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Review

Dynamics of Epithelial−Mesenchymal Plasticity: What Have Single-Cell Investigations Elucidated So Far?

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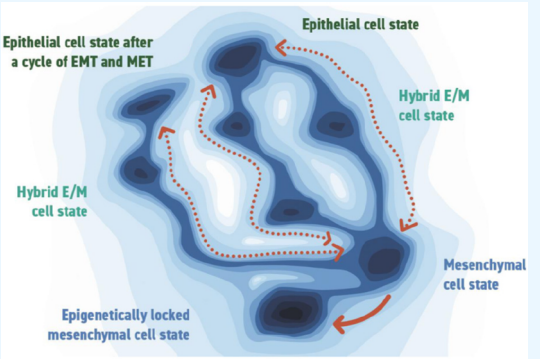
ABSTRACT: Epithelial−mesenchymal plasticity (EMP) is a key driver of cancer metastasis and therapeutic resistance, through which cancer cells can reversibly and dynamically alter their molecular and functional traits along the epithelial−mesenchymal spectrum. While cells in the epithelial phenotype are usually tightly adherent, less metastatic, and drug-sensitive, those in the hybrid epithelial/mesenchymal and/or mesenchymal state are more invasive, migratory, drug-resistant, and immune-evasive. Single-cell studies have emerged as a powerful tool in gaining new insights into the dynamics of EMP across various cancer types. Here, we review many recent studies that employ single-cell analysis techniques to better understand the dynamics of EMP in cancer both in vitro and in vivo. These single-cell studies have underlined the plurality of trajectories cells can traverse during EMP and the consequent heterogeneity of hybrid epithelial/mesenchymal phenotypes seen at both preclinical and clinical levels. They also demonstrate how diverse EMP trajectories may exhibit hysteretic behavior and how the rate of such cell-state transitions depends on the genetic/epigenetic background of recipient cells, as well as the dose and/or duration of EMP-inducing growth factors. Finally, we discuss the relationship between EMP and patient survival across many cancer types. We also present a next set of questions related to EMP that could benefit much from single-cell observations and pave the way to better tackle phenotypic switching and heterogeneity in clinic.



INTRODUCTION

Phenotypic plasticity—the ability of a genotype to enable multiple phenotypes in response to varying environmental conditions—is crucial for survival. A ubiquitous phenomenon seen across biological organisms and contexts, phenotypic plasticity can be considered as an evolutionary strategy for adapting to variable environments.[1] One popular graphical representation of phenotypic plasticity is Waddington's "epigenetic landscape" which conceptualizes embryonic development of multicellular organisms as a sequence of successive cell-fate decision-making bifurcations, where "master regulators" of specific cellular lineages at these branch points shape the dynamical trajectory of phenotypic differentiation.[2] Plasticity is not, however, restricted to development; it comes into play as cells take their reprogramming excursions during injury repair or also in pathophysiological conditions such as cancer and fibrosis. It is now considered as a key enabling hallmark of cancer aggressiveness.[3]

Phenotypic plasticity comes in various manifestations; one of the well-studied canonical instances is epithelial−mesenchymal plasticity (EMP). EMP involves reversible cell-state changes along the epithelial−hybrid−mesenchymal spectrum with varying molecular, functional, and cell morphological attributes.[4] It is observed in multiple stages of embryonic development, such as gastrulation, mammary gland development,[5] and neural crest formation,[6] and is implicated in cancer metastasis and somatic cell reprogramming[7,8] to induced pluripotent stem cells (iPSCs)[9] as well. Recent progress in single-cell investigations has unravelled novel insights into underlying molecular programs controlling the directionality and scope of phenotypic plasticity trajectories being traversed in a high-dimensional space. Here, we highlight the salient features of EMP dynamics in carcinomas that have been elucidated by single-cell investigations.

