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Review article

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Identification, functional insights and therapeutic targeting of EMT tumour states

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Abstract

Epithelial-to-mesenchymal transition (EMT) is a cellular process during which cells lose their epithelial characteristics and acquire mesenchymal features with enhanced migration capacities. EMT has key roles in different aspects of tumorigenesis, including tumour initiation, progression, metastasis and resistance to therapy. Here, we have reviewed the recent advances in our understanding of EMT in cancer. Instead of being a binary switch as initially proposed, EMT has been shown to be composed of multiple tumour states residing in specific niches with distinct functional properties that are controlled by different gene regulatory networks. We discuss how the types of oncogenic mutations, signalling pathways, transcription factors, epigenetic regulators and microenvironmental cues regulate the different EMT states. We also highlight the mechanisms by which EMT controls resistance to anticancer therapy and how new approaches to pharmacologically target EMT in clinical settings have recently been developed.

Sections

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Introduction

Epithelial-to-mesenchymal transition (EMT) is a biological process in which cells lose their epithelial features and acquire mesenchymal characteristics (Fig. 1). Originally discovered to occur during embryogenesis[1], EMT has also been shown to be associated with different aspects of tumour functions, including tumour initiation, malignant progression, metastasis and therapy resistance[1–5] (Box 1). Acquiring mesenchymal features allows tumour cells to leave their original niche, invade surrounding tissues, migrate and disseminate in the blood or lymphatic vessels and eventually establish distant metastasis (Fig. 2).

Cancer is not a uniform entity but rather a heterogeneous disease. Within a tumour, cells exhibit different cell states that are spatially and temporally regulated through intrinsic factors and extrinsic environmental cues. EMT equips tumour cells with a higher propensity to adapt to the environment and resist anticancer therapy[2–4]. Therefore, understanding the mechanisms regulating EMT is critical for developing more efficient strategies against tumour invasion, metastasis and resistance to chemotherapy, immunotherapy and targeted therapy.

EMT was long viewed as a binary process, in which the epithelial state is defined by the expression of epithelial markers such as epithelial cell adhesion molecule (EpCAM) and E-cadherin, whereas the mesenchymal state is defined by the absence of epithelial markers and instead the expression of mesenchymal markers such as vimentin and fibroblast-specific protein 1 (FSP1)[2]. However, recent findings suggest that EMT is much more heterogeneous than initially thought with the presence of a continuum of cell states from purely epithelial to purely mesenchymal passing through multiple intermediate states that co-express epithelial and mesenchymal transcriptional programmes at the single cell level[6–14]. Multiple studies have subsequently shown that the intermediate EMT states acquire stem cell features and possess the highest cell plasticity and metastatic potential compared with their epithelial and mesenchymal counterparts[6,7,10–12,15–22]. Different intermediate EMT states also reside in distinct niches within the tumour microenvironment (TME)[6,12,23,24] and follow a specific spatial organization with hybrid EMT cells located at the leading edge of the tumour[16,25,26].

Considering the complexity and depth of this rapidly evolving field, we discuss here recent findings regarding the identification and functional characterization of these distinct EMT states during tumour initiation, progression and metastasis. We then summarize the intrinsic and extrinsic regulators of different EMT states and finish by presenting novel therapeutic strategies targeting EMT for cancer treatment.

Detection of different EMT states

Most human malignant tumours are carcinomas originating from various epithelial cell types, which maintain cell–cell adhesion through different cell–cell junction complexes such as tight junctions or adherens junctions, ensuring the structural and histological characteristics of the different cancer types[27]. To invade neighbouring tissue and furthermore form metastasis, carcinoma cells must lose their cell–cell adhesion and acquire more invasive features. Loss of E-cadherin, a key gatekeeper of the epithelial state, is frequently observed in carcinomas[28] and correlates with the malignancy of the tumour[29]. Loss of E-cadherin can sometimes be accompanied by the acquisition of a spindle-shaped morphology and expression of mesenchymal markers such as neural cadherin (N-cadherin), fibronectin and vimentin[30], indicating a transition towards a more mesenchymal state (Fig. 1). Tumour cells with reduced E-cadherin expression are observed at the invasive front[31–33] and therefore are thought to be responsible for extravasation and metastasis formation[34].

For a long time, it was believed that EMT was a binary and reversible process, in which cells were either in the epithelial state or the mesenchymal state. However, recent studies have found that within a given tumour, some cells express both epithelial and mesenchymal markers[6,9,14], suggesting the presence of intermediate states of EMT. Subsequently, distinct intermediate states presenting specific characteristics were identified in multiple mouse tumour models[6,7,10–12,35] and were called hybrid EMT[6–8], partial EMT (p-EMT)[9,10] or a quasi-mesenchymal[35–38] state.

