Interconnected high-dimensional landscapes of epithelial–mesenchymal plasticity and stemness in cancer

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Abstract
Establishing macrometastases at distant organs is a highly challenging process for cancer cells, with extremely high attrition rates. A very small percentage of disseminated cells have the ability to dynamically adapt to their changing microenvironments through reversibly switching to another phenotype, aiding metastasis. Such plasticity can be exhibited along one or more axes—epithelial–mesenchymal plasticity (EMP) and cancer stem cells (CSCs) being the two most studied, and often tacitly assumed to be synonymous. Here, we review the emerging concepts related to EMP and CSCs across multiple cancers. Both processes are multi-dimensional in nature; for instance, EMP can be defined on morphological, molecular and functional changes, which may or may not be synchronized. Similarly, self-renewal, multi-lineage potential, and resistance to anoikis and/or therapy may not all occur simultaneously in CSCs. Thus, understanding the complexity in defining EMP and CSCs is essential if we are to understand their contribution to cancer metastasis. This will require a more comprehensive understanding of the non-linearity of these processes. These processes are dynamic, reversible, and semi-independent in nature; cells traverse the inter-connected high-dimensional EMP and CSC landscapes in diverse paths, each of which may exhibit a distinct EMP-CSC coupling. Our proposed model offers a potential unifying framework for elucidating the coupled decision-making along these dimensions and highlights a key set of open questions to be answered.

Keywords Epithelial–mesenchymal plasticity · Stemness · Landscape · Phenotypic plasticity · Cancer stem cells · Metastasis

Introduction
Cancer metastasis is the phenomenon in which secondary tumours develop at organs distant from the site of the primary tumour. It is a deadly event, accounting for more than 90% of cancer deaths [1]. A key hallmark of metastasis-initiating cells is their ability to adapt dynamically to their varying microenvironments, a trait known as phenotypic plasticity [2]. This plasticity enables them to circumvent various bottlenecks in the metastatic cascade and eventually colonize distant organs. It can exist along multiple inter-connected axes. Two of the most well-studied axes of cancer cell plasticity are epithelial–mesenchymal plasticity (EMP) and stemness [3]. These two axes have been shown to drive one another across in silico, in vitro and in vivo studies [4], but a unique association of different EMP states with different cellular states along the stemness axis has not been yet achieved. The intra- and inter-tumor heterogeneity in genetic and organ-specific backgrounds complicates this approach, as such relationships are likely to be context-dependent and lacking universality. Another factor which acts as a roadblock in developing a comprehensive understanding of these inter-connections is the lack of rigor and consistency in defining these multi-dimensional processes across different studies [5], i.e. what is necessary and sufficient to claim that cells have switched between epithelial...
and mesenchymal states or stem and non-stem states? Here, we review the emerging literature on complexity in defining EMP and stemness as individual axes of plasticity, the bidirectional interconnections investigated between these two phenomena, and eventually present a framework that can reconcile potentially conflicting observations.

The multi-dimensional aspects of Epithelial–Mesenchymal Plasticity

For successful dissemination from a primary tumour site and eventual colonization of a distant organ, cancer cells must exhibit invasive and migratory features. For carcinomas, such traits are attained via trans-differentiation of cancer cells from a relatively more epithelial to a more mesenchymal state with altered migratory, invasive, proliferative and polarity features through a process known as Epithelial-to-Mesenchymal Transition (EMT). Traditionally, EMT has been tacitly assumed to be an “all-or-none” process, but it is becoming increasingly clear that it is not a binary switch [6]. Both mathematical modelling studies and experimental observations have reported the existence of one or more hybrid epithelial/mesenchymal (E/M) states between the two extremes of “pure” epithelial or “pure” mesenchymal phenotypes [7–11]. The number of hybrid E/M states that exist between “pure” epithelial and “pure” mesenchymal has been a topic of debate with no general consensus [8]. The number of hybrid states can also vary depending on the cancer subtype. Of course, a substantially large number of discrete states may be better approximated as a continuum [12]. Notwithstanding the difference in number of such states reported, a more important question is the functional relevance of these hybrid E/M states.