EMP is comprised of epithelial-to-mesenchymal transition (EMT) and its reverse, mesenchymal-to-epithelial transition (MET). During EMT, epithelial cells partially or fully lose their typical features such as tight cell−cell adhesion and apicobasal polarity and gain migratory and invasive features. These changes can, in principle, be reversed during MET. The dynamics of EMT and MET are regulated by a complex network of transcription factors (TFs), microRNAs, epigenetic

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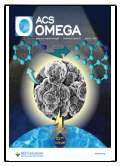

modulators, long noncoding RNAs, and external microenvironment signals such as hypoxia and matrix stiffness.[10] Most of the experimental investigations in EMP dynamics have been at a transcriptomic level,[11,12] but other axes such as morphology, proteomics, and epigenetics are becoming increasingly prevalent.[13−15]

Such multidimensional analysis of EMP has enabled visualizing it from a dynamical systems perspective, where a cell state can be specified using a set of state variables (expression levels, cell shapes, etc.). A stable cell state can then be viewed as an "attractor" in multidimensional cell-state space and can be specified by its state variables.[16] Cell-state transitions such as EMT/MET can be considered as a switch from one attractor to another in a multistable landscape (Figure 1). Single-cell investigations have begun to answer many salient features about cell-state transition dynamics: the existence of hybrid epithelial/mesenchymal (E/M) phenotypes, plurality of cell-state transition paths, reversibility, and symmetry of these transitions, etc.

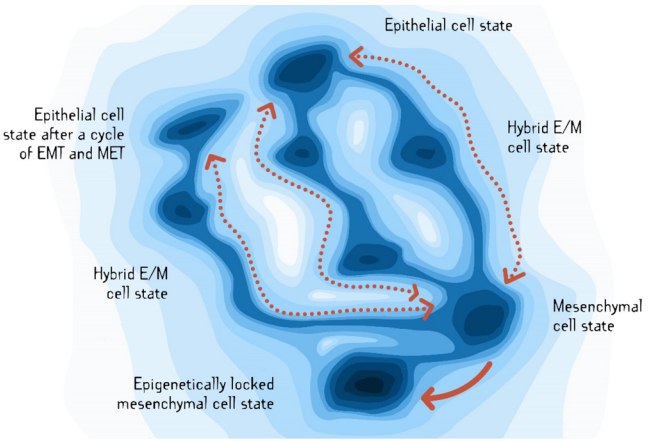

Figure 1. Schematic of a possible landscape showing cell states during EMP. Contour map showing different possible cell states and trajectories in the EMP landscape. Dotted arrows show reversible transitions while solid arrows show irreversible transitions.

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF HYBRID E/M STATES

Earlier thought of as a binary process, EMP is now understood as incorporating one or more hybrid E/M states, as demonstrated via multiple experimental and computational analyses.[17−20] One critical open question in the growing field of hybrid E/M cell states is identifying necessary and sufficient molecular or functional attributes of cancer cells to characterize them as a hybrid E/M phenotype. Traditionally, a hybrid cell type is indicated by coexpression of specific epithelial and mesenchymal marker genes in the same cell.[21] However, this set of markers to characterize the hybrid E/M states are often quite context-specific across multiple cancer types.[22−25]

Single-cell investigations have provided unprecedented insights into phenotypic heterogeneity along the EMP spectrum, revealing multiple hybrid E/M states with varied molecular and functional traits. For instance, multiple hybrid E/M subpopulations were identified in skin and mammary primary tumors by screening cell surface markers. These subpopulations displayed varying invasiveness, cellular plasticity, and metastatic potentials, which were distinct from those of the extreme epithelial or mesenchymal states.[26] The loss of function of cadherin Fat1 via mutation or deletion in mouse and human squamous cell carcinoma was found to promote the hybrid E/M state, enabling tumor initiation, progression, and metastasis.[27] Strikingly, despite different surface marker patterns, hybrid E/M cells were found to be more plastic relative to the epithelial and mesenchymal ones.[26] A possible explanation behind this observation was offered by recent computational analysis of multiple regulatory networks under-