Discovery of intermediate EMT states

The oversimplified ON and OFF model of EMT has raised debates and controversies concerning the involvement of EMT in tumour progression, such as metastasis formation[17,39–42]. Moreover, EMT does not progress to the fully mesenchymal state with the complete loss of epithelial markers in some tumours, especially in human cancer[16,25]. As such, the identification of intermediate states expressing both epithelial and mesenchymal markers provided novel insights into the mechanisms and functions of EMT in cancer[6,7,9,10,14,37,43–45] (Fig. 2). Early studies showed that co-expression of epithelial and mesenchymal markers could be observed in patients with breast cancer[46,47]. Subsequently, using a quantifiable, dual-colorimetric RNA in situ hybridization assay assessing the expression of transcripts of epithelial (E) and mesenchymal (M) markers, Yu et al.[14] identified the presence of rare hybrid E+/M+ cells in human primary breast tumours. By contrast, these E+/M+ cells are more abundant in circulating tumour cells (CTCs) in the peripheral blood of patients with breast cancer[14], suggesting a role for the intermediate EMT state during metastatic dissemination. Intermediate EMT cells have also been observed in CTCs from patients with non-small-cell lung cancer (NSCLC)[48], colon cancer[49,50], breast cancer[51] and prostate cancer[51].

The co-expression of epithelial and mesenchymal markers is also observed in different mouse tumour models, including pancreatic cancer, prostate cancer, breast cancer and skin squamous cell carcinoma (SCC)[6,13,52]. In addition, lineage tracing in mouse models makes it easier to distinguish mesenchymal tumour cells from their stromal compartments. Using a vimentin–GFP reporter, the intermediate EMT state can be identified in prostate cancer, where it maintains EpCAM expression while becoming positive for vimentin-GFP[13]. Similarly, in skin SCC derived from hair follicle stem cells (HFSCs), some EpCAM-positive populations continue to express both keratin 14 and vimentin in the same hybrid EMT cells[6,7]. Further characterization of this intermediate state has revealed a large heterogeneity within this population with distinct functional characteristics, which will be discussed in the following section[6].

The induction of EMT in vitro also recapitulates the intermediate state with co-expression of epithelial and mesenchymal markers. Transforming growth factor-β (TGFβ) is the best characterized exogeneous EMT inducer, which activates the SMAD signalling pathway to promote the expression of EMT transcription factors (EMT-TFs)[53]; therefore, it is widely used as an approach to study EMT dynamics in vitro. A systematic examination of core EMT regulators induced by treating a human breast cancer cell line with recombinant TGFβ protein has revealed three tumour states corresponding to epithelial, p-EMT and mesenchymal phenotypes during EMT, based on the expression of the epithelial marker E-cadherin and mesenchymal marker vimentin[9]. Interestingly, p-EMT cells revert back to an epithelial state upon withdrawal of TGFβ

