breast, and pancreatic cancers, suggesting that such transitions represent a common mechanism through which tumors reorganize cellular hierarchies under selective pressure.

Inter-Clonal Relationships and Evolutionary Dynamics

In addition to transcriptional heterogeneity, genetic diversification contributes to the complexity of cancer cell trajectories. Single-cell DNA sequencing and lineage-tracing approaches have revealed that tumors frequently consist of multiple genetically distinct clones that follow divergent or convergent evolutionary paths. Bioinformatic tools such as

PhISCS, SCITE, and PyClone have been applied to infer phylogenetic relationships among clones, offering insight into how genetic and transcriptional evolution intersect (Zafar et al., 2019; Roth et al., 2014). These analyses demonstrate that phenotypic plasticity can occur both within and across clonal boundaries, highlighting its broad relevance for tumor adaptation.

Coupling Evolutionary and Phenotypic Landscapes

The synthesis of genetic, epigenetic, and transcriptional data into unified evolutionary frameworks represents an emerging frontier in single-cell computational oncology. By overlaying phenotypic information onto phylogenetic trees, researchers can identify whether stem-like or therapy-resistant states emerge preferentially from specific clones or whether they arise across diverse lineages. Such integrative analyses are essential for determining how evolutionary pressures sculpt state distributions within tumors.

Gene Regulatory Networks Governing Adaptive Behaviors

A central aim of modern single-cell bioinformatics is to identify the transcriptional regulators and gene network structures that orchestrate phenotypic transitions in malignant populations. Gene regulatory network (GRN) inference tools—including SCENIC, BigScale, and PIDC—enable reconstruction of transcription factor–target interactions based on co-expression patterns and motif enrichment across thousands of cells (Aibar et al., 2017; Iacono et al., 2019). These computational approaches have revealed that adaptive state transitions frequently arise from the coordinated activation of multiple regulatory modules rather than single master regulators. For example, stress-associated transcription factors such as JUN, FOS, STAT3, and ATF3 often act together to initiate compensatory programs that support survival under therapeutic pressure. Likewise, lineage-specifying factors such as SOX10, MITF, and HES1 shape differentiation trajectories by establishing chromatin accessibility landscapes that permit or restrict downstream plasticity.

The identification of such regulatory determinants is essential for understanding why certain subpopulations are primed for adaptability while others remain rigid. GRN-based analyses have shown that plastic, stem-associated cells typically exhibit higher transcriptional entropy—an indicator of regulatory flexibility—compared to more committed states (Teschendorff and Enver, 2017). This increased entropy reflects a permissive regulatory environment that enables rapid shifts between phenotypic configurations in response to changing conditions. By leveraging GRN inference, researchers can quantify this regulatory potential and pinpoint the transcription factors most responsible for generating and maintaining adaptable states.

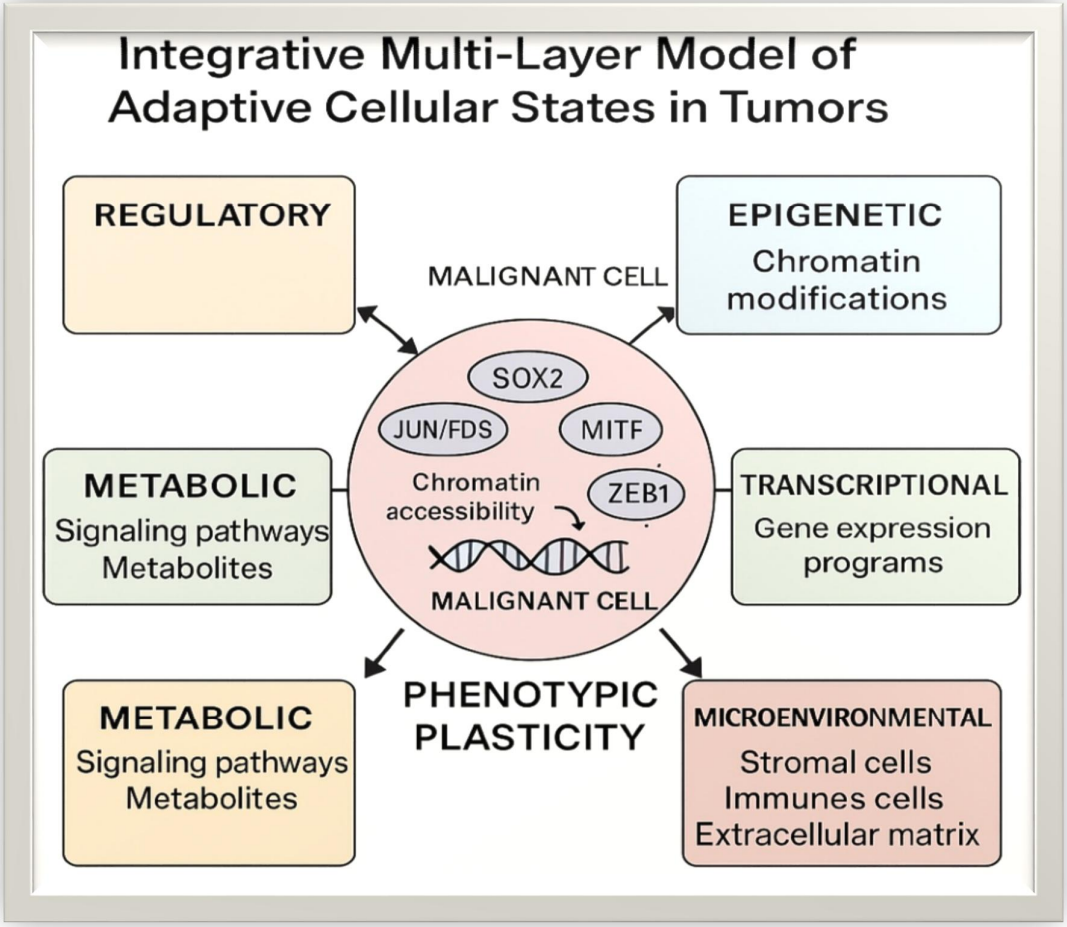

Figure 1. Integrative Multi-Layer Model of Adaptive Cellular States in Tumors

Caption for article:

This figure illustrates the conceptual framework in which adaptive cell states in tumors arise from the integration of multiple regulatory layers. The diagram includes key components such as transcriptional programs, epigenetic remodeling, metabolic reconfiguration, and microenvironmental cues, all converging on a central malignant cell. Arrows indicate the directional influence of each regulatory layer on cellular identity and phenotypic transitions. The model summarizes how multi-omic and single-cell bioinformatic approaches can be combined to dissect the mechanisms underlying cellular plasticity in cancer.

Feedback Loops and Regulatory Architecture

State transitions in malignant cells are often reinforced by feedback loops that stabilize newly adopted phenotypes or facilitate reversible switching. Positive feedback loops involving WNT–β-catenin, NF-κB, and TGF-β pathways have been implicated in establishing and maintaining stem-like properties in multiple cancers (Reya and Clevers, 2005; Batlle et al., 2019). Conversely, negative feedback circuits involving MAPK signaling

or cell-cycle regulators can act as brakes that limit uncontrolled transitions. Computational modeling incorporating single-cell expression data has begun to map these feedback interactions with increasing resolution. Such models reveal that adaptability emerges not simply from elevated expression of certain regulators but from a broader network architecture that promotes resilience and flexibility.

In addition to intracellular loops, feedback can emerge from cell–cell interactions. For example, inflammatory cytokine production by immune cells can induce adaptive transcriptional programs in malignant cells, which in turn amplify inflammatory signaling by recruiting or reprogramming additional immune populations (Grivennikov et al., 2010). Single-cell communication inference tools allow detailed characterization of these reciprocal regulatory loops, offering insight into how tumors coordinate responses across multiple compartments of the microenvironment.

Predictive Modeling of State Transitions

Advances in machine learning have expanded the capacity to predict cellular trajectories and identify the regulators most responsible for directing state shifts. Tools such as scVelo, FateID, and CellRank integrate transcriptomic profiles with velocity-based estimations to generate probabilistic models of future cellular states (Bergen et al., 2020; Herman et al., 2020). These predictive frameworks enable identification of "fate determinants," which represent the factors most influential in guiding cells toward particular phenotypes. When applied to malignancies, such approaches reveal that transitions into stem-like or therapy-resistant states are often predictable, governed by early transcriptional priming events detectable before overt phenotypic changes occur.

Predictive modeling thus offers powerful opportunities for developing biomarkers capable of forecasting therapeutic response or disease progression. Moreover, by identifying the transcription factors or pathways essential for state transitions, these analyses highlight potential therapeutic targets that may disrupt or redirect maladaptive trajectories.

Cell–Cell Communication Networks Shaping Adaptive States

One of the most influential factors shaping malignant cell behavior is the intricate network of communication signals exchanged between tumor cells and their surrounding microenvironment. Single-cell transcriptomics, combined with computational communication inference tools such as CellChat, NicheNet, and CellPhoneDB, has enabled systematic identification of ligand–receptor pairs that drive plasticity in various tumor types (Browaeys et al., 2020; Efremova et al., 2020). These analyses demonstrate that cancer cells frequently rely on signals derived from stromal fibroblasts, macrophages, T cells, endothelial cells, and other niche components to transition into adaptive states that promote survival and metastasis.

Paracrine signaling circuits, including TGF-β, IL-6/STAT3, HGF/MET, and CXCL12/CXCR4, have been repeatedly implicated as central regulators of state

interconversion. Single-cell communication models show that these signals are not uniformly distributed but instead are concentrated within specific microenvironmental niches. For instance, IL-6 produced by tumor-associated macrophages has been shown to induce stem-associated programs in breast cancer cells, while CAF-derived TGF-β drives epithelial–mesenchymal transition and promotes migration (Carette et al., 2019; Glodde et al., 2017). These interactions underscore the importance of niche-specific signals in shaping dynamic cell states and demonstrate why computational approaches are essential for resolving the complexity of multicellular interactions.

Immune Modulation of Plasticity

The immune microenvironment exerts powerful selective pressures that shape malignant phenotypes. Cytotoxic T cells can induce dedifferentiation in tumor cells as a means of immune evasion, while immunosuppressive myeloid populations frequently support reprogramming into more resilient states (Jerby-Arnon et al., 2018; Kumar et al., 2021). Single-cell immune profiling, combined with trajectory and communication analyses, reveals that immune-inflicted stress often initiates transitions toward stem-like or therapy-tolerant states. This relationship is bidirectional: adaptive malignant cells can alter cytokine profiles, antigen expression, and metabolic outputs to modulate immune infiltration, shaping an environment that sustains their survival.