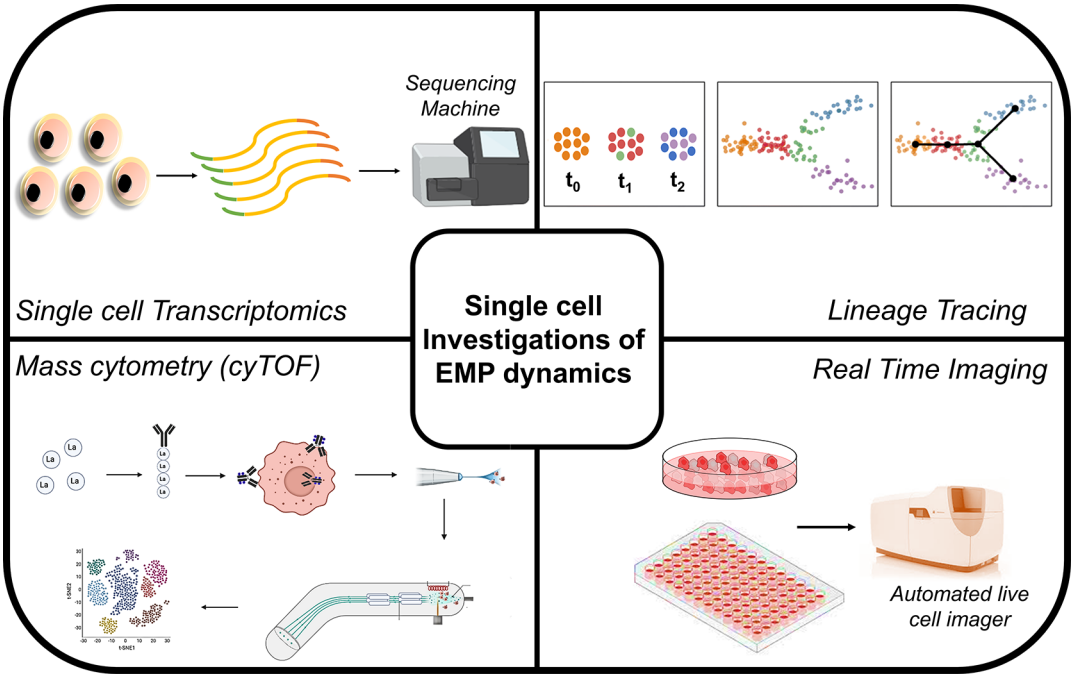

Figure 2. Summary of experimental methodologies for single-cell analysis of EMP. Single-cell RNA-Seq, single-cell lineage tracing, mass cytometry-based experiments, and real-time single-cell imaging are the most common laboratory methods to study EMP at a single-cell resolution.

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lying EMP. These networks contained "teams" of nodes that stabilized epithelial and mesenchymal phenotypes, but no such team was observed for hybrid E/M states, thus enabling their higher propensity to switch.[28] Another single-cell analysis tracked cancer cells undergoing full and partial EMT using live imaging, tamoxifen-inducible lineage-tracing systems, and 5-cell RNA sequencing in the MMTV−PyMT mouse model of metastatic breast cancer. While cells undergoing full EMT failed to colonize the lungs, partial EMT (or hybrid E/M) ones contributed majorly to lung metastasis and chemoresistance[29] (Figure 2). These trends are reminiscent of in vitro observations that hybrid E/M cells had almost 10 times higher mammosphere-forming ability than epithelial or mesenchymal cells.[20] A recent study establishing single-cell clones from the heterogeneous SUM149PT breast cancer line showed that clones exhibiting a hybrid E/M phenotype had both higher plasticity and tumor-initiating ability than the clones representing either end of the epithelial−hybrid−mesenchymal spectrum.[19] Together, these observations underscore that despite plurality and marker-specific identification of hybrid E/M phenotypes, higher plasticity and metastatic potential are their functional hallmarks.

One reason for multiple hybrid E/M phenotypes may be semi-independent changes along the epithelial and mesenchymal axes. For instance, single-cell RNA-seq (scRNA-seq) time-course transcriptomic profiles of TGF-β induction of MCF10A cells up to 10 days reported a delayed and gradual decrease of the canonical epithelial marker CDH1 but an immediate loss of S100 calcium-binding protein A9 (S100A9).[30] This observation raises an important question as to whether heterogeneity can be seen as a function of time or also as a function of dose of the EMT-inducing signal. To address this question, in a recent scRNA-seq experiment, MCF10A cells were treated with TGF-β at various doses and time points up to 14 days.[31] At a 14-day time point, with increasing dose, the cells progressed from (epithelial high, mesenchymal low) to (epithelial low, mesenchymal high) state and showed a saturating behavior at higher doses. A similar observation was made for characteristic epithelial (e.g., CDH1) and mesenchymal (e.g., VIM, FN1) genes. Intriguingly, time-dependent EMT demonstrated more significant separability in the continuum of cell states noted, as compared to dose-dependent behavior. When temporal resolution is missing explicitly from the experimental design, pseudotime analysis is often implemented to identify the cell-state transition trajectory. Such analysis for in vivo single-cell RNA-seq data from squamous cell carcinoma identified that the most likely EMP trajectory was dependent more on an increase in mesenchymal than on a concomitant decrease in epithelial.[32] These observations showcase that a decrease in epithelial and an increase in mesenchymal gene expression programs may be asynchronous, thus enabling a multitude of hybrid E/M states.