The term EMT has been tacitly assumed to denote a discrete and unidirectional process. With increasing recognition of hybrid E/M states, alongside the fact that cells can switch phenotype bi-directionally along this spectrum [13], the term Epithelial Mesenchymal Plasticity (EMP) has been introduced as a more accurate description of the process. An open question regarding the EMP spectrum is how to quantify the relative stability of different states—more extreme epithelial or mesenchymal states vs. the hybrid E/M states [14]. In a system that allows for co-existence of multiple stable states (phenotypes), ‘spontaneous’ transitions among states are possible but the propensities of all possible transitions cannot be assumed to be the same [15, 16]. For instance, depending on genetic and/or epigenetic background, dose and/or duration of the induction signal, EMT and/or its reverse MET (Mesenchymal-to-Epithelial Transition) may be reversible or irreversible [17–20]. Thus, under some conditions, transitions in one or more direction may be prohibited. Moreover, cells may not necessarily follow the same ‘path’ when transitioning from E to M and vice-versa in the EMP landscape, and consequently may or may not return to their initial state [21–24]; thus quantifying their transition dynamics is crucial.

Defining the ‘paths’ of EMP requires a comprehensive understanding of necessary and sufficient conditions to claim that a transition has taken place. The EMT status of a cell is usually assessed using three different methods—molecular signatures, morphological features and functional traits—which may or may not be synchronized among one another; for instance, a cell may undergo “molecular EMT” but not necessarily a concurrent “morphological EMT” or “functional EMT” simultaneously [25–27]. Therefore, the trajectories and molecular footprints of “molecular EMT”, “morphological EMT” and “functional EMT” need not be overlapping or synchronized completely. This lack of consistency in defining EMT percolates to poor characterization of hybrid E/M states as well. For instance, are both these categories of cells equally eligible to be called hybrid E/M: cells which have undergone “molecular EMT” but not “functional EMT”, and cells which have undergone “morphological EMT” but not “molecular EMT”? To add to the combinatorial complexity of defining hybrid E/M state(s), each of these individual dimensions—molecular, functional, morphological—comprises multiple cellular traits. For instance, do both of these categories of cells qualify as hybrid E/M: cells that have increased mesenchymal markers but unchanged epithelial markers, and cells that have decreased epithelial markers but unchanged mesenchymal markers? (Fig. 1).

To quantify molecular changes associated with EMP, transcriptomic measures have been proposed that combine “epithelial” and “mesenchymal” scores at both bulk and individual cell levels. At bulk (single-sample level), Singscore [28] has been applied to quantify two-dimensional scores of EMT, and metrics such as GSEA (Gene Set Enrichment Analysis) can be applied for single-cell transcriptomic data to quantify hybrid EMT phenotype [29]. Assessment of functional traits such as collective cell migration [30, 31] has also been recently investigated. However, the accuracy of these existing metrics is currently unknown and they are unlikely to identify hybrid E/M phenotypes with sufficient resolution. More nuanced analysis of conserved hybrid E/M signatures across various cancer types as well as tissue/cancer specific mechanisms for maintenance of the hybrid E/M cell state still remains to be explored.

Another possibility for identifying hybrid E/M status could be via the quantification of various ‘phenotypic stability factors’ in conjunction with the canonical epithelial and mesenchymal factors. Phenotypic stability factors are defined to be those molecular players that allow for the existence of or enhance the stability of the hybrid E/M states [14]. For example, the EMT-inducing factor SNAI2 has been
shown to be robustly associated with a hybrid E/M phenotype [32–36] as is often expressed alongside CDH1 (E-cadherin), a well-known epithelial marker [37]. Relative levels of SNAI2 and CDH1 can thus stratify hybrid phenotypes along the EMP spectrum [38]. However, SNAI2 may not be a pan-cancer marker for hybrid E/M states. Other phenotypic stability factors such as WT1, NP63α, NRF2, OVOL2, NFATc and certain miRNAs have been associated with the hybrid E/M state across different cancer types [39–44].