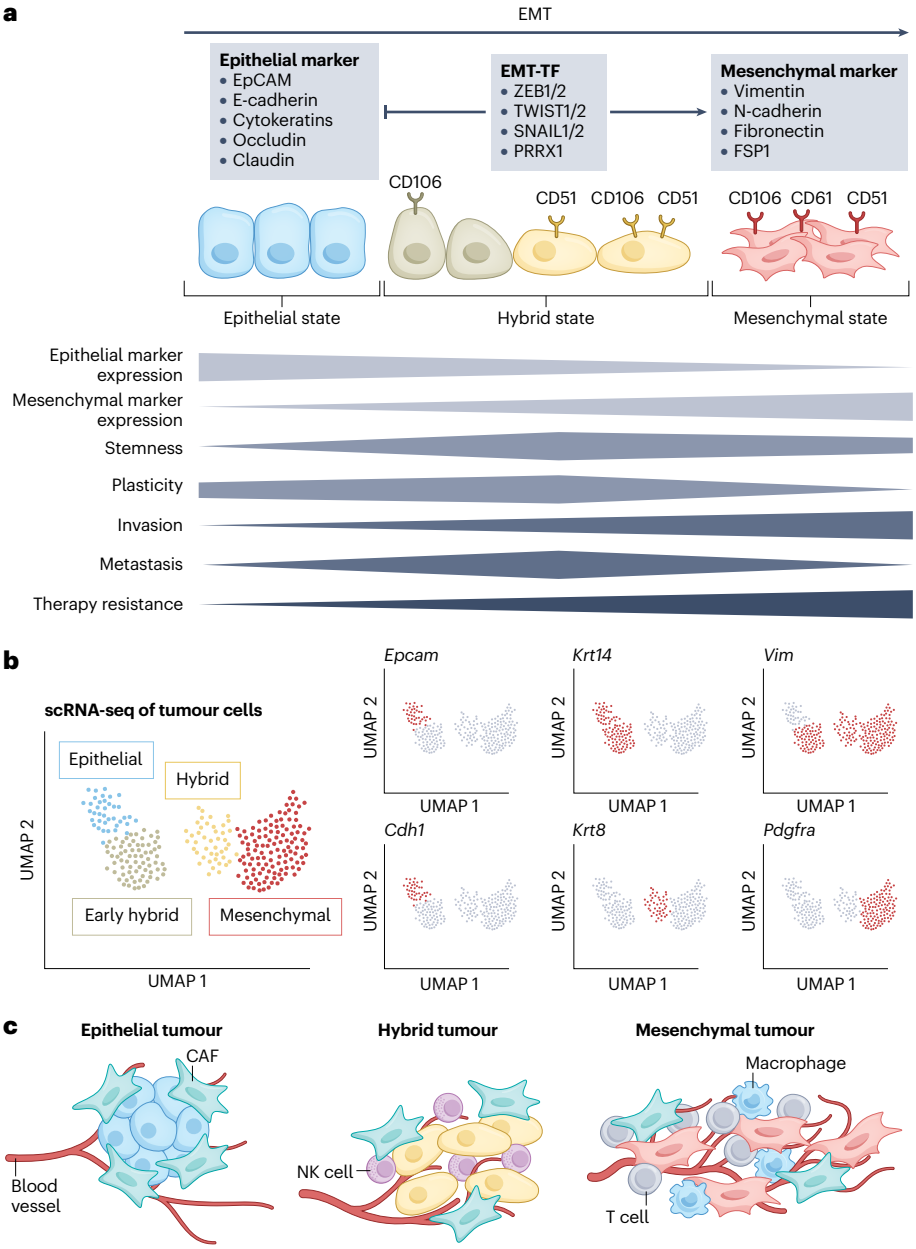


Fig. 1 | Distinct EMT tumour states. a, During epithelial-to-mesenchymal transition (EMT), epithelial cells lose their cell–cell junctions and epithelial gene expression (such as epithelial cell adhesion molecule (EpCAM), E-cadherin (encoded by *CDH1*), cytokeratins (encoded by KRT genes), occludin and claudin) through activating key EMT-transcription factors (EMT-TFs) ZEB, SNAIL, TWIST and PRRX1. The induction of EMT upregulates mesenchymal gene expression (such as vimentin, N-cadherin, fibronectin and fibroblast-specific protein 1 (FSP1)) and changes the cell morphology into a spindle-like shape with invasive capacity. Different EMT intermediate states have been identified between epithelial and mesenchymal states. These hybrid EMT states co-express epithelial and mesenchymal markers and express different cell surface markers. Different EMT states also exhibit different functional characteristics including stemness, plasticity, invasion, metastatic potential and therapy resistance. **b**, Different EMT states can be identified with single-cell RNA sequencing (scRNA-seq). **c**, Different EMT states reside in specific niches with distinct compositions of stromal cells. CAF, cancer-associated fibroblast; NK, natural killer; *PDGFRA*, platelet-derived growth factor receptor α; UMAP, uniform manifold approximation and projection.

treatment, whereas the mesenchymal state fails to do so, indicating that the p-EMT state has the highest plasticity[9].

Different EMT states uncovered by flow cytometry and scRNA-seq

With the identification of intermediate EMT states, multiple EMT scoring methods have been developed aiming to retrieve the features of the intermediate states across different tumour models[54–56]. These methods gather the transcriptomic signatures of epithelial and mesenchymal cells from different tumour models and use mathematical tools to calculate the EMT scores to predict where along the EMT spectrum tumour cells reside. However, as these methods only calculate the EMT scores based on the bulk transcriptomic data, it takes extra effort to distinguish the pure intermediate EMT state from the mixture of epithelial and mesenchymal states[57]. In addition, these scoring methods have limited ability to detect the heterogeneity within the intermediate state. Therefore, the identification of novel methods and markers that can distinguish the different intermediate states is required.

The tumours that arise following $Kras^{G12D}$ expression and *Trp53* deletion in HFSCs and their progeny present different degrees of EMT, with some tumour cells losing EpCAM expression and beginning to express the mesenchymal marker vimentin[6,58]. Transcriptional profiling of $EpCAM^{+}$ and $EpCAM^{-}$ tumour cells demonstrated that they express canonical markers of epithelial or mesenchymal states. The bimodal expression of EpCAM ($EpCAM^{+}$ or $EpCAM^{-}$) in this tumour mouse model suggested that EMT was a binary process. However, by assessing the

Box 1 | The canonical functions of EMT in cancer

Pioneering works investigating the function of epithelial-to-mesenchymal transition (EMT) in tumour progression have been mostly performed through modulating the expression of the core EMT transcription factors (TFs), namely, SNAIL (also known as SNAI1), TWIST1, ZEB1 and ZEB2 (ref. 2), which function through repressing E-cadherin expression and activating the EMT programme[244–249]. The first report demonstrating EMT as essential for metastasis showed that knockdown of *Twist1* in a mouse mammary tumour cell line strongly reduced its ability to metastasize to the lung[250]. Deletion of another EMT-TF, *Zeb1*, in pancreatic tumour cells also strongly inhibits metastasis[52]. Interestingly, although EMT is observed in circulating tumour cells from patients[51,251,252], distant metastases of human carcinoma often present with histologically epithelial characteristics[30,253–255]. The mesenchymal-to-epithelial concept has therefore been proposed to solve this paradox, that upon seeding in distant organs, mesenchymal tumour cells need to revert to an epithelial state to form macrometastasis (Fig. 2). Supporting this concept, transient overexpression of *Twist1* in mouse skin cancer is sufficient to promote lung metastasis, whereas sustained expression of *Twist1* reduces metastasis[256]. Similarly, knockdown of *Prrx1* (ref. 257) or transient *Snail* expression[258] is required for establishing metastasis in a breast tumour mouse model, indicating a strict and time-dependent regulation of EMT for metastasis initiation.