Another possible reason enabling multiple hybrid E/M phenotypes is varying levels of coordination among the various axes of EMT—molecular EMT, morphological EMT, and functional EMT.[33] For instance, single-cell mechanical investigation and spatial EMT-related gene expression profiling illustrated that leader cells in collective cell migration of A549 lung cancer cells were more elongated and softer (lower Young's modulus) than the follower ones. These cells expressed mesenchymal markers (SNAI1, VIM) while still retaining epithelial (CDH1) ones, exhibiting a partial EMT phenotype.[34] Another study using image quantification to track EMT progression revealed a partial EMT phenotype with a unique cytoskeletal signature, consistent with decreased stiffness compared to mesenchymal cells.[35] Tracking the morphological changes upon treatment with EMT-inducing growth factors has helped infer transition rates among different morphological cell states and has been used to develop classifiers to segregate epithelial vs mesenchymal states with 92% accuracy.[36−38] Such characterization can be coupled with scRNA-seq data to extract information about E−M heterogeneity. An example would be the development of a functional single-cell selection pipeline that allowed screening based on time-varying cellular dynamics or morphology and selective labeling of cells among a heterogeneous population.[39] Monitoring the migration trajectories of individual cells in the MCF10 cell line, fast-moving cells were identified and phototagged. These cells were spindle-shaped, exhibiting a mesenchymal-like phenotype. They were later segregated for single-cell transcriptomic analysis, which revealed an upregulation of EMT-driving pathways TGF-β and NF-κB in these cells, compared to the slow-moving ones. This study thus demonstrated how cells identified to be mesenchymal using image analysis (Figure 2) exhibited concomitant gene expression profiles too, i.e., well-coordinated molecular and morphological EMT.

How many phenotypes exist along the epithelial−hybrid−mesenchymal spectrum is an open question. While scRNA-seq data can help identify prevalent heterogeneity, pinpointing an optimal number of meaningful clusters in such high-dimensional data is a challenge from both the methodical and conceptual standpoints. A scRNA-seq study in genetic mouse models of pancreatic ductal adenocarcinoma (PDAC) revealed ∼50 cancer cell clusters spanning the epithelial−mesenchymal continuum; they observed similar trends in human PDAC samples.[40] Simulations for large regulatory networks underlying EMP support the idea of a spectrum of hybrid E/M states.[41,42] However, it remains to be understood whether these states are functionally very distinct from one another or that at least some of them can be considered as "microstates" within a "macrostate".[43,44]

PLURALITY OF TRAJECTORIES ENABLING VARYING LEVELS OF EMP

Plasticity refers to the change of phenotype either spontaneously (i.e., without an obvious external trigger) due to biological noise or driven by specific microenvironmental cues. In cell biology, intrinsic factors such as epigenetic variations or stochastic fluctuations in gene expression or levels of signaling molecules can lead to changes in the phenotype. For instance, microRNA−mRNA interaction-driven oscillations seen without any explicit feedback loops can explain observed cell-state transitions on a slower time scale. Such scenarios may explain intrinsic and reversible EMP.[45] As cells change phenotypes, they can be conceptualized as traveling along diverse paths in a high-dimensional gene expression space. Thus, characterizing cellular trajectories in this landscape is crucial in determining the allowed and forbidden cell states/phenotypes and may point toward a more rational design of differentiation therapy in cancer.[46]

In the quest for finding a common EMT gene expression program, Cook and Vanderhyden performed scRNA-seq across 12 EMT time-course experiments to measure the gene expression profiles of 103 999 cells from 960 samples.[47] They assessed four different cancer cell lines (A549, lung; DU145,