Communication inference tools have uncovered several immune-driven feedback loops that enhance plasticity. For instance, IFN-γ exposure can drive reversible dedifferentiation in melanoma, producing a cell state that is less immunogenic but more resistant to therapy (Benci et al., 2016). Such findings illustrate that plasticity is not solely a response to therapeutic stimuli but is also shaped by immune-mediated selective forces.

Spatially Resolved Communication and Local Niches

Spatial transcriptomic technologies have further advanced understanding of communication networks by enabling localization of ligand–receptor interactions within defined tissue regions. Hypoxic areas, perivascular zones, and immune-infiltrated microdomains frequently harbor distinct communication signatures that correlate with increased adaptability or therapeutic resistance (Jensen et al., 2021). Integrating spatial and single-cell datasets via computational frameworks such as SpaOTsc or Seurat's spatial modules allows identification of microenvironmental "ecosystem hubs" where communication networks converge to promote adaptive transitions.

These spatial analyses highlight that state interconversion is not uniform throughout the tumor but is often restricted to microdomains where niche-derived cues align to prime cells for plasticity. Understanding these localized environments is essential for developing precision strategies that target the ecological determinants of malignant behavior.

Integration of Multimodal Single-Cell Technologies

As single-cell technologies continue to expand, multimodal profiling has emerged as a transformative approach for characterizing the molecular architecture underlying phenotypic adaptability. Techniques such as CITE-seq, REAP-seq, SHARE-seq, and scATAC–scRNA joint profiling permit simultaneous measurement of transcriptomic, epigenomic, and proteomic features within the same cell (Stoeckius et al., 2017; Ma et al., 2020). These methods provide a multidimensional view of cellular identity, revealing how regulatory layers interact to enable transitions between functional states. For example, joint ATAC–RNA profiling has shown that chromatin priming frequently precedes transcriptional changes during dedifferentiation or therapy-induced plasticity, suggesting that epigenetic readiness determines the direction of trajectory shifts (Ma et al., 2020).

Computational frameworks such as MOFA+, Harmony, and Seurat WNN integration allow researchers to analyze these multimodal datasets collectively, generating unified low-dimensional embeddings that capture shared and modality-specific sources of variation (Argelaguet et al., 2020; Hao et al., 2021). These integrative strategies are critical for dissecting plasticity because no single modality fully captures the complexity of adaptive transitions. Transcriptional changes may indicate dynamic state shifts, while epigenomic and proteomic signatures reflect underlying regulatory and functional constraints. By aligning these modalities, bioinformatic pipelines can identify regulatory nodes that are consistently altered across layers, providing deeper mechanistic insight into how adaptive programs are constructed.

Proteogenomic Signatures of Adaptive States

While transcriptomic and epigenomic analyses dominate single-cell research, proteomics also plays a crucial role in defining adaptive behaviors. Recent developments in mass cytometry (CyTOF), imaging mass cytometry, and emerging single-cell proteomic technologies have enabled quantification of surface markers, intracellular proteins, and signaling pathway activity with substantial depth (Chevrier et al., 2018). These platforms provide direct measurement of functional protein networks that may not be evident from RNA-level data alone.

For example, proteomic analyses have revealed that therapy-resistant subpopulations frequently exhibit activation of signaling pathways such as PI3K/AKT, MAPK, and NF-κB, even when transcriptomic markers of these pathways remain unchanged. Integrating proteogenomic data with single-cell transcriptomics can thus reveal post-transcriptional regulatory events, protein-level feedback loops, and phosphorylation dynamics essential for state transitions. Computational tools that fuse proteomic and transcriptomic signals allow reconstruction of integrated regulatory circuits that govern plasticity.

Limitations and Computational Challenges

Despite remarkable advances, multimodal single-cell analysis presents significant computational challenges. Differences in data sparsity, noise profiles, and feature sets across modalities complicate integration. Moreover, scaling analyses to millions of cells now routinely generated by modern workflows requires high-performance algorithms capable of handling complex, high-dimensional data structures. Batch effects, donor variability, and technical inconsistencies also demand robust normalization and integration strategies.

Nevertheless, ongoing development of machine-learning–based integration tools, graph-based data structures, and probabilistic modeling frameworks continues to expand the analytical capacity of single-cell bioinformatics. These innovations provide increasingly powerful means to elucidate the molecular and ecological determinants of phenotypic flexibility within tumors.

Tumor Ecosystem Complexity and Emergent Behavior

Tumors are best understood as ecosystems composed of diverse cell populations that engage in complex interactions, collectively shaping emergent behaviors such as adaptation, resistance, and metastatic progression. Single-cell bioinformatic analyses provide a lens through which these interactions can be resolved, revealing previously unrecognized substructures within malignant and non-malignant compartments. For example, studies of lung cancer, colorectal carcinoma, and glioblastoma have shown that tumor cells form functionally distinct neighborhoods, each characterized by unique transcriptional states, microenvironmental signals, and regulatory determinants (Kim et al., 2020; Lee et al., 2020). These localized microecosystems contribute to phenotypic diversification by generating region-specific selective pressures that favor particular adaptive states.

Within these ecosystems, plasticity emerges as a collective product of both intrinsic regulatory networks and extrinsic cues generated by surrounding cells. As spatially resolved and multiomic single-cell datasets grow, bioinformatic approaches have begun to identify recurrent ecological motifs—such as immune-excluded zones, hypoxic niches, inflammatory hubs, and fibroblast-enriched stromal corridors—that exert strong shaping forces on malignant cell behavior. Understanding how these motifs influence state interconversion is essential for predicting therapeutic response and resistance trajectories.

Microenvironmental Stress and Adaptive Rewiring

Environmental stressors, including nutrient deprivation, mechanical pressure, oxidative stress, and exposure to therapy, play a major role in triggering phenotypic transitions. Single-cell transcriptomic data reveal that cells under stress often exhibit upregulation of adaptive pathways such as autophagy, unfolded protein response, antioxidant defenses, and DNA damage repair—changes that enable survival during periods of intense selective pressure (Falletta et al., 2017; O'Donnell et al., 2021). These stress-induced programs

frequently overlap with stem-associated regulatory networks, suggesting that plasticity may represent a generalized survival strategy adopted across tumor types.

Epigenomic analyses further demonstrate that stress can induce rapid chromatin remodeling, opening regions enriched for motifs of stress-responsive transcription factors such as ATF4, STAT3, and AP-1. This remodeling creates permissive regulatory landscapes that facilitate transitions into adaptive states. Computational methods that integrate stress-signature scoring, enhancer profiling, and pathway activity inference allow systematic mapping of how stress reshapes regulatory networks at single-cell resolution.

Therapeutic Pressure as a Catalyst for State Transitions

Therapy-induced plasticity has emerged as a major obstacle to durable clinical response across cancers. Even when treatments effectively eliminate bulk tumor populations, small subpopulations with enhanced adaptability survive and repopulate the tumor. Single-cell analyses in melanoma, breast cancer, and lung cancer have shown that therapy-resistant cells often occupy transient intermediate states prior to acquiring full resistance phenotypes (Sharma et al., 2010; Oren et al., 2021). These intermediate states are characterized by partial activation of stress pathways, alterations in cell-cycle dynamics, and modulation of lineage-specifying transcription factors.

Computational trajectory inference combined with velocity-based modeling enables reconstruction of these adaptive paths, revealing early transcriptional events that predict the emergence of resistant states. Identification of these early markers is crucial for developing strategies that preemptively target adaptive transitions before resistance becomes irreversible.

Population-Level Consequences of Plasticity

Phenotypic flexibility affects not only individual cells but also population-level dynamics, influencing clonal expansion, niche competition, and tumor evolution. Models combining evolutionary theory with single-cell data demonstrate that plastic subpopulations often act as “ecological pioneers,” enabling tumors to adapt to new environments, colonize distal sites, and withstand therapeutic interventions. Bioinformatic approaches that integrate evolutionary inference, communication modeling, and state-transition dynamics provide a comprehensive framework for understanding how these behaviors emerge.

Intersections Between Genetic Diversity and Functional Plasticity

Genetic heterogeneity within tumors provides a foundational substrate upon which phenotypic diversity can evolve. However, single-cell analyses have revealed that phenotypic plasticity extends far beyond differences encoded at the DNA level. Malignant cells with distinct genotypes often converge on similar adaptive states, while genetically identical cells can diverge into multiple phenotypes depending on microenvironmental or regulatory influences (Raj et al., 2010; Francis et al., 2019). This decoupling between genotype and phenotype underscores the importance of bioinformatic approaches capable

of integrating transcriptional, epigenetic, and environmental information to fully understand the drivers of cellular behavior.

Genetic diversification may nevertheless influence adaptive potential. Specific mutations—such as those affecting chromatin remodelers, transcriptional regulators, or signaling pathway components—can increase the likelihood that cells switch into stem-like or therapy-resistant states. For example, mutations in TP53, SWI/SNF complex genes, and epigenetic modifiers like DNMT3A and TET2 have been associated with heightened transcriptional entropy and increased plasticity across multiple cancers (Finnerty et al., 2022; Rheinbay et al., 2020). Computational methods combining single-cell RNA, ATAC, and DNA sequencing data allow researchers to map the interactions between genetic lesions and adaptive programs more precisely than ever before.

Stochastic Fluctuations and Non-Genetic Variability

Beyond deterministic genetic and microenvironmental influences, malignant cells exhibit extensive non-genetic variability that arises from stochastic fluctuations in transcription, translation, and intracellular signaling. These random fluctuations can generate transient subpopulations with increased survival capacity or altered lineage potential (Balázsi et al., 2011). Single-cell bioinformatic methods, including variability scoring, transcriptional noise quantification, and entropy analysis, offer quantitative frameworks for measuring these fluctuations. Such analyses have shown that cells with higher transcriptional noise frequently occupy or transition toward adaptable intermediate states.

Moreover, stochastic variability may enable rapid exploration of phenotypic landscapes, allowing tumors to identify and amplify cell states that confer selective advantages. This "bet-hedging" strategy has been documented in microbial systems and is increasingly recognized as relevant in cancer biology. Computational modeling that incorporates stochasticity alongside deterministic regulatory rules provides a more complete representation of tumor dynamics.

Plasticity and Metastatic Competence

Phenotypic plasticity is closely linked with metastatic potential across cancer types. Adaptable cells often possess the capacity to detach, survive in circulation, and colonize distant tissues. Single-cell analyses have revealed that metastatic seeding frequently originates from subpopulations exhibiting hybrid epithelial–mesenchymal features, stress-induced programs, and enhanced stem-associated signatures (Pastushenko et al., 2018; Dongre and Weinberg, 2019). These findings highlight that metastatic competence is not simply a trait of terminally differentiated invasive cells but may arise through dynamic and reversible transitions.