EMT is also believed to contribute to therapy resistance, as the acquisition of mesenchymal features is often detected in clinical samples after standard cancer treatments[5,54,259]. Inhibition of EMT through conditional knockout of *Twist1* or *Snail* in mouse pancreatic cancer sensitizes tumours to chemotherapy[40]. This ability to promote therapy resistance is proposed to be associated with the stem cell features of EMT cells[3,260]. In line with this, ectopic expression of the EMT-TFs *Snail* or *Twist* in mouse mammary epithelial cells increases stem cell marker expression and increases tumour incidence following transplantation[94,261]. Consistently, deletion of *Zeb1* in mouse pancreatic tumour cell lines reduces their tumour-initiating capacity in vivo[52].

expression of ~200 cell surface markers, Pastushenko et al.[6] found that although the epithelial EpCAM$^+$ population comprises a relatively homogeneous population, the mesenchymal EpCAM$^-$ population is very heterogeneous, with more than 50% of the cell surface markers heterogeneously expressed, suggesting that the mesenchymal state is composed by different EMT subpopulations. By combining the different markers that were heterogeneously expressed, they could identify at least six different EMT subpopulations using EpCAM and three additional cell surface markers (CD106, CD51 and CD61)[6]. Interestingly, these distinct tumour populations are associated with different tumour functions. The most proliferative population is the epithelial state. Surprisingly, the most metastatic cell populations are the hybrid states that have both epithelial and mesenchymal features, but not the late EMT cells that lose all epithelial characteristics, and are the most locally invasive. Although the different EMT states present similar stemness as defined by their ability to form a secondary tumour upon transplantation, they present with different plasticity and ability to transit from one state to another, with the most stable state being the late EMT state[6,8] (Fig. 2). Using the same cell surface marker combination, different transition states are also detected in metaplastic-like mammary tumours and MMTV-PyMT mammary luminal tumours[6]. These cell surface markers were also reported to regulate EMT and promote tumour stemness and invasion in human breast[59,60], lung[61,62] and pancreatic[62] tumour cells, suggesting that some mechanisms regulating the different EMT states are conserved across different tumour models. Heterogeneity within the mesenchymal population of a tumour is also observed in triple-negative breast cancer (TNBC) cell lines[38]. Integrin-β4 (also known as CD104) has been identified to label the more epithelial-like cells in the mesenchymal TNBC cell lines, suggesting the existence of the intermediate epithelial/mesenchymal (E/M) state in this cancer type[38], which was later referred to as the quasi-mesenchymal state[35]. With the same marker, the quasi-mesenchymal state can also be identified in the human mammary epithelial cell model[37], expressing both epithelial and mesenchymal markers and associated with the malignant phenotype.

The use of the single-cell RNA-sequencing (scRNA-seq) technique has revolutionized the understanding and definition of cell states including common and tumour-specific cell states[63,64]. The recent advances in single-cell technology have enabled the exploration of the molecular heterogeneity across tumour states, the distinct stages during EMT, as well as characterizing the complexity in the composition of the TME across different tumours. For example, a combination of a multiplexed lineage tracing system with scRNA-seq has revealed the spectrum of EMT in the pancreatic ductal adenocarcinoma (PDAC) mouse model[11]. The canonical epithelial markers, such as *Epcam*, mucin 1 (*Muc1*) and E-cadherin (*Cdh1*), are enriched in non-aggressive clones, whereas mesenchymal markers, such as *Sparc*, *Zeb2* and the α1 chain of type III collagen (*Col3a1*), are enriched in aggressive clones. Multiple hybrid states reside between the E–M spectrum and possess different proliferative and metastatic potentials, with the late hybrid state being the most metastatic[11]. The heterogeneity within the hybrid state has also been identified with scRNA-seq of breast cancer[65,66] and NSCLC[67] cell lines, as well as of MMTV-PyMT[12] and skin SCC[6,68] mouse models. These different studies demonstrate the existence of a continuum of cell states during the EMT process. The first transition corresponds to the transition from a pure epithelial state to the early hybrid EMT state, which is characterized by the loss of EpCAM or E-cadherin, the acquisition of few mesenchymal markers including TWIST1 and vimentin, and a sustained expression of many epithelial markers such as keratin 5 or keratin 14. As EMT progresses, cells lose the expression of canonical epithelial markers and begin to express epithelial markers of simple epithelia such as keratin 8, which is expressed in the skin during the early steps of embryonic skin formation[69]. Finally, the last transition gives rise to full EMT tumour cells that lose all epithelial markers and exclusively express mesenchymal markers[2].

Although a full mesenchymal state is observed in multiple mouse tumour models[7], the complete loss of all epithelial traits and full acquisition of EMT features are rare in human carcinoma samples[16,25]. In human head and neck squamous cell carcinoma (HNSCC), a tumour state presenting features of a p-EMT have been identified[16]. Similar to the intermediate EMT state identified in mouse tumours, this p-EMT state expresses multiple mesenchymal genes such as *VIM* and *ITGA5* (encoding integrin-α5), while the overall epithelial markers are maintained. When cultured in vitro, these p-EMT cells exhibit increased invasiveness, accompanied by reduced proliferation. However, the typical EMT-TFs such as *ZEB1* and *ZEB2*, *TWIST1* and *TWIST2*, and *SNAI1*

are not detected by scRNA-seq. Several transcription factors including GRHL2 (ref. 70), ΔNp63α[71,72], nuclear factor erythroid 2-related factor 2 (NRF2)[73,74] and nuclear factor of activated T cells, cytoplasmic (NFATC)[75] have been shown to promote the hybrid EMT state in human cancer cell lines in vitro. Further studies would be required to assess whether these factors also regulate the hybrid state in mouse models and in patients with cancer.