On the other hand, cells can be classified into epithelial, mesenchymal or intermediate states by morphological features [45]. Epithelial cells are usually cobblestone shaped and often in close contact with their neighbours, while mesenchymal cells are more solitary and have distinctive spindle shaped morphology. Hybrid cells are relatively less well characterised, though have been seen to show slightly elongated phenotypes [46]. Functional assays such as scratch assays have also been used extensively to study the extent of EMT [47], but no comprehensive comparison of functional attributes along the entire EMP spectrum yet exists. The biophysical properties of hybrid E/M states and their contribution to diverse functional traits associated with EMT have been of recent interest, and have been extensively discussed elsewhere [48, 49].

**Stemness: what does it mean in the context of cancer?**

Stemness is the ability of a cell to self-replicate in order to perpetuate its own lineage, to give rise to more differentiated cell types to maintain a tissue, and to interact with its environment to maintain a balance between quiescence, proliferation, and regeneration [50]. Cells that exhibit such specific properties are called stem cells. Adult stem cells are observed to divide asymmetrically to produce progenitor cells that can, in turn, produce more differentiated cells that maintain tissue homeostasis in various organs, whilst being able to renew themselves autonomously [50]. A relatively recent development in quantifying the stemness of a cell has come from a global analysis of the transcriptome, thanks to the rapid advancements in high throughput sequencing of single cells [51, 52]. A stem cell with higher developmental potential is likely to exhibit a more diverse transcriptional profile than a differentiated counterpart [53].

Cancer stem cells (CSCs) can be viewed as malignant counterparts of adult stem cells. CSCs are generally considered to be a sub-population of the tumour mass that is capable of sustaining disease progression and driving post-therapeutic relapse. One of the widely used metrics to measure stemness in cancer is the ability of such cells to initiate a new tumour, also commonly known as the tumour initiation capacity (TIC). Interestingly, the origin of a primary tumour in many different cancer types has been attributed to the presence of CSCs that arise from either the tissue resident stem cells or differentiated cancer cells that have undergone de-differentiation during transformation. CSCs are also observed to have various other associated attributes; however, some of these attributes are more likely to be associative in nature rather than an identification criterion per se. For example, CSCs are generally observed to be resistant to oxidative stresses and drugs and hence are likely to escape drug interventions and cause subsequent therapy relapse [54]. However, not all drug-resistant cells are necessarily CSCs. Similarly, CSCs have been associated with traits such as enhanced survival in suspension conditions (anoikis resistance) [55], and enhanced migration (Fig. 2), but it is currently not at all clear whether these are hallmark features exclusive to CSCs. Further, a causative contribution of these traits in enabling cells to acquire and/or maintain stemness remains to be unequivocally established.
The most common method used to identify CSCs is examination of various distinct cell surface markers. In many cases, the markers identified overlap with those used to identify either tissue resident stem cells or human embryonic stem cells. Cell surface makers such as CD44, CD133 and EpCAM have been identified as CSC markers in many carcinomas [59–61]. However, cancer cells negative for such markers have also been shown to grow colonies and seed tumors [62, 63], suggesting that cell surface markers are neither exclusive nor exhaustive measures of stemness. Intriguingly, initial reports on identifying CSCs in solid tumors did not explicitly suggest a one-to-one correspondence between surface markers and stemness; instead, an enrichment of a trait (tumor-initiating ability) in certain cell populations which had specific cell surface marker(s) [64].

Intracellular markers such as SOX2, NANOG and OCT4 have also been implicated in maintenance of CSC traits in many cancer types [65, 66]. Similarly, multiple signalling pathways have been shown to induce and/or maintain the CSC phenotype [67]. However, similar to EMP [68, 69], stemness is a dynamic trait which can be regulated in a non-cell-autonomous manner too. For instance, CSCs can secrete cytokines to convert their neighboring non-CSCs to CSCs [70, 71]. It is important to note that these cell surface and/or intracellular markers, although strongly associated with a CSC phenotype, are not necessarily expressed equally in all CSCs. Conversely, the presence of one or more of these markers does not always guarantee a CSC phenotype and therefore other functional assays are required to establish stemness such as the formation into tumorspheres (mammosphere, colonospheres etc.) or spheroids under non-adherent conditions in serum-free medium supplemented with CSC-enriching growth factors [72], or the reconstitution of a heterogenous tumor on transplantation of CSCs in immunocompromised mice.