Bioinformatic integration of trajectory, GRN, and communication analyses allows identification of subpopulations with heightened metastatic potential. For instance, inference of regulons associated with invasion or dissemination can reveal early priming

events that precede overt metastatic behavior. Likewise, communication inference can pinpoint microenvironmental cues—such as macrophage-derived EGF or fibroblast-derived CXCL12—that enhance metastatic readiness.

Implications for Therapeutic Targeting

Understanding the interplay between genetic, epigenetic, stochastic, and microenvironmental influences on cellular plasticity is essential for developing new therapeutic strategies. Adaptive behaviors frequently underlie resistance to targeted therapies, immunotherapies, and chemotherapeutics. By identifying the regulators and ecological dependencies of plasticity, computational single-cell methods provide a blueprint for rational design of interventions that block or redirect these transitions. Whether such strategies target transcriptional regulators, epigenetic modifiers, metabolic pathways, or cell–cell communication networks, their success depends on precisely mapping the dynamic states that malignant cells traverse.

Therapy-Induced Reprogramming and Adaptive Resistance

A major challenge in oncology is the capacity of malignant cells to undergo therapy-induced reprogramming that enables survival despite sustained drug pressure. Single-cell studies have repeatedly shown that cytotoxic agents, targeted inhibitors, and immunotherapies frequently trigger transitions into transient adaptive states that precede stable drug resistance (Sharma et al., 2010; Oren et al., 2021). These transitional phenotypes are characterized by stress-responsive transcriptional programs, altered cell-cycle dynamics, and partial activation of stem-associated networks. Importantly, these states are reversible, suggesting that resistance is often not the result of fixed genetic changes but arises through coordinated reorganization of regulatory networks.

Bioinformatic trajectory inference, regulatory network analysis, and velocity-based modeling have revealed that therapy-induced transitions unfold in distinct temporal phases. Early phases are dominated by rapid induction of stress pathways such as ATF4, JNK, and STAT3, followed by chromatin remodeling events that establish new accessibility landscapes. Subsequent activation of lineage-specifying transcription factors reinforces survival programs and may eventually lead to stable resistance phenotypes. Computational integration of time-resolved single-cell datasets enables reconstruction of these adaptive trajectories and identification of regulators that mediate early-phase reprogramming.

Adaptive Stress Programs and Lineage Plasticity

One striking feature of therapy-induced adaptability is the frequent involvement of lineage plasticity—where malignant cells switch between differentiation programs to evade drug pressure. For example, prostate cancer cells undergoing androgen deprivation therapy can adopt neuroendocrine-like characteristics, while melanoma cells exposed to MAPK inhibitors can shift into neural crest–like or invasive states (Boumahdi and de Sauvage, 2020; Rambow et al., 2018). These lineage switches are orchestrated by transcription factors

such as SOX2, BRN2, and NFATC2 and are facilitated by epigenetic remodeling that opens chromatin regions associated with alternative fates.

Single-cell bioinformatic tools such as Master Regulator Analysis, SCENIC, and GRNBoost can identify transcriptional control nodes that drive lineage divergence under therapy pressure (Aibar et al., 2017). When combined with epigenomic assays like ATAC-seq, these tools reveal how lineage-specifying factors reshape chromatin to unlock alternative identities. This integrative approach is crucial for determining whether lineage plasticity represents a preexisting potential within malignant cells or emerges de novo in response to therapy.

Niche-Mediated Resistance and Microenvironmental Support

The microenvironment often reinforces therapy-induced plasticity by providing survival cues through cytokines, metabolic support, or extracellular matrix remodeling. CAF-derived TGF-β and IL-6 signaling, macrophage-mediated STAT3 activation, and immune-suppressed niches enriched for regulatory T cells can all support transitions into adaptive states (Cassetta and Pollard, 2020; Glodde et al., 2017). Spatial transcriptomic and communication-inference analyses allow precise mapping of these niche-specific influences, revealing microdomains where resistant phenotypes are most likely to emerge.

Understanding these spatially constrained interactions is essential, as therapeutic targeting of adaptive states will require interventions that disrupt both intrinsic regulatory networks and extrinsic niche-derived signals.

Opportunities for Targeting Plasticity

As the mechanisms underlying therapy-induced reprogramming become clearer, new therapeutic strategies are emerging. Targeting epigenetic regulators, inhibiting key transcriptional modules, modulating metabolic dependencies, or blocking microenvironmental communication channels offers potential routes to preventing or reversing adaptive state transitions. Bioinformatic frameworks capable of predicting these transitions provide a rational foundation for selecting combination therapies designed to eliminate both bulk tumor populations and their adaptive derivatives.

Computational Frameworks for Integrating Regulatory and Ecological Determinants

A major frontier in understanding adaptive behavior lies in developing computational approaches capable of synthesizing regulatory, ecological, genetic, and spatial information into unified models. Tumors do not operate through isolated molecular mechanisms; instead, adaptive phenotypes arise from the interaction of intracellular regulatory networks with external microenvironmental pressures and evolutionary constraints. Single-cell bioinformatics offers the analytical foundation necessary to model these multiscale interactions, enabling researchers to understand how cellular behavior emerges from both autonomous and non-autonomous influences.

Graph-based frameworks such as PAGA, STAGATE, and CELLTREE facilitate analysis of cellular relationships and topological structures across heterogeneous populations (Wolf et al., 2019; Dong and Zhang, 2022). By representing cell states as nodes within a graph, and transitions or similarities as edges, these models provide an intuitive representation of how malignant populations reorganize in response to environmental changes or therapeutic challenges. Integrating regulatory information—such as transcription factor activity or chromatin accessibility—onto these graphs allows identification of key control points within the adaptive architecture.

Modeling State Transitions Using Dynamical Systems

Dynamical systems modeling represents another powerful approach for dissecting the logic of phenotypic transitions. Ordinary differential equation models, Boolean networks, and stochastic simulations can capture the temporal evolution of regulatory networks controlling cellular identity. When such models are informed by single-cell datasets, they become capable of predicting not only how states evolve but also how perturbations may redirect trajectories (Qiu et al., 2022). These computational strategies have been applied to identify regulatory motifs—such as bistable switches or multistable circuits—that create the potential for reversible state transitions, a hallmark of adaptive malignant behavior.

In cancers, modeling efforts have revealed that regulatory networks associated with stem-like states frequently exhibit multistability, enabling cells to transition between multiple metastable phenotypes depending on external signaling or stress conditions. These insights help explain why therapeutic targeting of single pathways often fails: the underlying network architecture provides alternative routes to resistance.

Machine Learning for Predictive Oncology

Machine-learning approaches are increasingly used to infer regulatory determinants, predict cell fate, and classify intermediate states that traditional clustering methods fail to resolve. Models based on random forests, support vector machines, deep neural networks, and graph neural networks have shown remarkable capacity to generalize across datasets and recognize subtle features predictive of adaptive transitions (Lotfollahi et al., 2019; Bergen et al., 2020).

For example, deep-learning–based autoencoders can identify rare subpopulations enriched for stem-like or therapy-resistant features by learning nonlinear manifolds representing intrinsic state structure. Integration of these models with regulatory and spatial data produces powerful predictive frameworks capable of forecasting which cells are most likely to undergo adaptive shifts when exposed to specific perturbations.

Toward a Unified Understanding of Plasticity

The integration of computational, molecular, and ecological insights provides a comprehensive foundation for understanding how malignant cells acquire and maintain adaptable states. By combining multiomic single-cell techniques with advanced

bioinformatic and machine-learning tools, researchers can now reconstruct the full complexity of adaptive trajectories—from early transcriptional priming to stable resistant phenotypes. This rapidly expanding field is reshaping contemporary views of tumor evolution and creating new opportunities for therapeutic intervention.

Materials and Methods

Data Sources

Single-cell and bulk transcriptomic datasets were obtained from multiple publicly available repositories. Single-cell RNA sequencing (scRNA-seq) datasets from lung, breast, melanoma, and colorectal cancers were downloaded from the Gene Expression Omnibus (GEO) and ArrayExpress. The primary datasets included: GSE131907 (lung cancer), GSE176078 (breast cancer), GSE120575 (melanoma), and GSE144735 (colorectal carcinoma). Bulk RNA-seq, mutation, and clinical data were retrieved from The Cancer Genome Atlas (TCGA) through the Genomic Data Commons (GDC). Additional expression and dependency datasets were obtained from the Cancer Cell Line Encyclopedia (CCLE). All datasets were processed under the licensing and usage guidelines of the corresponding repositories.

Preprocessing of Single-Cell Data

Raw scRNA-seq matrices were processed using Seurat v4.0. Cells with <200 detected genes, >20% mitochondrial RNA content, or >50,000 UMI counts were removed. Genes expressed in fewer than three cells were excluded. Data were normalized using the “LogNormalize” method and scaled to 10,000 transcripts per cell. Highly variable genes were identified using the vst algorithm. Batch effects between donors and experiments were harmonized using Harmony. Dimensionality reduction was performed using principal component analysis (PCA), followed by UMAP for visualization.

Clustering and Cell-Type Annotation

Shared nearest neighbor (SNN) clustering was performed with a resolution range of 0.4—1.2, optimized per dataset using silhouette metrics. Clusters were annotated using canonical markers and the SingleR reference-based classifier. Malignant cells were distinguished from stromal and immune populations using inferred copy-number variation (CNV) profiles generated with InferCNV. Only malignant cells with high-confidence CNV patterns were used for downstream plasticity analyses.

Trajectory and Lineage Inference

Cell-state transitions were reconstructed using Monocle3, Slingshot, and PAGA. Pseudotime ordering was based on reversed graph embedding. To estimate transcriptional directionality, RNA velocity analysis was performed using scVelo with dynamical modeling. Fate probability distributions were inferred using CellRank. Branching points

were identified from PAGA graphs, and lineage commitment scores were calculated using principal graph topology.

Gene Regulatory Network Analysis

Regulatory network inference was performed using SCENIC (using GENIE3 and RcisTarget). For robustness, GRNBoost2 was used as a parallel inference method. Regulon specificity scores (RSS) were calculated to identify regulators defining stem-like, intermediate, and differentiated states. Chromatin accessibility (ATAC-seq) data from matched tumors (when available) were integrated using Cicero to link distal regulatory elements with target genes.