In another multimodal profiling analysis comparing human cutaneous squamous cell carcinoma (cSCC) with site-matched normal skin, a keratinocyte population that is uniquely present in the tumour sample and possesses invasive features has been revealed[25]. Similar to the p-EMT cells, this tumour-specific keratinocyte population does not upregulate typical EMT-TFs. Pan-cancer analysis of scRNA-seq from human tumour cell lines and tumours of patients has also identified consensus EMT cellular states, which contribute to intratumoural heterogeneity[76–78]. All these data suggest the existence of intermediate EMT states that are shared by multiple tumour types.

Different EMT trajectories

To study the underlying dynamics of a biological process with the scRNA-seq data, trajectory inference methods have been developed to generate a graph-like structure along a pseudo-timescale, which allows

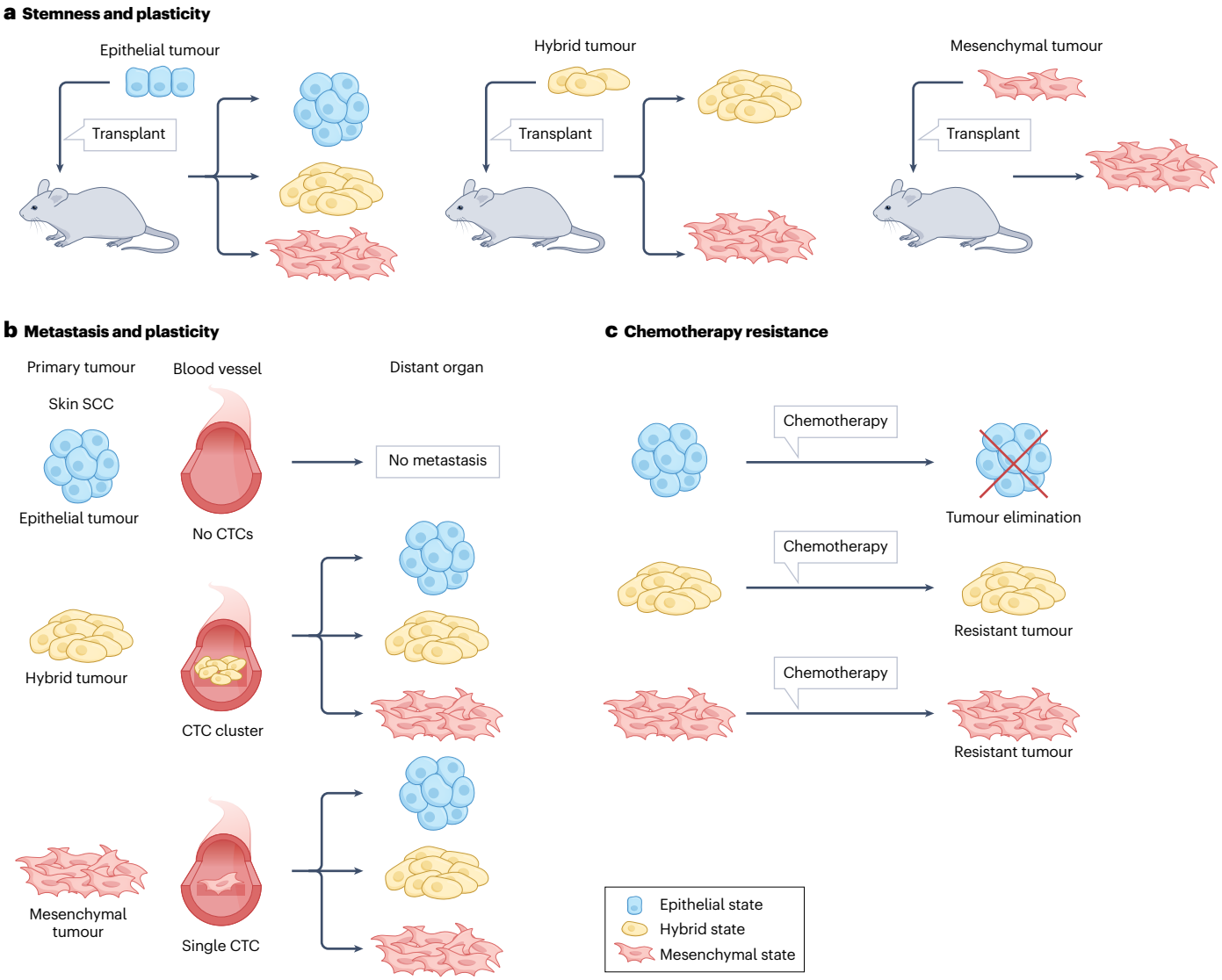


Fig. 2 | Functional characteristics of different EMT states. a, Tumour cells from epithelial, hybrid and mesenchymal states can all give rise to tumours after transplantation. Upon subcutaneous transplantation, epithelial cells can give rise to epithelial, hybrid and mesenchymal tumours; hybrid epithelial-to-mesenchymal transition (EMT) tumour states can give rise to hybrid and mesenchymal tumours; whereas late EMT tumour states can only give rise to late EMT tumours. **b**, In mouse skin squamous cell carcinomas (SCCs), primary tumours composed exclusively of the epithelial state do not metastasize. Hybrid and mesenchymal tumours can both intravasate into blood and generate metastases composed of epithelial, hybrid and mesenchymal states. However, hybrid cells tend to migrate in cell clusters for better dissemination, whereas mesenchymal cells migrate more often as single cells. **c**, Epithelial cells are sensitive to therapy, whereas hybrid and mesenchymal cells are therapy-resistant. CTC, circulating tumour cell.