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prostate; MCF7, breast; and OVCA420, ovarian) and exposed them to three EMT-inducing factors (TGFB1, EGF, and TNF). Among the three EMT inducers, TGFB1 was found to be the most potent. The study highlighted the context specificity of transcriptional dynamics of EMT, where only 22% of response genes, on average, were shared between any two of the 12 EMT time courses. Moreover, the single-cell transcriptomic profiles were grouped based on cell lines rather than by the inducing growth factor, revealing that the genetic background of cells can govern the manifestation of EMP. In another study, they analyzed scRNA-seq data spanning eight different cancer types from 266 tumors.[48] They reported a high degree of intratumoral heterogeneity, which could be influenced by the tumor microenvironment and signaling associated with combinations of common regulatory pathways. Using a general EMP signature to query samples from TCGA (The Cancer Genome Atlas), they found EMP to be associated with reduced progression-free intervals and changes in immune cell proportions within the tumor microenvironment. scRNA-seq data can also be used to deconvolute the extent of the EMP signature coming from cancer cells vs that from stromal cells,[49] thus possibly reconciling conflicting reports of clinicopathological association of EMP.[50] Clarifying the contribution of cancer cells vs stromal cells in bulk transcriptomic readouts of EMP is crucial because recent lineage-tracing experiments in mouse models of metastatic breast cancer have suggested that a complete transition to a mesenchymal state is rare; rather, cells often underwent a partial EMT.[29] Such preclinical observations reinforce the trends witnessed in three-dimensional reconstruction of the primary tumor samples that single-cell migration is an extremely rare phenomenon; instead, cells migrated mostly as "tumor buds" with simultaneous expression of both E-cadherin (epithelial cell−cell adhesion molecule) and Zeb1 (EMT-inducing transcription factor), thus displaying hybrid E/M state(s).[51]

The prevalence and plurality of hybrid E/M phenotypes raise the question of how many paths can cells take en route to EMT in a high-dimensional space. To address this question, Wang et al. recorded single-cell trajectories of A549 lung cancer cells labeled endogenously with Vimentin-RFP that underwent EMT for varying concentrations of TGF-β.[52] They identified two parallel trajectories in a multidimensional cell-feature space, thus indicating that the transition dynamics proceeds via parallel trajectories. They conjectured that with increasing TGF-β concentrations, the initial epithelial attractor collides with two saddle node points in a sequential manner, and a new mesenchymal attractor emerges. How similar or different the transcriptomic profiles of cells moving along these two paths are yet remains to be elucidated. Nonetheless, such temporal mapping allows investigating the high-dimensional EMT landscape from a dynamical systems theory lens, as applied earlier to reveal independent trajectories for evolution of drug-tolerant cells in individual melanoma cells treated with the BRAF inhibitor vemurafenib.[53,54] Parallel activation of EMT signaling pathways was also reported in scRNA-seq analysis of MCF10A cells treated with TGFβ up to a duration of 8 days,[30] and cells were found to progress through EMT at different speeds despite identical duration and dose of the inducing signal, illustrating dynamic nongenetic heterogeneity. Computational modeling has predicted that parallel progression through alternative transition paths can accelerate EMT,[43] but whether and how future experiments can test this prediction rigorously are worth delving into.

The diverse trajectories seen in the EMT landscape can not only underlie the heterogeneity of hybrid E/M states but also explain the "exit" from hybrid E/M phenotypes stochastically toward a more epithelial or mesenchymal one. Upon experiencing homogeneous physical compression, the H1975 lung cancer cells, identified as a hybrid E/M phenotype,[55] exhibited increased gene expression noise and consequent diversification into either an epithelial or a mesenchymal phenotype, as identified through single-cell sequencing and single-molecule fluorescent in situ hybridization (smFISH)[56] of canonical EMT and MET markers. smFISH measurements of gene expression, together with lineage trees, have been instrumental in inferring reversible and stochastic dynamics of cell-state transition in multiple biological contexts.[57,58] Similar approaches can facilitate measuring transition rates along different trajectories in the multidimensional EMP landscape.