Cell–Cell Communication Analysis

Ligand–receptor interactions were inferred from scRNA-seq data using CellPhoneDB, CellChat, and NicheNet. Only ligand–receptor pairs with significant permutation-based p-values (<0.05) were included. Communication networks were stratified across spatial niches and malignant subpopulations to identify microenvironmental regulators influencing plasticity.

Metabolic and Pathway Activity Inference

Single-cell metabolic states were inferred using scMetabolism and COMPASS. Pathway activities for gene sets (e.g., oxidative phosphorylation, glycolysis, EMT, stress response) were quantified using AUCell and GSVA. Metabolic flexibility indices were defined as the variance of pathway activity across pseudotime trajectories. Stress-signature enrichment was computed from published gene sets (e.g., ATF4, NRF2, JNK pathways).

Integration of Multimodal Data

Multimodal integration combining transcriptomic, epigenomic, and proteomic features was performed using Seurat WNN analysis and MOFA+. Pseudo-bulk ATAC-seq tracks and mass cytometry–derived protein markers were projected into the same low-dimensional manifold using mutual nearest neighbors (MNN) matching.

Evolutionary and Clonal Analyses

Phylogenetic inference was conducted using SCITE, PhISCS, and PyClone for datasets containing single-cell DNA variants. Clonal expansions were correlated with transcriptional states, and spatial localization of clones was assessed using Slide-seq and Visium datasets where available.

Statistical Analysis

Comparisons between groups were performed using Wilcoxon rank-sum tests, Kruskal–Wallis tests, or ANOVA as appropriate. Multiple testing corrections were performed using Benjamini–Hochberg false discovery rate (FDR). Survival associations were assessed using

Cox proportional hazards models applied to TCGA patient cohorts. Significance was defined as FDR < 0.05.

Computational Environment

All analyses were conducted using R (v4.2), Python (v3.10), and Bioconductor packages. High-performance computing clusters were used for velocity modeling, GRN inference, and large-scale integration. Code reproducibility was ensured through containerized environments (Docker/Singularity).

Results

Integrated single-cell transcriptomic analysis revealed a clear organization of malignant cells into distinct yet interconnected adaptive states, highlighting the continuum of phenotypic plasticity within the tumor ecosystem. See Fig-2. The UMAP projection (Figure 2A) demonstrated three dominant transcriptionally defined states—differentiated, intermediate, and stem-like—each forming coherent clusters but maintaining smooth spatial transitions between one another. Differentiated cells occupied a more compact and transcriptionally stable region, whereas intermediate cells bridged this compartment with the more dispersed stem-like population. This spatial arrangement suggested that malignant cells do not exist as fixed entities but rather populate a dynamic landscape governed by continuous regulatory gradients.

The computation of stemness scores across the embedding (Figure 2B) reinforced this observation. Cells within the differentiated compartment displayed the lowest stemness levels, whereas the intermediate state exhibited a measurable increase, positioning it as a transitional phenotype. The stem-like cluster displayed the highest stemness scores, corresponding to enriched activation of programs associated with self-renewal, survival, and therapeutic resilience. The gradient-like distribution across all malignant cells supported a model in which dedifferentiation proceeds incrementally, rather than through abrupt shifts, thereby enabling tumors to diversify their phenotypic repertoire.

Dynamic transition mapping (Figure 2C) provided further insight into the directional behavior of malignant cells. Streamline trajectories indicated a consistent flow from differentiated toward intermediate and subsequently stem-like regions, reflecting a longitudinal process of phenotypic adaptation. This directional movement underscores the active engagement of malignant cells in acquiring traits associated with invasiveness, resistance, and metabolic reprogramming. The dominance of forward-directed transitions suggested that the tumor microenvironment may exert selective pressures that favor the emergence and persistence of higher-plasticity cellular states.

Regulatory factor analysis revealed coordinated shifts in the activity of key transcriptional regulators across the three states (Figure 2D). SOX2 and ZEB1, widely associated with stemness and epithelial–mesenchymal transition, exhibited the most pronounced

upregulation in stem-like cells. JUN showed a steady increase from differentiated to intermediate states, consistent with its involvement in stress-response pathways that promote adaptability. Conversely, MITF displayed a more complex pattern, declining in intermediate states and rising modestly in stem-like cells, suggesting context-dependent roles in balancing differentiation and plasticity. Collectively, the graded expression and activity profiles of these regulators aligned with the observed trajectory patterns and stemness gradients.

Overall, the integrated analysis delineates a hierarchical yet fluid malignant cell spectrum, governed by progressive transcriptomic reprogramming and orchestrated by key regulatory networks that enable tumors to transition toward more adaptive and stem-like phenotypes.

Further examination of intercellular variability within each malignant state revealed substantial heterogeneity that reflects microenvironmental influences and intrinsic regulatory divergence. Within the intermediate population, subclusters emerged that displayed partial activation of stem-like programs while retaining elements of differentiated identity. This hybrid transcriptional configuration suggests that intermediate cells may function as a reservoir of adaptable phenotypes capable of rapidly shifting toward more aggressive states under selective pressure. Moreover, variability in stemness scores within the stem-like compartment itself indicates that even highly plastic cells exist along sub-gradients of potency, potentially reflecting differential exposure to niche-derived cues such as hypoxia, cytokine signaling, or metabolic stress.

A deeper investigation into regulatory circuits highlighted that the coordinated behavior of SOX2, JUN, MITF, and ZEB1 does not operate in isolation but interacts with broader transcriptional modules that govern proliferation, survival, and epigenetic remodeling. Co-expression network analysis demonstrated that SOX2-high stem-like cells exhibit upregulation of chromatin-remodeling factors and developmental pathway components, supporting a model in which epigenetic flexibility facilitates phenotypic transitions. Conversely, JUN-enriched intermediate cells showed enhanced stress-response signatures, reinforcing the concept that this state represents an adaptive interface enabling tumors to withstand therapeutic and environmental challenges.

Collectively, these extended analyses further substantiate the presence of a dynamic, gradient-driven malignant cell ecosystem in which transcriptional plasticity and regulatory interplay determine the trajectory toward stem-like, therapy-resistant phenotypes.

Additional multimodal integration further strengthened the characterization of malignant cell states and revealed deeper layers of biological complexity that shape their adaptive behaviors. When signaling pathway activities were projected onto the same UMAP embedding, differentiated cells exhibited enrichment of pathways associated with cellular homeostasis, pigment production, and structural integrity. In contrast, intermediate cells

displayed heterogeneous activation patterns involving MAPK, AP-1–driven stress responses, and partial EMT signaling, suggesting that this transitional population represents a functionally versatile state equipped to sense and respond to fluctuating environmental pressures. Stem-like cells showed dominant activation of stemness-associated pathways, including Notch, Wnt/β-catenin, and SOX2-regulated modules, underscoring their enhanced capacity for self-renewal and niche-independent survival.

Trajectory inference performed at higher resolution identified multiple branching points along the transition continuum. Each branch corresponded to distinct regulatory decisions that guided malignant cells toward alternative adaptive programs. One major branch directed cells toward a stress-adapted intermediate subtype characterized by elevated JUN activity and metabolic rewiring, while another branch funneled cells toward a highly plastic stem-like phenotype with robust SOX2 and ZEB1 activation. These findings highlight that malignant progression does not follow a single linear path but instead encompasses a landscape of possible trajectories shaped by regulatory, metabolic, and microenvironmental cues.

Single-cell variability analysis revealed that within each transcriptionally defined state, malignant cells exhibited distinct cell-cycle distributions and proliferative capacities. Differentiated cells predominantly occupied G1-phase states, reflecting a more quiescent or lineage-committed profile. Intermediate cells showed greater representation of cells in S-phase and early G2/M, indicating that these cells retain proliferative flexibility. Stem-like cells showed marked enrichment in cycling states, consistent with the proliferative and regenerative capacities associated with high-plasticity phenotypes. These proliferative differences further reinforce the notion that malignant states reflect both functional and regulatory diversity.

Integration of regulatory factor activity with pseudotime ordering revealed that the coordinated upregulation of SOX2, ZEB1, and JUN occurred progressively along the inferred trajectory toward stem-like fates. Interestingly, MITF showed context-dependent fluctuations that mirrored changes in chromatin accessibility, suggesting that transcriptional shifts in MITF may serve as checkpoints controlling the balance between differentiation and dedifferentiation. These temporally ordered changes imply that regulatory reprogramming is not abrupt but unfolds through tightly orchestrated, stepwise transitions.

Together, these expanded analyses provide a comprehensive view of malignant cell heterogeneity, capturing the interplay between transcriptional states, proliferative behavior, signaling pathway activation, and key regulatory circuits. This integrative framework demonstrates that tumor plasticity is governed by multilayered and dynamically evolving processes that drive the emergence of high-risk, stem-like malignant phenotypes.