the visualization of how cells may evolve from one state to another and how cell fate is determined[79]. Multiple pseudo-time trajectory analyses have been performed to study the dynamics of EMT and have revealed how tumour cells pass through the different EMT states.

Profiling of MCF10A human mammary epithelial cells undergoing confluence-dependent or TGFβ-induced EMT has revealed a continuum of EMT progression[60]. Rather than a direct gain or loss of EMT-associated genes, cells exhibit continuous waves of gene expression along the EMT progression, with a gradual loss of epithelial markers such as *CDH1*, *CRB3* and desmoplakin (*DSP*), accompanied by a gradual gain of mesenchymal markers including *VIM*. This linear progression across the EMT continuum is also observed in patient samples of HNSCC[16,60], and a similar EMT continuum is identified through single-cell lineage tracing in the PDAC mouse model[11]. Interestingly, comparing the trajectory from spontaneous and TGFβ-induced EMT shows that KRAS is sustained during EMT. Interrupting the KRAS pathway disrupts the EMT continuum and traps cells in discrete EMT states corresponding to previously identified partial or hybrid EMT states[6,10,37,38,60,68], suggesting a role for KRAS signalling in promoting EMT progression.

In some circumstances, EMT can happen through distinct trajectories, leading to different 'end points'. The pseudo-time trajectory analysis of the mouse skin SCC model, which undergoes spontaneous EMT, has revealed that EMT can occur from two divergent EMT trajectories, leading towards a hybrid and late EMT state, respectively[6,68]. Similar observations have been obtained in the mouse MMTV-PyMT breast cancer model[80]. Using RNA velocity analysis, Youssef et al.[80] identified two distinct trajectories towards EMT states with inflammatory features and invasive features, respectively. The maintenance of the distinct trajectories is tightly regulated. *Snail* is activated in both trajectories, and conditional knockout of *Snail* reduces the number and size of primary tumours and generates highly differentiated tumours with low-grade malignancy, suggesting its pioneer role in mediating EMT. By contrast, *Prrx1* is essential for the progression towards the invasive EMT state, and conditional knockout of *Prrx1* reduces lung metastasis and induces the expression of markers for the inflammatory EMT state[80]. Interestingly, the invasive EMT trajectory recapitulates the EMT programme during embryonic development, whereas the inflammatory trajectory resembles the fibrosis process, highlighting different paths controlling EMT with distinct features.

The entry into different EMT trajectories can also be regulated epigenetically[81]. Genome-wide CRISPR screening on human mammary epithelial cells revealed that different epigenetic regulators have distinct roles during EMT progression. Loss of two epigenetic regulators, polycomb repressive complex 2 (PRC2), which regulates the histone H3 lysine 27 trimethylation (H3K27me3) repressive mark, and histone-lysine *N*-methyltransferase (KMT2D) complex of proteins associating with SET1 (COMPASS), which regulates the monomethylation of H3K4, leads to different EMT states. Specifically, trajectory analysis with a loss-of-function approach shows that loss of PRC2 and KMT2D–COMPASS unlocks two distinct EMT trajectories with different functional characteristics. PRC2 inhibition leads to a hybrid EMT state with the more metastatic potential, whereas KMT2D–COMPASS inhibition leads to the fully mesenchymal EMT state. Interestingly, restoration of PRC2 function is sufficient to induce mesenchymal-to-epithelial transition (MET) in the intermediate EMT state, highlighting the epigenetic plasticity of this state, and suggesting that epigenetic reprogramming may be a potential approach for targeting the intermediate EMT states.

An in vitro study mimicking the MET process by the removal of TGFβ in NSCLC cell lines reveals that the MET trajectory differs from the original EMT path[67], suggesting that MET does not simply proceed through the reverse path of the original EMT trajectory. Similarly, another in vitro study inducing MET by withdrawing doxycycline (DOX)-induced *SNAI1* expression in a human prostate cancer cell line also shows that MET leads to a distinct epithelial state compared with the original parental epithelial cell state with some memory that the cells had previously experienced EMT[82]. The transcriptional signature of the reversed epithelial state overlaps with gene signatures upregulated in patients with metastasis and is associated with poor prognosis.

Taken together, all these lineage trajectory analyses raise the possibility that rather than a 'one-way road', EMT can be achieved through multiple paths with distinct hybrid states, different probability of transition, through specific gene regulatory networks and eventually leading to different EMT destinations with distinct plasticity and tumour functions. The transitioning paths may also be context-dependent and differentially regulated by the microenvironment.