REVERSIBILITY AND HYSTERETIC BEHAVIOR OF EMP DYNAMICS

EMT is a reversible process during many stages of embryonic development. Similarly, during metastasis, the reverse of EMT—MET—is considered to drive metastatic colonization.[59] In vitro MET is often studied via treatment of epithelial cells with an EMT-inducing signal (such as TGFβ or inducible expression of EMT-inducing transcription factors such as SNAIL), followed by its withdrawal. For instance, inducible SNAIL expression in LNCAP prostate cancer cells drove transcriptomic changes concomitant with EMT. Also, while the EMT-induced cells exhibited reduced proliferation and increased invasion, the reverting cells reinitiated proliferation and formed multicellular spheroids, thus recapitulating traits often driving metastatic colonization. Interestingly, upon reversal, not all genes returned to their pretreatment expression levels,[11] thus enabling a "transcriptional memory". However, because this analysis was at a population level, it could not be distinguished as to whether this "memory" was restricted to a subpopulation of cells or more equitably distributed across cells. Other experimental analyses have shown that the extent of memory can depend on at least these factors—the heterogeneity in the degree of EMT induced in different cells in a population,[60] the duration after which MET was assessed,[61] and markers used for MET.

Single-cell investigations are well-positioned to address some of these questions. For instance, through TGFβ treatment and its subsequent withdrawal, eight distinct cell states were identified in lung cancer, using a mass cytometry-based time-course analysis with single-cell resolution in vitro.[62] Using this high-dimensional temporal data, they developed an algorithm to project the malignant cells on a lung cancer reference map called PHENOtypic STAte MaP (PHENOSTAMP) (Figure 2). They observed that cells undergoing MET took a different trajectory in this map as compared to EMT trajectories, showcasing hysteresis—a hallmark feature of EMT predicted by many computational models.[63,64] Such hysteretic response can enable a short-term stimulus to trigger cell-state transition in a subpopulation, as witnessed in flow cytometry-based single-cell analysis for TGFβ-driven EMT. Hysteresis is often seen in multistable systems; in the NmuMG mouse mammary epithelial cells undergoing TGFβ-driven EMT, for TGFβ concentrations between 10 and 25pM, flow cytometry and immunofluorescence experiments revealed two subpopula-

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tions: E-cadherin-low (mesenchymal) and E-cadherin-high (epithelial),[65] thus explaining the underlying basis of hysteretic behavior. Intriguingly, upon CRISPR/Cas9-mediated disruption of the ZEB/miR-200 feedback loop that enabled such multistability, the bimodality of E-cadherin and hysteretic response was largely lost in vitro. Further, the mutant cells with the disrupted ZEB/miR-200 loop had dramatically reduced metastatic potential as compared to the wild-type (with an intact ZEB/miR-200 feedback loop) one. This decrease in metastasis in vivo was observed despite no significant reduction in migration on invasion in vitro, demonstrating the key importance of multistability and consequent cell plasticity in establishing metastasis in vivo. This analysis further endorses previous in vitro observations about the role of the miR-200/ZEB1 feedback loop in mediating the reversibility of EMT[66,67] and explains how ZEB1-mediated epigenetic rewiring can control the rate of MET.[61,68,69]

Single-cell time-course observations have also helped map the extent of reversibility of EMT as a function of the duration of induction of EMT. For instance, MCF10A cells were cotransduced with two sensors—destabilized green fluorescent protein (GFP) that is regulated by EMT-transcription factor ZEB1 and red fluorescent protein (RFP) which is driven by the promoter of E-cadherin (CDH1). Counting the number of GFP+ and RFP+ cells when exposing cells to varying durations of TGF-β revealed that cells exposed for a shorter duration (3−6 days) reverted to being epithelial in a similar time frame. However, for cells exposed for a longer duration (12−15 days), they underwent a stronger degree of EMT measured by CDH1 and ZEB1 levels, and not all of them reverted to being epithelial even after 15 days post-TGF-β removal, suggesting that at least a subpopulation of cells exhibited an "effectively irreversible" EMT; i.e., they can maintain a mesenchymal state without exogenous TGF-β.[70] Such "irreversibility" and consequent lack of plasticity displayed attenuated metastatic outgrowth, while coexpression of epithelial and mesenchymal programs enabled cell plasticity (i.e., more reversible) and consequent metastasis.[60] Further longer-term wait time experiments later unraveled that cells post-EMT induction can revert back to being epithelial after an initial delay.[61]