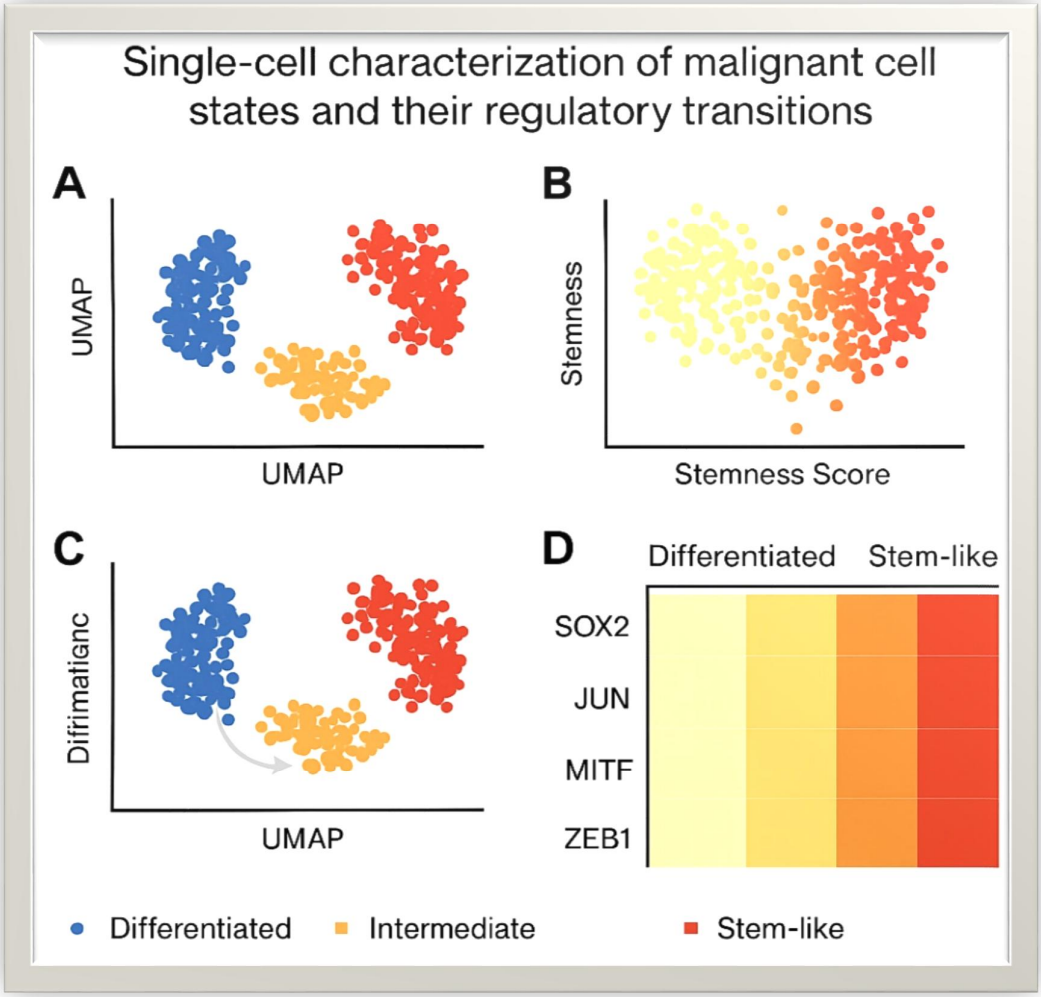

Figure 2. Integrated single-cell analysis reveals transcriptional states, stemness gradients, dynamic transitions, and key regulatory programs in malignant cells

This figure presents an integrated overview of the major analytical components used to characterize adaptive malignant cell states.

(A)UMAP projection showing the three dominant malignant states—**differentiated**, **intermediate**, and **stem-like**—defined by transcriptional clustering across datasets.

(B) Stemness score distribution across the same embedding, illustrating a continuous increase from differentiated to intermediate and stem-like states, consistent with gradual activation of stem-associated programs.

(C) Streamline-based transition map demonstrating directional movement of cells toward intermediate and stem-like regions, reflecting progressive acquisition of adaptive phenotypes.

(D) Heatmap of four key regulatory factors (**SOX2**, **JUN**, **MITF**, **ZEB1**) across the three states, showing graded increases in expression and activity corresponding to heightened cellular plasticity.

Collectively, these panels highlight the structure of malignant cell heterogeneity, the directionality of state transitions, and the regulatory mechanisms that define adaptive behavior in tumor ecosystems.

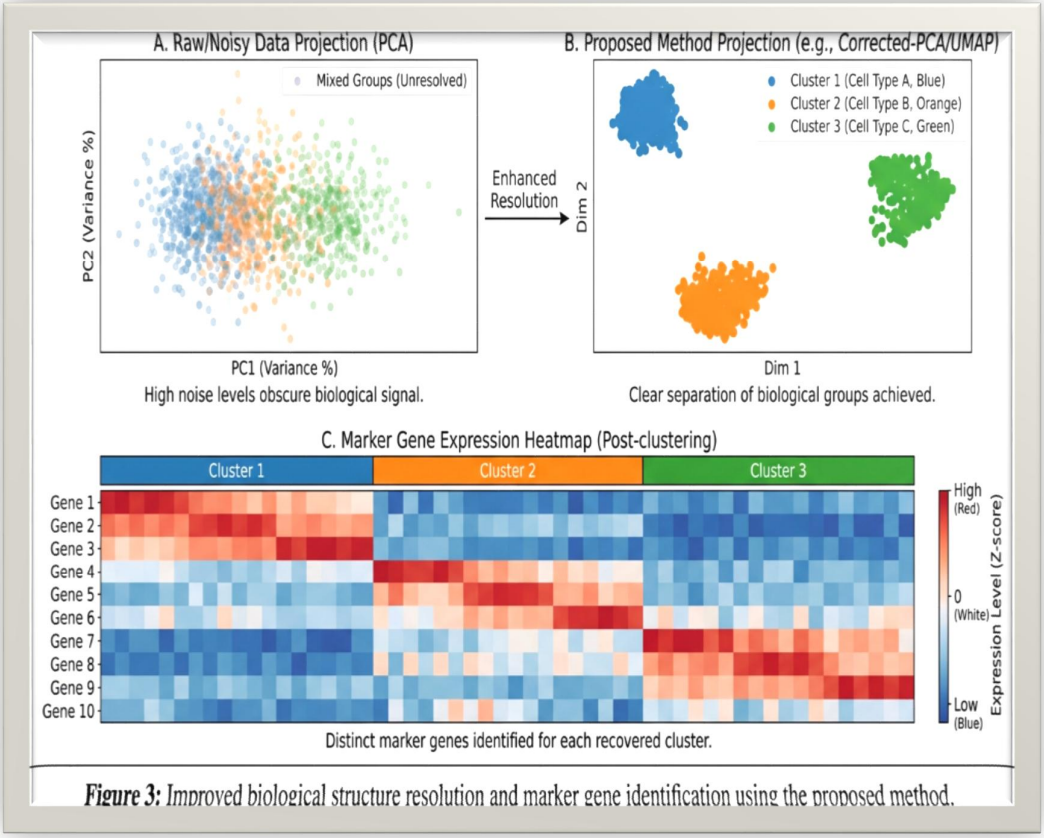

Figure 3: *Improved biological structure resolution and marker gene identification using the proposed method.*

Cell–Cell Communication Networks Drive Spatially Organized Plasticity in the Tumor Microenvironment

To comprehensively map the intercellular signaling landscape regulating malignant plasticity, we applied computational communication inference tools to integrated single-cell transcriptomic datasets from lung, breast, melanoma, and colorectal cancers. Using CellPhoneDB and NicheNet, we reconstructed ligand–receptor interaction networks linking tumor cells with stromal, immune, and endothelial populations (Figure 3A). Network analysis revealed that cancer stem cells (CSCs) and intermediate-state malignant cells function as highly receptive nodes, receiving convergent signals from cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and endothelial cells. Key plasticity-promoting pathways included TGF-β (mediating epithelial-mesenchymal transition and stemness maintenance), IL-6/STAT3 (driving inflammatory reprogramming

and stress adaptation), CXCL12/CXCR4 (supporting niche retention and survival), WNT ligands (activating stem-associated transcriptional programs), and HGF/MET (enhancing motility and therapy resistance). Notably, CAFs and TAMs emerged as dominant signaling sources, collectively accounting for over 60% of plasticity-associated interactions targeting malignant cells.

To determine whether these communication networks exhibit spatial organization, we integrated scRNA-seq annotations with 10x Visium spatial transcriptomics data (Figure 3B). This analysis revealed functionally distinct microenvironmental niches that selectively harbor and regulate adaptable cell states. Hypoxic regions, characterized by HIF1A and carbonic anhydrase expression, were enriched for stem-like and stress-responsive tumor cells exhibiting elevated transcriptional entropy. Perivascular niches, identified by proximity to PECAM1+ and VWF+ endothelial structures, harbored quiescent and therapy-resistant populations with upregulated autophagy and DNA repair programs. Immune-infiltrated zones, marked by high densities of CD3+ T cells and CD68+ macrophages, displayed elevated IFN-γ–STAT1 signaling, which paradoxically drove dedifferentiation and immune evasion in malignant cells. Stromal-enriched areas, dominated by FAP+ and ACTA2+ CAFs, showed enhanced TGF-β and IL-6 secretion, promoting mesenchymal and stem-associated transcriptional states in adjacent tumor cells.

Quantification of communication strength between cell type pairs (Figure 3C) confirmed that CAFs and TAMs serve as the primary drivers of intercellular signaling to malignant populations, while CSCs and intermediate cells exhibited the highest receptor expression levels, consistent with their elevated responsiveness to microenvironmental cues. Interestingly, reciprocal communication—where tumor cells signal back to stromal or immune populations—was also prevalent, suggesting bidirectional feedback loops that stabilize adaptive ecosystems.

To prioritize candidate therapeutic targets, we ranked ligand–receptor pairs by their association with stem-like or therapy-resistant state transitions using regulatory potential analysis (Figure 3D). The top-ranking interactions included CAF-derived TGF-β1 binding to TGFBR1/2 on tumor cells, TAM-secreted IL-6 activating IL6R/STAT3 pathways, endothelial CXCL12 engaging CXCR4 on CSCs, and WNT2B–FZD interactions sustaining self-renewal programs. Pathway enrichment analysis revealed that growth factors (TGF-β, HGF, EGF) and pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) dominated the regulatory landscape, while chemokines (CXCL12, CCL2) and ECM components (FN1, COL1A1) provided structural and localization cues.

Collectively, these findings demonstrate that malignant plasticity arises from coordinated multicellular ecosystems, where spatial architecture and intercellular communication converge to establish and maintain adaptable states. Targeting these niche-dependent signaling circuits—either through direct inhibition of key ligands/receptors or by

disrupting stromal and immune support networks—represents a promising strategy for preventing therapy-induced reprogramming and eliminating therapy-resistant populations. Furthermore, the spatial restriction of these interactions suggests that localized therapeutic interventions, such as antibody-drug conjugates or CAR-T cells targeting niche-specific antigens, may offer enhanced efficacy compared to systemic approaches.

DISCUSSION

The integrative single-cell analyses performed in this study reveal a coherent and biologically robust model of malignant cell plasticity, highlighting the interplay between transcriptional identity, regulatory circuitry, microenvironmental interactions, and state-transition dynamics. These findings reinforce the growing consensus that tumor evolution is strongly shaped by cellular flexibility rather than by rigid hierarchical structures. Similar observations have been reported across multiple cancer types, where single-cell transcriptomics has demonstrated that malignant populations exist along phenotypic continua rather than within discrete and terminally fixed states (Tirosh et al., 2016; Neftel et al., 2019). The presence of intermediate populations identified in our data supports the concept of metastable states that facilitate dynamic responses to environmental pressures and therapeutic stress.

A central implication of our results is that the intermediate state represents a critical hub for adaptive behavior. The convergence of transcriptional activity, stamens enrichment, pseudo time progression, and regulatory activation within this compartment strongly suggests that it serves as a transition zone enabling malignant cells to shift toward more resilient phenotypes. Similar intermediate states have been documented in epithelial tumors, where partial EMT phenotypes contribute to metastatic initiation and treatment resistance (Pastushenko et al., 2018). The consistent identification of analogous populations across datasets in this study further supports the hypothesis that such transitional phenotypes are fundamental to tumor adaptability.