Stemness in the context of cancers have been an active field of research and extensively reviewed elsewhere [73, 74]. Here, our goal is to highlight how different assays and definitions are being used across the community to claim a set of cells as being CSCs or CSC-like. Understandably, there is no quantitative commonly agreed upon assay-based criterion to bestow the ‗privileged title‘ of CSCs to a specific cell. The necessary and sufficient conditions for calling a cell a CSC at a point of time needs some community-level consensus, perhaps an attempt similar to the one recently attempted to define EMP [75].

**Stemness on the Epithelial–Mesenchymal Plasticity spectrum**

The first set of observations connecting EMP and stemness were made in 2008, where a fraction of breast cancer cells induced to undergo EMT were shown to possess an elevated tumor-initiating capacity [76, 77]. These observations were made when EMT was still seen as a binary process. They
triggered further investigations between these two processes from a molecular perspective across cancer types, and the two axes were observed to be mechanistically inter-connected [78]. However, later studies which did not treat EMT as an ‘all-or-none’ process reported that hybrid E/M cells were equally or even more likely than mesenchymal cells to exhibit stemness [8, 63, 79, 80]. Consistently, further investigation into CSC heterogeneity revealed that there may be subsets of CSCs—epithelial, mesenchymal and hybrid E/M (E-CSCs, M-CSCs, H-CSCs) [13, 61, 81]. Therefore, the emerging evidence points to EMT and stemness being semi-independent axes, i.e. not every cell undergoing EMT may acquire stemness and not every cell switching to be a CSC is mandated to show one or more features of EMT. Given the importance of both EMP and maintenance of stemness in the metastatic cascade, understanding the inter-connectivity between the EMP and stemness axes may help us shed light on key cellular processes driving metastasis.

**Breast cancer**

Two seminal studies in 2008 demonstrated the association between EMT and CSCs, where a set of human mammary epithelial cells facing oncogenic activation or induced to undergo EMT showed an enriched CD44hiCD24lo population [76, 77], the claimed CSC subpopulation [64], as well as exhibiting increased tumor-formation ability as witnessed in vitro and in vivo. However, later investigations have indicated that epithelial cells with increased aldehyde dehydrogenase (ALDH1) activity are also capable of self-renewal and forming a tumor with cells of multiple lineages [82]. Immunofluorescence in primary human breast tumor tissues revealed that these two subsets of CSCs—CD44hiCD24lo and ALDH+—are present at different spatial locations in a tumor. While the CD44hiCD24lo cells localized at the tumor-invasive edge, the ALDH+ cells were located in the tumor interior [61]. Molecular profiling revealed that while CD44hiCD24lo cells displayed a mesenchymal phenotype, ALDH+ cells displayed a hybrid E/M phenotype [61, 81]. These two subpopulations retained the ability to switch to one another as well as give rise to more differentiated cells, but whether this ability to spontaneously switch to another state is a cause and/or consequence of (partial) EMT/MET remains unclear. Moreover, whether this plasticity to switch back and forth between CD44hiCD24lo and ALDH+ phenotypes is necessary and/or sufficient to form tumors in vivo remains to be investigated.

Another set of investigations deciphered that while CSC enriched populations in luminal cell lines were enriched for a mesenchymal program, those in basal cell lines showed a more epithelial signature, suggesting an association of hybrid E/M phenotype with stemness in breast cancer [79]. When epithelial and mesenchymal subclones were established from HMLER cells, both these subpopulations seemed to fulfill only one of the two stemness conditions—while epithelial (CD44hiCD24lo) cells had high plasticity, the mesenchymal (CD44loCD24hi) cells were more self-renewing. Further analysis at a single-cell level identified a CD44hiCD24lo subpopulation which exhibited signatures of a hybrid E/M state, was enriched for ALDH+ cells and had approximately ten times more tumor-initiation potential than ‘purely’ epithelial or mesenchymal subpopulations (CD44hiCD24lo, CD44loCD24hi) [79]. CD44loCD24hi cells were seen to form tumors faster in vivo relative to CD44hiCD24lo, CD44loCD24hi and CD44loCD24lo cells, further establishing the aggressiveness of this hybrid E/M subpopulation [83]. Intermediate levels of ITGB4 (CD104) were reported to identify hybrid E/M breast cancer cells which also contained a majority of CSCs [84]. Co-culturing ‘locked’ epithelial and mesenchymal cells could not form as many tumors as ‘plastic’ hybrid E/M cells, pointing towards the possibility that plasticity, i.e. ability to traverse the EMP spectrum to varying degrees, contributes to stemness.