Computational modeling simulations suggest nongenetic heterogeneity in terms of reversal time, a prediction that remains to be tested experimentally through single-cell dynamical investigations. Epigenetic reprogramming has been proposed to underlie such effectively irreversible EMT, and longer waiting periods may allow for sufficient cell divisions causing gradual loss of such memory (epigenetic reprogramming). Given that most of such analysis has been at a population level,[61] future studies should track dynamic changes in the epigenetic status driven by key EMT/MET regulators at the single-cell level to establish causative connection(s) between the rate of EMT reversibility and chromatin reprogramming.

Another important aspect while investigating MET dynamics is to design experiments where MET is induced in a dose- and/or duration-dependent manner, similar to analysis of EMT in MCF10A cells.[31] Given that EMT and MET may not follow the same trajectories and that many MET drivers such as GRHL2 can drive epigenetic remodeling,[71] the trajectories of EMT reversal and of MET induction may not necessarily overlap, especially in a high-dimensional space.

SPATIAL HETEROGENEITY ALONG THE EMP SPECTRUM

Most in vitro investigations on EMP have considered only temporal dynamics, ignoring the spatial component. However, heterogeneity along the EMP status is observed spatially in tumors, with the mesenchymal cells preferentially located at the invasive edge while epithelial ones are present in the interior, thus indicating an "EMT gradient".[72,73] Computational models have revealed how diffusing molecules such as TGF-β, secreted mostly by stromal cells, coupled with cell−cell communication (for instance, through Notch-Jagged signaling), can explain the existence of such gradients. Importantly, Notch-Jagged signaling can stabilize cells in hybrid E/M phenotypes,[74] thus possibly explaining their observed strong cell−cell communication traits as seen in scRNA-seq by quantifying the degree to which a given cell type can send and receive signals.[32]

Similar to pseudotime, pseudospatial trajectories have been generated for cells seeded within cloning rings in the center of a tissue culture dish; these rings were removed to allow for spontaneous EMT.[75] These trajectories showed opposing gradients of CDH1 and VIM, reflecting a cell-state continuum. Recent advancements in in vivo intravital imaging have allowed a more comprehensive mapping of spatial heterogeneity in the EMP status. Using Fsp1 (fibroblast specific protein 1) as a marker to identify cells that have undergone EMT in vivo, cells exhibiting a more mesenchymal state were found to localize preferentially closer to blood vessels and also had longer membrane extensions oriented toward the vessels.[76] How accurate and sensitive Fsp1 is as a marker of cells in hybrid epithelial/mesenchymal states is still under debate,[77] given the multifaceted context-specific display of EMP.

ASSOCIATION OF EMP WITH PATIENT SURVIVAL

Initial reports, positing EMT as a binary process, proposed EMT and its associated transcription factors to be associated with worse clinical outcomes.[78,79] However, further pan-cancer analysis revealed that association of EMT with patient survival metrics was largely cancer subtype-specific.[80,81] Particularly, coexpression of epithelial and mesenchymal signatures was found to be associated with the worst patient outcomes in luminal and basal breast cancer,[20] thus highlighting the aggressive behavior of hybrid E/M cells. Consistently, a recent sequential immunohistochemistry analysis demonstrated that the presence of hybrid E/M cells was strongly associated with poor prognosis. This analysis suggested that the presence of a minimum percentage of tumor cells in the hybrid E/M state (E/M score <2%) was sufficient to confer poor overall and disease-free survival in patients.[82] Such strong association of hybrid E/M cells with patient survival can be at least partly attributed to their salient features—enhanced stemness, drug-resistance, and immune-evasion.