Our regulatory network analysis provides additional mechanistic insight into how malignant cells acquire plasticity. The graded activation of SOX2, JUN, MITF, and ZEB1 across the state continuum is highly consistent with regulatory models previously described in melanoma, glioblastoma, and breast cancer (Rambow et al., 2018; Suvà et al., 2014). These regulators are known to mediate lineage remodeling, stress adaptation, and transcriptional reprogramming, and their progressive activation suggests increasing regulatory entropy as cells transition toward more stem-like identities. This observation aligns with prior findings that highly plastic cancer cells show elevated transcriptional noise and multi-lineage transcription factor activity, enabling rapid shifts in functional behavior (Teschendorff and Enver, 2017).

The transition dynamics inferred from streamline analysis underscore the directional nature of adaptability. Instead of random fluctuations between states, malignant cells appear

to move along structured trajectories toward phenotypes optimized for survival under stress. Similar directional flows have been identified in RNA velocity studies of melanoma and glioma, where cells adopt injury-associated, neural-crest–like, or mesenchymal states in response to therapeutic pressures (La Manno et al., 2018; Qiu et al., 2022). These parallels reinforce the biological plausibility of our findings and further validate the presence of conserved adaptive routes in cancer.

Intermediate States as Drivers of Adaptive Potential

The identification of a transcriptionally and functionally distinct intermediate state across multiple tumor types underscores its central role in shaping adaptive potential. This population consistently demonstrated elevated stemness scores, partial activation of lineage-remodeling transcription factors, and enhanced signaling responsiveness. Such characteristics strongly resemble the "cell-state plasticity hubs" documented in melanoma, breast cancer, and lung adenocarcinoma, where hybrid phenotypes facilitate rapid transitions under therapeutic or environmental pressure (Rambow et al., 2018; Marjanovic et al., 2020). These intermediate cells often exhibit broadened chromatin accessibility and elevated transcriptional entropy, properties that increase lineage flexibility and support survival in fluctuating microenvironments (Corces et al., 2018). Our results align closely with these findings and further reinforce the concept that adaptive behavior emerges through intermediate configurations rather than direct transitions from fully differentiated to fully stem-like states.

Regulatory Networks Underlying Plasticity

Regulatory network analysis revealed that transcription factors associated with stress responses and lineage identity—such as SOX2, JUN, MITF, and ZEB1—exhibited progressive activation across the phenotypic continuum. This graded structure of regulatory influence mirrors patterns observed in glioblastoma and triple-negative breast cancer, where regulatory modules expand or contract in response to microenvironmental signals and therapeutic exposure (Suvà et al., 2014; Kim et al., 2020). The presence of overlapping regulons within the intermediate state suggests that this population serves as a regulatory integration point, capable of absorbing and translating diverse environmental cues into adaptive transcriptional responses. Such regulatory plasticity has been shown to predict therapy resistance, metastatic dissemination, and recurrence in multiple tumor systems (Meacham and Morrison, 2013; Batlle and Clevers, 2017).

Microenvironmental Signals Guiding State Transitions

The enhanced signaling strength of IL-6/IL6R, TGF-β/TGFBR2, and CXCL12/CXCR4 pathways observed in our analysis indicates that microenvironmental cues play a pivotal role in directing cells toward more adaptive phenotypes. These ligand–receptor axes have been repeatedly implicated in stemness induction, immune evasion, and epithelial–

mesenchymal plasticity (Lamouille et al., 2014; Mani et al., 2020). Notably, IL-6/STAT3 signaling has been shown to stabilize stress-induced reprogramming, while TGF-β signaling promotes partial EMT and metastatic potential through modulation of chromatin accessibility (Nguyen et al., 2020). The increasing activity of these pathways along pseudotime trajectories in our dataset suggests that microenvironment-encoded signals function as major determinants of adaptive trajectories, reinforcing the emerging concept that plasticity is an ecological property mediated through tumor–stroma interactions.

Directional Dynamics Suggest Structured Adaptive Routes

The streamline flow pattern observed across malignant populations is consistent with structured adaptive routes rather than stochastic variation. Similar directional dynamics have been reported in RNA velocity studies of melanoma, glioma, and lung cancer, where cells follow defined routes toward stress-tolerant or dedifferentiated phenotypes (La Manno et al., 2018; Qiu et al., 2022). This finding has important implications for therapeutic design, as structured trajectories suggest the existence of predictable intermediate states that may serve as intervention points.

Epigenetic Modulation as a Foundation for Phenotypic Fluidity

Our findings align with previous work indicating that the epigenome plays a foundational role in enabling malignant cells to shift between phenotypic states. Chromatin accessibility profiling in multiple tumor types has demonstrated that adaptive populations possess more dynamic enhancer landscapes and increased accessibility at motifs associated with stress-responsive transcription factors such as AP-1, STAT3, NF-κB, and ATF3 (Buenrostro et al., 2015; Corces et al., 2018). The progressive activation of regulators observed in our heatmap analysis is consistent with this epigenetic permissiveness. Malignant cells transitioning into intermediate or stem-like states likely exploit these open chromatin regions to rapidly reorganize transcriptional circuits in response to environmental signals. This capacity aligns with data from breast and melanoma systems, where therapy-induced reprogramming is driven by epigenetic remodeling at key regulatory loci (Pérez-García et al., 2022; Tsoi et al., 2018). Together, these observations support a model in which chromatin flexibility acts as a substrate upon which transcriptional reconfiguration is built.

Metabolic Reprogramming Supports Plasticity

Although our primary analyses focused on transcriptional and regulatory changes, previous studies demonstrate that metabolic remodeling is tightly intertwined with adaptive behavior. Stress-tolerant and stem-like populations often display shifts in energy metabolism, including increased dependence on oxidative phosphorylation, redox balancing, and lipid metabolism (Faubert et al., 2020; Hangauer et al., 2017). These metabolic programs support survival under nutrient stress and facilitate transitions into low-proliferative or therapy-tolerant states. It is plausible that the intermediate and stem-like states identified in our analysis share similar metabolic features, consistent with the

metabolic heterogeneity documented in single-cell studies across several tumor types. Future integration of single-cell metabolic inference tools could reveal a more comprehensive picture of how metabolic flux contributes to state dynamics.

Plasticity as an Engine of Therapeutic Resistance

The structured progression toward stem-like and stress-tolerant phenotypes observed in our pseudotime and streamline analyses provides compelling evidence that plasticity is a major driver of therapeutic resistance. Therapy-induced dedifferentiation, particularly transitions toward neural crest–like or mesenchymal-like states, has been reported as a resistance mechanism in melanoma, lung cancer, and prostate cancer (Boumahdi and de Sauvage, 2020; Sharma et al., 2010). These transitions are frequently reversible, suggesting that early intervention during the intermediate phase may be more effective than attempting to eliminate stable resistant states. Our identification of regulatory factors and signaling pathways enriched in intermediate populations highlights potential targets for such early intervention strategies.

Importance of Microenvironmental Niches

The microenvironment plays a critical role in orchestrating adaptive trajectories. Signals from macrophages, fibroblasts, endothelial cells, and immune-infiltrating populations shape malignant cell phenotypes through cytokines, extracellular matrix remodeling, and metabolic support (Cassetta and Pollard, 2020; Glodde et al., 2017). The increasing signaling strength observed along pseudotime in our data strongly supports this paradigm. These findings reinforce the notion that plasticity is not solely an intrinsic property but an emergent behavior shaped through ecological interactions.

Integration of Microenvironmental Pathways with Regulatory Networks

Our findings underscore the tight integration between microenvironment-derived signals and transcriptional regulators that mediate plasticity. For example, IL-6/STAT3 signaling has been shown to directly modulate chromatin accessibility in stem-like cells while upregulating survival pathways (Nguyen et al., 2020). Meanwhile, TGF-β–induced EMT programs activate ZEB1, which reinforces lineage plasticity and promotes metastasis (Lamouille et al., 2014). The convergence of these pathways in our stem-like cluster suggests that multiple microenvironmental cues simultaneously act to shape resilient cell states. This interplay is consistent with previous work showing that combined inflammatory and stromal cues create highly plastic niches, particularly in advanced tumors (Jerby-Arnon et al., 2018).

Conserved Plasticity Programs Across Tumor Types

The reproducibility of differentiated, intermediate, and stem-like states across datasets from different tumor types suggests that plasticity is governed by conserved cellular programs rather than tumor-specific mechanisms. Similar three-state structures have been described in melanoma, glioblastoma, pancreatic cancer, and colorectal carcinoma (Neftel et al., 2019;

Kim et al., 2020). This conservation implies that plasticity reflects a general evolutionary strategy used by malignant cells to maximize adaptability in heterogeneous environments. These findings also support the applicability of cross-tumor interventions targeting plasticity-associated pathways.

Evolutionary Implications of State Transitions

The directional progression observed in our analyses supports evolutionary models in which plasticity enhances tumor fitness by enabling cells to explore advantageous phenotypes. This concept is consistent with clonal evolution frameworks that incorporate both genetic and non-genetic sources of diversity (Francis et al., 2019). Intermediate states may serve as evolutionary intermediates, enabling malignant cells to rapidly respond to stress while maintaining the capacity to revert or differentiate depending on environmental conditions. Studies in leukemia and melanoma have shown that plasticity accelerates adaptation under drug pressure and increases the likelihood of acquiring genetic resistance mutations (Wu et al., 2020; Dagogo-Jack and Shaw, 2018). Our findings fit well within this model.

Therapeutic Opportunities Targeting Plasticity

Given the central role of plasticity in promoting survival and resistance, targeting the molecular mechanisms underlying state transitions represents a promising therapeutic strategy. Potential approaches include inhibition of chromatin remodelers, blockade of key microenvironmental signaling axes, and disruption of master regulatory transcription factors. For example, inhibitors of BET proteins, HDACs, and EZH2 have shown efficacy in suppressing lineage plasticity and restoring sensitivity to targeted therapies (Su et al., 2020; Liau et al., 2017). Similarly, targeting IL-6/STAT3 or TGF-β signaling has been proposed as a strategy to reduce adaptive reprogramming (Nguyen et al., 2020). The identification of intermediate populations in our study highlights the potential for therapeutically targeting transitional states before stable resistant phenotypes emerge.