Different niches for the different EMT states

The TME consists of immune cells, cancer-associated fibroblasts (CAFs) and other stromal cells such as endothelial cells and lymphatic cells as well as different compositions of the extracellular matrix (ECM). It has been shown that different EMT states reside in distinct niches[6] (Fig. 1c). For example, the niche for the most mesenchymal state is enriched for monocytes, macrophages, $CD8^+$ and $CD4^+$ T cells[6,12,23,24] and associated with a highly vascularized environment[6,83]. Increased matrix stiffness is also associated with EMT progression in breast tumours, suggesting EMT cells reside in a collagen-rich area[84–87]. Although EMT cells secrete their own ECM proteins such as fibronectin and collagens, CAFs also contribute to the ECM remodelling and the stiffness of the TME[88]. A spatial transcriptomic analysis of human breast tumours has revealed a myofibroblastic CAF-dominant phenotype with the full EMT state[23]. Co-culture of CAFs with mesenchymal lung adenocarcinoma cells promotes cancer cell invasion in vitro[26,89], and their co-transplantation enhances the metastatic ability of the mesenchymal tumour cells to the lymph node[26]. Full EMT cells also secrete different soluble proteins to recruit specific stromal cell populations[90,91]. SNAI1, when acetylated by CREB-binding protein (CBP), activates the transcription of CC-chemokine ligand 2 (*CCL2*) and *CCL5* to promote the recruitment of tumour-associated macrophages[90]. Mesenchymal PDAC cells also produce different cytokines and chemokines such as CCL5 and macrophage colony-stimulating factor 1 (CSF1) to recruit macrophages with an M2-like phenotype[91]. Correspondingly, the inhibition of macrophage recruitment prevents EMT progression and restricts tumour cells in a hybrid EMT state[6].

Hybrid EMT cells reside in a distinct inflammatory environment compared with full EMT cells, with more dendritic cells and polymorphonuclear leukocytes and a specific enrichment of natural killer (NK) cells[23]. Although CAFs are less abundant in tumours with hybrid EMT states, they exhibit both inflammatory CAF and myofibroblastic CAF phenotypes[23]. The hybrid EMT states are found at the invasive leading edge with an inflammatory, fibrovascular environment[16,25,26]. In human renal cell carcinomas, the leading edge also shows an enrichment of IL-1β-expressing macrophages[92], suggesting that macrophages may promote EMT through an IL-1β-mediated pathway. Additionally, metastasis-initiating cells, which display p-EMT features, can activate surrounding fibroblasts upon seeding to enable efficient metastatic

establishment[93]. The activated CAFs in turn promote tumour cell plasticity to a more epithelial state for better colonization, suggesting a reciprocal interaction between tumour cells and CAFs during EMT and MET.

The designated organization of the TME suggests that EMT occurs within a dynamic ecosystem, in which tumour cells actively communicate with their surrounding environment to facilitate their survival and invasion. A comprehensive understanding of the nature of the cell–cell communication network that spatially and temporally regulates the composition of the TME during EMT will be important to define a novel therapeutic approach targeting EMT in cancer.

Functional characteristics of EMT states

EMT has been associated with tumour initiation, progression, metastasis and therapy resistance. With the identification of distinct EMT states along the EMT progression, it is becoming increasingly clear that distinct states may contribute differently to tumours, and numerous efforts have been made to better define the functional characteristics associated with the distinct EMT states.

Contribution of EMT states to tumour stemness, plasticity and therapy resistance

In humans, most tumours originate from epithelial tissues, owing to the accumulation of mutations during cell proliferation. EMT attenuates proliferation and induces motility and invasion. Other characteristics such as stemness, plasticity and therapy resistance are also co-opted by EMT cells to facilitate tumour outgrowth, dissemination, metastasis, and resistance to anticancer therapy[5] (Fig. 2).

Stemness is considered the key feature of EMT cells[6,37,94]. The stemness of tumour cells is defined by the ability to self-renew to sustain the tumour population and give rise to tumour cells with more distinct functions. It can be evaluated through transplantation, lineage tracing and lineage ablation assays[95]. Although both malignant epithelial and EMT cells can give rise to tumours after transplantation, EMT cells have a higher tumour-propagating potential[6]. In skin SCCs, $EpCAM^-$ EMT cells present with increased tumour propagation frequency compared with the $EpCAM^+$ epithelial state, and the different EMT subpopulations based on CD51, CD61 and CD106 cell surface marker expression exhibit similar stemness upon transplantation[6,58]. Acquisition of the hybrid EMT state through deletion of *FAT1* in both human and mouse SCC cells promotes tumour stemness in transplantation assays[8]. In other tumour types such as ovarian cancer[96] and breast cancer[37,38,97], hybrid EMT cells possess the highest stemness.