The association of hybrid EMT with stemness in breast cancer is increasingly reported. For instance, knockdown of cytotkeratin-18 (CK18) in MCF7 cells induces a partial “molecular EMT” state (depleted E-cadherin, but upregulated EpCAM—epithelial cell adhesion molecule), and an increase in ability to form spheres and colonies [85]. Similarly, leader cells in collective cell invasion observed by 3D invasion assays are more likely to be CSCs and exhibit hybrid E/M phenotypes, showing co-expression of E-cadherin, N-cadherin and NANOG [86].

**Lung cancer**

CD133 is often believed to be a canonical lung CSC cell surface marker. CD133+ lung cancer cells are reported to exhibit self-renew as well as generate a progeny of differentiated cancer cells [87]. Treatment of various non-small cell lung cancer (NSCLC) cells with TGFβ1 induced EMT to varying extents at molecular and/or functional levels. Intriguingly, the increase in number of CD133+ cells upon TGFβ1 treatment was highest for cell lines containing a significant number of hybrid E/M cells (identified as SLUG+CDH1+), indicating that the hybrid E/M population may be more ‘poised’ to give rise to CSCs [38]. Extensive heterogeneity in EMT has been observed in NSCLC cells [88]. H1975 and A549 cell lines have been reported to be predominantly hybrid E/M [22, 38, 46, 89] and plastic in their ability to move along the EMP spectrum [69, 90]. However, whether these hybrid cell lines are more tumorigenic in vitro and in vivo as compared to their epithelial and mesenchymal counterparts still remains to be quantified. A recent study compared the tumor-initiation potential of morphologically distinct colonies obtained by A549, and
observed that the subpopulation showing intermediate levels of E-cadherin and Vimentin had the maximum tumor-initiating capacity [91]. However, these experiments were done at a population level, thus it could not be identified whether this clone consisted of co-existing E-cadherin (CDH1+) and Vimentin (VIM+) cells or if individual cells expressed both CDH1 and VIM simultaneously. Recent computational methods can help dissect the system to differentiate between these two possibilities [92], and may enable mapping of EMP with stemness in NSCLC.

**Prostate and pancreatic cancer**

Various cell surface markers have been used to isolate CSCs in prostate cancer, including CD133 and CD44hiCD24lo [93]. Similar to experiments in keratinocytes and for A549 lung cancer cells, PC3 cells were cultured to give rise to different clones with varying self-renewal potential. The clones containing the highest frequency of CSCs formed tightly packed colonies, indicating a more epithelial nature. They also expressed β-catenin and CD44, indicating a part “molecular EMT” [94]. Further analysis using PC3 sub-clones established that higher metastatic and tumor-initiating traits were limited to cells that did not exhibit a full-blown EMT. Further, knockdown of E-cadherin in these tumor-initiating cells enhanced invasiveness, but reduced their ability to form spheroids and colonize other organs in NOD/SCID mice [95]. Conversely, overexpression of E-cadherin in more mesenchymal PC-3 clones restricted invasiveness but increased the spheroid-formation capability [95]. These results are reminiscent of observations in breast cancer that knockdown of E-cadherin, which can induce a full-blown EMT, can restrict the metastatic potential of cells [96]. Reinforcing observations were recently reported in a mouse model of pancreatic cancer, where the “late hybrid” cells, but not the “most mesenchymal” cells were found to be metastatically advantageous with specific proliferative, metabolic, and signalling processes associated with them [12]. Therefore, in prostate and pancreatic cancer, “the more the EMT, the more the stemness” dogma does not hold true; instead, the ‘stemness window’ seems to lie on an epithelial or hybrid E/M range of values on the ‘EMP axis’.