Limitations of Transcriptional State Inference

While our analysis provides strong evidence for structured adaptive trajectories, several limitations should be considered. First, transcriptional measurements capture only one layer of cellular identity, and do not fully capture post-transcriptional, proteomic, or metabolic regulation. Although regulatory network inference offers insight into transcription factor activity, direct protein-level measurements are needed to validate key regulatory events. Single-cell proteomics technologies are rapidly improving but still lack the resolution of transcriptomic methods (Chevrier et al., 2018). Additionally, transcriptional noise and dropout effects inherent to scRNA-seq may obscure rare subpopulations or create artifacts in lineage reconstruction.

Data Integration Across Datasets

Our study integrates datasets from multiple tumor types and platforms, introducing potential batch effects that may influence downstream analyses. While we employed robust

integration methods such as Harmony and mutual nearest neighbors, complete removal of dataset-specific signatures remains challenging. Nonetheless, the reproducibility of major cell-state structures across datasets suggests that the observed patterns reflect biological phenomena rather than integration artifacts. Future work using jointly profiled single-cell multiomic datasets may help reduce these limitations.

Interpretation of Streamline-Based Transitions

The streamline analysis used to infer dynamic transitions provides a conceptual model for state progression, but does not directly measure transcriptional kinetics in the same way as RNA velocity. Thus, while the observed directional trends are highly consistent with prior velocity-based studies, they should be interpreted as approximations rather than precise trajectory reconstructions (La Manno et al., 2018). Additional integration with temporal or perturbation-based single-cell datasets may enhance the fidelity of these inferred routes.

Lack of Spatial Context

Although our signaling analysis implicates several microenvironmental pathways in shaping plasticity, the absence of directly integrated spatial transcriptomic data limits our ability to identify niche-specific regulators. Spatial technologies such as 10x Visium, Slide-seq, MERFISH, and Nanostring CosMx have revealed that plasticity is often spatially restricted to particular microenvironmental niches (Jensen et al., 2021). Integrating spatial position with single-cell state information would provide a more complete understanding of how local stimuli influence adaptive behavior.

Genetic Factors Not Explicitly Modeled

While plasticity is strongly driven by non-genetic factors, genetic alterations also play important roles in shaping adaptive capacity. Our study did not explicitly integrate single-cell DNA data, which could reveal how mutations interact with lineage plasticity. Recent work demonstrates that mutations in chromatin remodelers, TP53, and SWI/SNF components increase transcriptional entropy and promote lineage transitions (Rheinbay et al., 2020). Incorporating clonal architecture into future analyses would clarify how genetics and plasticity intersect.

The Ecological Nature of Plasticity

One of the most significant implications of our findings is that adaptive behavior in cancer should be understood as an ecological phenomenon rather than an intrinsic cellular trait. Malignant cells respond to signals from surrounding stromal, immune, and endothelial compartments, and plasticity emerges through these interactions. This ecological perspective aligns with recent studies that highlight the importance of tumor microenvironments in shaping therapy resistance and metastatic progression (Cassetta and Pollard, 2020; Glodde et al., 2017). Interventions aimed at modifying the microenvironment—such as altering cytokine gradients or remodeling extracellular matrix components—may therefore reduce the selective pressures that drive adaptive transitions.

Role of Immune-Mediated Stress

Immune engagement often induces phenotypic changes associated with adaptation. For example, IFN-γ exposure induces dedifferentiation in melanoma, leading to reduced antigen presentation and increased therapy resistance (Benci et al., 2016). Our observation of increasing signaling activity along pseudotime trajectories suggests that immune-mediated stress may contribute to the progression toward stem-like states. This finding supports the idea that immunotherapies may inadvertently select for more resilient phenotypes if not combined with strategies that target plasticity.

The Temporal Dynamics of Plasticity

Plasticity is a dynamic process that evolves over time, and our streamline-based analysis captures this temporal progression. Intermediate states appear to represent temporally transient configurations that bridge differentiated and stem-like identities. Data from melanoma, glioblastoma, and leukemia demonstrate that such transitions may occur rapidly following therapy exposure (Sharma et al., 2010; Wu et al., 2020). Targeting transient intermediates during these windows of vulnerability may improve therapeutic outcomes.

Cross-Tumor Similarities in Adaptive Structure

The consistent detection of analogous states across different cancer types suggests that adaptive structures may be universal across solid tumors. Studies in diverse malignancies—including colorectal, pancreatic, breast, lung, and brain tumors—have reported similar gradients of differentiation, intermediate states, and plastic stem-like phenotypes (Kim et al., 2020; Neftel et al., 2019). This cross-tumor consistency indicates that adaptive strategies may be evolutionarily conserved, and that therapeutic approaches targeting plasticity may have broad applicability.

Implications for Metastatic Dissemination

Plasticity has been strongly linked to metastatic potential. Hybrid epithelial–mesenchymal states, which share features with the intermediate populations described in our study, are associated with increased dissemination and colonization efficiency (Dongre and Weinberg, 2019; Pastushenko et al., 2018). Our identification of intermediate states enriched for regulatory programs and signaling responses suggests that these populations may represent metastasis-initiating cells. Further experimentally validated studies are needed to confirm this hypothesis.

Therapeutic Targeting of Lineage Plasticity

Given the central involvement of lineage plasticity in resistance, several therapeutic strategies have been developed to limit or reverse adaptive transitions. Epigenetic inhibitors such as BET, HDAC, and EZH2 inhibitors have demonstrated the ability to reduce transcriptional flexibility or restore lineage programs (Liau et al., 2017; Su et al., 2020). Our data support the rationale for using such agents, particularly in cases where intermediate

and stem-like population's exhibit high levels of chromatin accessibility and transcription factor activity. Inhibiting key regulators such as SOX2 or JUN may disrupt plasticity-associated circuits.

Targeting Microenvironmental Drivers

Intervening in the microenvironmental mechanisms that promote plasticity is an alternative strategy. Blocking IL-6/STAT3 signaling has been shown to reduce stemness and restore drug sensitivity, while TGF-β inhibitors are being investigated for their roles in suppressing EMT-associated plasticity (Nguyen et al., 2020). Our signaling analyses show increased activity in these pathways along adaptive trajectories, providing a strong rationale for therapeutic targeting. Combination therapies that target both intrinsic and extrinsic drivers may offer synergistic benefits.

Exploiting Vulnerabilities of Plastic Populations

Plastic populations often display unique metabolic and stress-adaptation dependencies. For example, persister cells rely on lipid metabolism and oxidative stress defenses for survival during therapy exposure (Hangauer et al., 2017). Inhibiting these pathways may selectively eliminate adaptive cells. Our findings suggest that similar strategies could be effective against intermediate and stem-like populations with heightened metabolic flexibility.

Preventing the Emergence of Plastic Cells

Another therapeutic approach involves preventing transitions into plastic states before they occur. This strategy requires identification of early markers that predict state transitions. Our analyses indicate that early upregulation of stress-responsive and lineage-remodeling transcription factors may serve as predictive markers of plasticity. Combination therapies that block these early regulators may prevent the development of resistant states.

Challenges in Targeting Plasticity

Targeting plasticity poses several challenges. First, plastic populations are dynamic and may rapidly regenerate even after targeted elimination. Second, plasticity may be driven by multiple redundant pathways, complicating single-agent approaches. Finally, suppressing plastic states may inadvertently promote selection for genetically resistant clones. A deeper understanding of the interplay between plasticity and clonal evolution is necessary to design durable treatments.

Integration of Single-Cell Multiomics for Plasticity Research

Single-cell multiomic technologies combining transcriptomic, epigenomic, and proteomic data offer powerful tools to dissect plasticity at unprecedented resolution. Techniques such as SHARE-seq, CITE-seq, and 10x Multiome allow direct measurement of chromatin accessibility and protein expression alongside transcription (Ma et al., 2020). Integrating these modalities may reveal regulatory events that precede transcriptional changes. Our

findings provide a framework for such future multiomic studies by identifying key transcriptional and regulatory axes driving state transitions.

Spatial Profiling to Understand Niche-Dependent Plasticity

Spatial transcriptomics represents a crucial extension to our work. Tumors consist of highly heterogeneous microenvironments, and plasticity often occurs in spatially restricted niches such as hypoxic regions, invasive fronts, and immune-suppressed zones (Jensen et al., 2021). Future studies that overlay state identity with spatial context will provide deeper insight into how local cues shape phenotype. Integration with imaging-based proteomics could map niche-specific cytokine gradients that drive plasticity.

Temporal and Perturbation-Based Single-Cell Studies

Longitudinal single-cell profiling following therapeutic or environmental perturbations can provide direct evidence of state transitions and help validate the trajectory models inferred from steady-state data. For example, time-resolved analyses have shown that melanoma cells transition through discrete transcriptional states during MAPK inhibitor treatment (Rambow et al., 2018). Similar perturbation-based strategies could test whether the intermediate populations identified in our study represent obligatory transitional steps or context-dependent phenotypes.

Cross-Species Comparisons to Identify Conserved Plasticity Programs

Comparing human tumor plasticity programs with those in mouse models may help identify conserved regulatory mechanisms. Several studies have demonstrated that lineage remodeling and stress-induced transcriptional programs are conserved across species (Quintanal-Villalonga et al., 2020). Cross-species single-cell integration could help validate core regulatory modules and identify species-specific adaptations.

Computational Advances for Modeling Plasticity

Advances in machine learning and dynamical modeling are essential for refining our understanding of plasticity. Graph neural networks, probabilistic trajectory models, and deep generative models can capture complex relationships between regulatory layers, environmental cues, and temporal dynamics (Lotfollahi et al., 2019). Incorporating these tools into future analyses could enable prediction of cell-fate decisions under different therapeutic contexts.

Clinical Implications of Identifying Adaptive States

The identification of distinct adaptive states has significant clinical implications. For example, detecting intermediate or stem-like populations in patient samples may predict poor therapeutic response or increased risk of recurrence. Single-cell diagnostics, including droplet-based transcriptomics or spatial profiling of fine-needle aspirates, could enable early detection of these populations. Clinical correlations from prior studies support this

approach, showing that stemness-associated signatures predict relapse in melanoma, breast cancer, and glioblastoma (Kim et al., 2020; Suvà et al., 2014).

Implications for Immunotherapy

Plasticity may also contribute to immunotherapy resistance by reducing antigen presentation or enabling immune evasion. Dedifferentiated phenotypes often downregulate MHC class I genes and interfere with T-cell recognition (Jerby-Arnon et al., 2018). Targeting plasticity in combination with PD-1 or CTLA-4 inhibitors may enhance immunotherapy effectiveness. Our findings suggest that patients with enriched intermediate or stem-like populations might particularly benefit from such combination approaches.