Beyond these prognostic observations based on a few markers, computational methods to connect single-cell RNA-sequencing data to EMT have been developed, such as *scPrognosis*. It integrates EMT-associated pseudotime data and dynamic gene coexpression networks and suggests that most breast cancer signature genes so identified a peak during hybrid E/M state(s) acquisition.[83] Similarly, another metric overlays single-cell RNA-sequencing data on targets of specific miRNAs to improve patient risk stratification in colorectal cancer.[84] Future attempts to test the prognostic power of hybrid E/M

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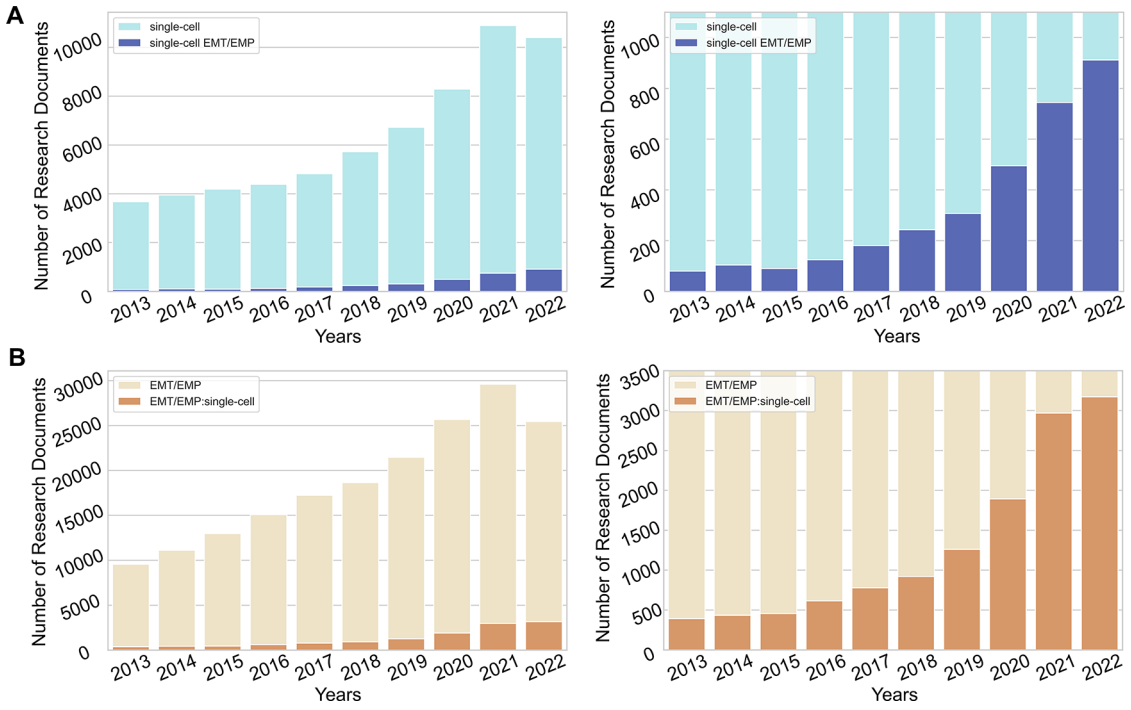

Figure 3. Bar-plot depicting the increasing focus on EMP single-cell research documents in a decade. (A) Light blue bars represent the number of articles with major keywords as "single-cell", and dark blue bars denote articles with keywords "EMT", "EMP", "Epithelial−Mesenchymal Transition", or "Epithelial−Mesenchymal Plasticity" within the "single-cell" domain (right), zoomed-in view (left). (B) Light brown bars represent number of articles with major keywords "EMT", "EMP", "Epithelial−Mesenchymal Transition", or "Epithelial−Mesenchymal Plasticity"; dark brown bars represent those with keyword "single-cell" within the EMT domain (right); zoomed-in view (left) (data taken Scopus on Nov. 8, 2022).

signatures shall depend on identifying the tissue-specific signatures for EMT.

CONCLUSION

Single-cell investigations of EMP have begun to elucidate the complexity of phenotypic plasticity in the context of cancer metastasis. With the advent of single-cell technologies at transcriptomic, proteomic, live-cell imaging, and lineage-tracing levels, there has been a burst in employing them to improve our understanding of underlying dynamical patterns involved in cell decision-making. Although the emphasis to study EMP has been steadily growing in the past few years, EMP-based studies still form a minor portion of the total set of single-cell-based studies (Figure 3). We envision that further single-cell analysis of EMP will be instrumental in uncovering the mechanistic basis of the complex dynamics of EMP and its functional relevance in tumor progression and metastasis.

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Notes

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