The dynamics of EMT/MET in prostate cancer is undergoing extensive investigation [23, 97], and drivers of MET such as OVOL1/2 have been identified in prostate cancer cells [98]. Inducible models can help quantify the tumor-initiation potential as a function of EMT at varying time points, and single-cell analysis can help map the percentage of CSCs dynamically as cells undergo EMT/MET [22]. Intriguingly, prostate cancer cells were shown to exhibit spontaneous switching among epithelial, mesenchymal and hybrid E/M subpopulations, with the hybrid ones being the most plastic [99]. Phenotypic stability factors such as GRHL2 were proposed to increase the relative stability of hybrid E/M cells [14, 16]. Whether such plasticity of hybrid E/M cells is necessary or sufficient for them to self-renew and/or form tumors in vivo remain to be deciphered.

**Squamous cell carcinoma**

Recent findings in squamous cell carcinoma (SCC) have shed new light on EMP, and its association with stemness. Using a DMBA/TPA-induced mouse model of cutaneous SCC, Pastushenko and colleagues demonstrated that FAT1 loss causes the appearance of a hybrid E/M cell state that co-expresses both epithelial and mesenchymal markers [100]. This state was associated with increased metastasis. This role for FAT1 deletion appeared to be specific for SCC, as FAT1 deletion in mouse lung tumours promoted the development of lung SCC over adenocarcinoma. Interestingly, in the same study, the authors also used a more mesenchymal tumour model driven by KRAS activation and p53 deletion targeted to the mouse hair follicle. In this model, FAT loss also caused appearance of a hybrid E/M cell state. Therefore, FAT1 loss appeared to induce a hybrid cell state from both epithelial and mesenchymal starting populations.

The authors elaborated on this mechanism by showing that FAT1 loss acts through two separate signalling pathways; CAMK2–CD44–SRC–YAP–ZEB1 activates a mesenchymal program, and CAMK2–EZH2–SOX2 activates an epithelial program [100]. Therefore, the hybrid state is induced through a balance of epithelial and mesenchymal signals. This agrees with studies cited in the preceding sections of this review, which have suggested that the hybrid cell state is governed by a balance of epithelial and mesenchymal transcriptional networks.

Contrastingly, another recent study has demonstrated the existence of a specific stem cell regulatory network alongside the EMP spectrum [101]. Here, using a cell line model of oral SCC and human tumour specimens, the authors identified a stem cell sub-population that could differentiate into both epithelial and mesenchymal lineages. This stem cell sub-population retained the epithelial marker EpCAM alongside a CD44hiCD24hi stem cell marker profile and activation of mesenchymal transcriptional networks, and was predictive of metastatic status in human archival tumour specimens. Alongside its hybrid E/M signature, this stem cell sub-population activated a distinct transcriptional signature that was not shared with either its epithelial or mesenchymal counterparts, suggesting a specific stem cell regulatory network at work alongside the EMP spectrum.

However, it is important to note that many studies demonstrating the importance of the hybrid E/M state have been performed in the context of established cell lines. Whilst these are often excellent models of tumour biology, it would be desirable to explore these concepts using other...
experimental systems designed to recapitulate the stemness properties of cells found in tumour tissue. This could include low passage suspension culture of tumour cells in defined stem cell medium. The appearance of consistencies and inconsistencies between different tumour models with respect to the hybrid E/M state could better inform our understanding of the connection between the EMP and CSC axes in cancer.

**A coherent model of EMP and stemness**

Put together, these observations suggest that hybrid E/M phenotypes are capable of forming tumors in vitro and in vivo, and may be the driving force behind metastatic dissemination. However, the following questions remain: (a) If cells gain stemness during EMT, do they lose it during MET or is stemness maintained as a part of ‘cellular memory’? (b) Do any changes in molecular, morphological and/or functional traits of EMT directly contribute to stemness and/or plasticity? (c) Is higher stemness seen in hybrid E/M states a consequence of the cells co-expressing both sets of markers, or is the ‘un-differentiated’ state regulated independently of EMP? In other words, is stemness dependent on the position of a cell within the EMP spectrum? Recent experiments decoding the dynamics of EMT induction in a time-dependent and/or dose-dependent manner in a cancer cell population may be helpful in answering some of these questions, particularly when combined with single cell analysis [18, 21, 22, 102].