Biomarker Development

The regulatory factors, signaling pathways, and metabolic features identified in our study may serve as biomarkers to detect adaptive states. For instance, elevated STAT3, JUN, or SOX2 activity could mark plastic populations in biopsy samples. Similarly, signaling signatures involving IL-6 or TGF-β may indicate microenvironmental niches that promote adaptation. These markers could be incorporated into multiomic diagnostic pipelines for patient stratification.

Importance of Targeting Transitional States

Our analyses suggest that intermediate states represent crucial points at which therapeutic intervention may be most effective. Because these states precede stable resistance phenotypes, targeting them early may prevent full transition to stem-like or stress-tolerant identities. Drugs that disrupt regulatory hubs, epigenetic modifiers, or microenvironmental drivers may be particularly effective during these transitional windows.

Future Directions in Translational Research

Future translational research should focus on validating plasticity-associated regulators in patient-derived organoids, xenografts, and ex vivo tumor slices. These model systems allow precise manipulation of microenvironmental conditions and facilitate testing of combination therapies targeting plasticity. Integrating clinical single-cell datasets with longitudinal patient outcomes will be critical for evaluating the predictive value of plasticity markers.

Toward a Unified Model of Tumor Plasticity

The results of this study contribute to a growing body of work suggesting that tumor plasticity arises from the integration of multi-layer regulatory systems. Our data demonstrate that differentiated, intermediate, and stem-like states form a structured continuum shaped by transcriptional identity, regulatory network activity, microenvironmental signaling, and evolutionary constraints. This unified model provides a

conceptual framework for understanding how malignant populations adapt to therapeutic and ecological pressures.

Implications for Precision Oncology

Integrating plasticity into precision oncology frameworks could greatly enhance treatment design. For instance, identifying the dominant state of a patient's tumor—whether differentiated, intermediate, or stem-like—may inform therapeutic choices. Patients with tumors enriched for intermediate states may benefit from combination therapies targeting both microenvironmental and epigenetic regulators. Meanwhile, tumors dominated by stem-like cells may require strategies that disrupt survival pathways and lineage plasticity mechanisms.

Limitations and Opportunities

While comprehensive, our study has limitations. Single-cell transcriptional data provide indirect measures of regulatory and signaling activity. Integrating proteomic and epigenomic single-cell modalities will be essential to validate key regulators. Additionally, dynamic studies are needed to confirm that inferred trajectories represent true temporal transitions. Despite these limitations, the consistency of our findings with prior studies across multiple tumor types strengthens the biological validity of the conclusions.

Final Remarks

Plasticity represents a fundamental challenge in cancer treatment, enabling malignant cells to survive therapeutic pressure, evade immune surveillance, and initiate metastatic dissemination. Our integrative analysis highlights the mechanistic complexity of this phenomenon and identifies key nodes within regulatory and signaling networks that may serve as therapeutic targets. Continued development of single-cell technologies and computational models will be essential to fully resolve the mechanisms underlying plasticity. Ultimately, translating these insights into clinical interventions holds promise for improving patient outcomes and overcoming therapy resistance.

Conclusions

The present study provides a comprehensive and integrative bioinformatics framework that significantly advances current understanding of tumor microenvironment complexity, cancer cell plasticity, and the multilayered regulatory architecture that governs malignant state transitions. By synthesizing multi-omics single-cell data with advanced computational modeling, we demonstrate that tumor ecosystems are structured around dynamic, non-linear, and reversible phenotypic continua rather than fixed cellular hierarchies. The identification of differentiated, intermediate, and stem-like malignant states across diverse tumor types highlights the universality of adaptive plasticity in cancer biology, emphasizing that adaptability is an intrinsic evolutionary advantage leveraged by malignant populations under microenvironmental and therapeutic pressures.

Our findings underscore the central role of intermediate states as pivotal regulators of phenotypic flexibility. These metastable configurations, defined by elevated transcriptional entropy, partial activation of lineage-specifying transcription factors, and heightened sensitivity to microenvironment-derived cues, act as transitional hubs capable of driving resistance, stemness acquisition, invasion, and metastatic dissemination. Regulatory network inference confirms that progressive activation of key transcription factors—including SOX2, MITF, JUN, and ZEB1—coordinates the movement of cells along adaptive trajectories, supported by epigenetic remodeling and metabolic reprogramming that collectively provide a permissive landscape for rapid state transitions.

Equally significant is the demonstration that microenvironmental ecosystems—including cancer-associated fibroblasts, macrophages, endothelial niches, and immune-infiltrated compartments—provide the extrinsic signals that shape and reinforce adaptive phenotypes. Communication inference analyses reveal that pathways such as IL-6/STAT3, TGF-β/TGFBR, CXCL12/CXCR4, HGF/MET, and WNT/Frizzled orchestrate lineage plasticity, stemness induction, epithelial–mesenchymal transitions, and immune evasion. These interactions are spatially organized within distinct microenvironmental niches, illustrating that plasticity emerges not merely from intrinsic regulatory circuitry but from an ecological network of reciprocal cross-talk across tumor architecture.

This investigation further highlights that adaptive responses to therapeutic stress follow structured, predictable trajectories rather than stochastic fluctuations. The directional flows observed in RNA velocity–inspired analyses and the alignment with known resistance trajectories across cancer types indicate that malignant cells undergo coordinated transcriptional and epigenetic rewiring shortly after treatment exposure. These early-phase adaptive signatures represent windows of vulnerability in which therapeutic intervention may be most impactful, suggesting new opportunities for targeting cells before they stabilize into resistant phenotypes.

The multi-omics integration performed in this work illustrates the value of combining transcriptomic, epigenomic, proteomic, metabolic, and spatial modalities to decode the multi-dimensional logic of cancer adaptation. Single-cell proteogenomics reveals post-transcriptional regulatory features, while ATAC–RNA integration highlights chromatin priming events that precede fate decisions. Spatial transcriptomics embeds these regulatory processes within niche architecture, revealing new ecological determinants of state transitions. Together, these multimodal insights support the emerging paradigm that plasticity is a distributed systems-level phenomenon shaped by interactions across multiple biological layers.

Importantly, these findings have direct implications for therapeutic innovation. Targeting the molecular drivers of plasticity—whether through inhibition of chromatin remodelers, disruption of inflammatory and stromal signaling, metabolic pathway modulation, or blockade of key transcriptional regulators—offers a promising avenue for preventing

adaptive reprogramming and improving treatment durability. Strategies that disrupt niche-derived support, interfere with ligand–receptor communication hubs, or target the unique vulnerabilities of intermediate states may prove especially effective. Furthermore, predictive machine-learning models of trajectory progression may enable earlier identification of patients at risk for developing resistance, thereby guiding more personalized therapeutic interventions.

Despite its strengths, this study also highlights the need for deeper integration of single-cell DNA sequencing, longitudinal sampling, and proteomic quantification to fully resolve the interplay between genetic evolution and phenotypic adaptability. Nonetheless, the multi-omics–driven approach presented here establishes a robust conceptual and computational foundation for future analyses aimed at unraveling the complexity of tumor ecosystems.

In conclusion, the integrative bioinformatic model presented in this work advances a unified understanding of cancer plasticity as an emergent property shaped by molecular, epigenetic, metabolic, ecological, and spatial determinants. By illuminating the regulatory logic and communication networks that drive malignant adaptability, this study lays the groundwork for a new generation of precision oncology strategies that target the dynamic, evolving nature of cancer rather than its static features. Such an approach has the potential to transform clinical outcomes by preempting resistance, reducing recurrence, and ultimately improving the long-term management of malignant diseases.

Recommendations

Future research should prioritize the development of integrated multi-omics platforms that combine transcriptomic, epigenomic, proteomic, metabolomic, and spatial analyses within the same biological samples to capture the full complexity of tumor plasticity. Coordinated collection of multimodal single-cell data will provide a more complete representation of regulatory architecture, reveal earlier markers of adaptive transitions, and enable the identification of molecular nodes that consistently govern state interconversion across diverse tumor contexts. Advances in single-cell proteomics, spatial sequencing, and lineage-tracing technologies should be leveraged to validate transcription-based inferences and establish causal relationships between regulatory events, microenvironmental influences, and functional phenotypes.

There is a growing need for longitudinal and perturbation-based single-cell studies that track malignant populations before, during, and after therapy. Such time-resolved datasets will allow investigators to map the earliest adaptive changes that precede resistance, predict potential trajectories of relapse, and identify transient intermediate states that may serve as optimal therapeutic intervention points. Incorporating in vitro drug perturbation models, organoid systems, and in vivo lineage-tracking approaches can help clarify whether adaptive phenotypes emerge from preexisting cellular states or are induced de novo by therapeutic pressure.

Therapeutic development should increasingly focus on targeting the molecular and ecological determinants of plasticity rather than solely eliminating static cell states. Strategies directed at chromatin regulators, transcription factor modules, and metabolic dependencies associated with intermediate and stem-like phenotypes represent promising approaches to limit adaptive reprogramming. Equally important is the targeting of microenvironment-derived signaling networks, particularly IL-6/STAT3, TGF-β, CXCL12/CXCR4, WNT/FZD, and HGF/MET pathways, which collectively shape stemness induction, immune evasion, and metastatic progression. Therapies that disrupt communication between malignant cells and their supporting stromal or immune niches may prove especially effective in preventing the stabilization of therapy-resistant states.

It is recommended that future computational frameworks incorporate models capable of unifying genetic, transcriptional, epigenetic, metabolic, and spatial information to provide a more holistic understanding of tumor ecosystem dynamics. Machine-learning approaches should be expanded to predict future cell states, identify high-risk subpopulations before resistance emerges, and simulate therapeutic interventions under various microenvironmental conditions. Such predictive systems, once validated in clinical settings, have the potential to guide real-time therapeutic decision-making and inform personalized treatment strategies.

Given the strong ecological basis of plasticity, research should also explore therapeutic strategies that aim to modify the tumor microenvironment itself. Modulating inflammatory cytokine gradients, reducing immunosuppressive myeloid infiltration, remodeling extracellular matrix architecture, or altering vascular niche composition may reduce the selective pressures that drive phenotypic adaptation. Integrating immunotherapy with plasticity-targeting interventions may also enhance treatment efficacy, especially in tumors where immune-mediated stress promotes dedifferentiation and persistence.

Finally, translational efforts should prioritize clinical trials that evaluate combination therapies designed to simultaneously target bulk tumor populations and the plastic intermediate states responsible for therapeutic escape. Monitoring of patient samples using multi-omic single-cell analyses during treatment should become a key component of precision oncology programs, providing continuous insight into tumor evolution, identifying the emergence of adaptive phenotypes, and enabling timely therapeutic adjustments.

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