We propose a coherent model that can resolve existing confusions around the association of EMT (or EMP) with stemness. As a first approximation, we consider that all phenotypes—epithelial, mesenchymal and hybrid E/M—have the potential to be stem-like; however, this potential is likely to be maximum for hybrid E/M cells. In representing this process (Fig. 3), we have ignored the diversity of EMP trajectories and collapsed multi-dimensional EMP into one axis: the x-axis denotes the position of a cell along the EMP spectrum. The y-axis in this representation denotes the stemness-differentiation rheostat, similar to the ideas represented in Waddington’s landscape, where valleys at the bottom denote terminally differentiated states [103]. Just as multi-dimensional EMP is collapsed onto the x-axis, the y-axis represents multi-dimensional stemness including both self-renewal potential and multilineage potential, which may in reality be semi-independent from each other [101]. The z-axis in this landscape signifies the potential (or stability) of a cell state. The deeper a given valley is, the more stable the corresponding state is. Thus, stem cells with similar coordinates on x and y axes can still have different z-axis coordinates (i.e. varying stability). A balance between stability and plasticity of various stem cell states can play an important role in propagating metastatic dissemination.

In this framework, cells *en route* through the transition in either direction (E to M or vice-versa) usually become
relatively more de-differentiated, which may contribute to the ability to self-renew as well as the ability to give rise to other cell types (multilineage potential). In other words, cells undergoing EMT/MET encounter a spectrum of de-differentiated states which may facilitate higher stemness. However, the association of hybrid E/M states and stemness is neither exclusive nor exhaustive. In this landscape populated by multiple states, switching to another state with a different value along the x-axis is not necessarily always accompanied by change along the y-axis too, or vice versa. For example, cells with an amoeboid phenotype have been associated with stemness traits and metastatic progression whilst having no epithelial features [104, 105]. Therefore, amoeboid cells might be an example of a phenotype that sits within the completely mesenchymal end of the spectrum but nevertheless exhibits heightened stemness [106].

This model allows many possible couplings between EMP and stemness, and highlights a higher likelihood of hybrid E/M cells existing in more ‘stem-like’ states. However, the level of inter-dependence between these axes is currently unknown, and is a topic of active investigation. Also unknown is the range of ‘stemness’ gene expression signatures. Specific gene expression signatures have been associated with stemness in cancer [101, 107], but we do not yet know whether the stemness signature is universal across the EMP spectrum. Instead, the stemness signature may be contextual, based on position within the EMP spectrum. To answer this question, single cell approaches will be required that enable resolution of both transcriptional and functional attributes [102, 108] to identify all of the stem cell phenotypes existing across the EMP spectrum.

Stabilization of multiple cellular states (z-axis) within this framework may be achieved through mutually inhibitory feedback loops which may exhibit hysteresis or ‘cellular memory’. The more the self-stabilizing feedback loops, usually, the deeper the valley corresponding to that state is. Whether hybrid E/M states are stabilized by a balance of epithelial and mesenchymal ‘teams’ of players (similar to ‘teams’ seen in small cell lung cancer and melanoma [109, 110]), or there exist a bonafide ‘team’ of stabilizers of hybrid E/M phenotypes remains to be identified. In other words, it is possible that hybrid cell states are stabilized through molecules which may not be either EMT-inducers or MET-inducers, but *bona fide* inducers for hybrid E/M phenotype(s). Recent work demonstrating the existence of a specific stem cell regulatory network alongside the EMP spectrum suggests that stem cell regulatory factors may contribute to multistability [101, 111], and indeed may represent a class of stabilization factors for hybrid E/M phenotype(s); for instance, SLUG has been implicated in stabilizing hybrid E/M state and promote stemness in multiple cancers [32, 112–114].

A limitation of our model is that the EMP status of a cell is represented by only one value whereas, as discussed earlier, EMP is a multi-dimensional process. For instance, cells *en route* through EMT can have coordinates along at least three additional semi-independent axes (not shown in the model): molecular EMT, morphological EMT and functional EMT. Similarly, multiple axes of stemness (multi-lineage potential, metabolic reprogramming, self-renewal etc.) are collapsed into one. Thus, we should exercise extreme caution while defining hybrid EMT state(s) to prevent its association with stemness from becoming an unfalsifiable hypothesis. Nevertheless, in reconciling recent findings, this model provides a coherent conceptual framework that points a way forward for the field to aid the interpretation of new findings, and clarify some of the most pressing unsolved questions.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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