Tumour dormancy is also associated with stemness features and can contribute to tumour relapse after therapy. EMT-TFs have been reported to contribute to tumour dormancy through regulating cell cycle arrest[5,98]. For example, ZEB2 induces a G1 cell cycle arrest in the A431 SCC cell line through repressing cyclin D1, accompanied by a mesenchymal phenotype[99]. ZEB2 expression is also associated with quiescence in colorectal cancer[100]. Similarly, SNAI1 mediates a G0–G1 cell cycle arrest through regulating CDK1 in breast cancer cells[101]. Metastatic tumour cells can remain in a dormant or quiescent state for a long period of time to escape therapy treatment, immune surveillance and metabolic stress[102]. Features of dormancy, such as cell cycle arrest, can be detected in disseminating oestrogen receptor positive (ER^+) breast tumour cells with EMT[103]. Forced expression of E-cadherin is sufficient to awaken disseminating tumour cells from dormancy and generate macrometastases. Single-cell analyses of metastatic breast cancer patient-derived xenograft (PDX) models also demonstrated the enrichment of stemness and dormancy and/or quiescence signatures in metastatic cells[104]. However, the detailed mechanisms of how different EMT states contribute to dormancy and how the reversed MET drives metastatic outgrowth remain unclear.

Cellular plasticity is defined by the ability of cells to switch between cell states and change identity[105]. Epithelial cells possess a certain degree of plasticity, as they can undergo EMT to give rise to mesenchymal cells, and can also give rise to both epithelial and mesenchymal tumour cells after transplantation[58]. By contrast, full EMT cells can only give rise to cells with mesenchymal phenotype at the primary tumour site following subcutaneous injection[6]. Among all the EMT states, the hybrid EMT state possesses the highest plasticity. The reversal of early hybrid EMT cells induced by treatment with recombinant TGFβ protein back to the epithelial state is observed upon withdrawal of TGFβ from cell culture[9]. Tracing p-EMT cells using the early EMT marker tenascin C in the MMTV-PyMT mouse model has also revealed the reversal of early EMT cells to the epithelial state[12]. Hybrid cells are also able to give rise to lung metastases with an epithelial phenotype upon intravenous injection[17,21]. However, upon subcutaneous transplantation with matrigel, hybrid EMT cells only give rise to hybrid and full EMT cells but not epithelial cells[6], suggesting that the microenvironment of the metastatic site also has a role in regulating EMT reversion.

The contribution of EMT cells to therapy resistance is well recognized[39,40]. Residual human breast tumour cells following endocrine therapy or chemotherapy display a mesenchymal phenotype and tumour-initiating features[106]. Mouse skin SCC tumours that are resistant to standard chemotherapy are also enriched with EMT cells[107]. Although both hybrid and full EMT cells are enriched in primary tumours upon chemotherapy, a dramatic metastatic outgrowth is observed from full EMT cells in the context of chemotherapy[12], suggesting that the full EMT cells could be more chemo-resistant.

Hybrid EMT as the most metastatic tumour state

The formation of metastases in distant organs requires multiple critical steps[108]. Tumour cells from the primary tumours need to invade the neighbouring tissues, disseminate through the blood or lymphatic vessels and seed cells in distant organs, which will eventually colonize the metastatic site, proliferate and form macrometastases. For a long time, there has been a debate about the necessity of EMT for metastasis, with reports showing the absence of fully mesenchymal tumour cells in metastatic organs[39,40]. However, the recent findings showing that hybrid EMT cells, rather than full EMT cells, largely contribute to metastasis formation may account for the conflicting reports[6,7,10,12,15–20].

The retention of epithelial features benefits hybrid EMT cells in dissemination and migration[10,15,19]. The co-expression of epithelial and mesenchymal markers is observed in CTC clusters but not in single CTCs[10,12,71,109–111], suggesting that hybrid EMT cells tend to migrate in clusters, possibly owing to their ability to be adhesive with the expression of epithelial adhesion proteins to disseminate more efficiently, increasing their metastatic potential[110]. Consistent with this notion, the injection of breast cancer cell clusters results in a higher metastatic burden compared with a fully dissociated single-cell suspension[12,19,110]. PDX models of colorectal cancer also showed that hybrid EMT clusters generate metastasis more frequently than single tumour cells[112]. An in vitro culture of pancreatic cancer cell spheroids also demonstrated that hybrid EMT spheres retain cell–cell contacts and move as a collective cluster, whereas tumour spheres from full EMT cells migrate as single cells[10].

The 'leader–follower' model is one of the mechanisms explaining the collective cell migration[113]. Leader cells tend to display distinct morphologies, interact with the ECM and facilitate the movement of the follower cells. Hybrid EMT cells are reported to be the leader cells of collective invasion cell clusters and localized at the front of the migration group in vitro[72,114]. Accordingly, hybrid cells are also observed at the leading edge of human HNSCC and cSCC[16,25]. In another example, ΔNp63α expression induces a hybrid EMT state[71], which promotes the collective invasion of breast cancer in vitro, and a leader–follower relationship is also identified within the ΔNp63-induced invasive hybrid EMT cell clusters in vitro[72]. The ΔNp63-low leader cells pioneer in creating the microtracks in the surrounding ECM to facilitate the migration of ΔNp63-high follower cells, highlighting the importance of the step-wise regulation of EMT to facilitate invasion, migration and metastasis.

Apart from maintaining the adhesive feature, retaining epithelial markers also helps the survival of hybrid cells within the systemic circulation[15]. Upon detachment from the primary tumour site, disseminating cells experience massive stress, leading to the accumulation of reactive oxygen species (ROS) and eventually cell death[15]. In addition, acquiring the mesenchymal features through EMT-inducing signals such as TGFβ also generates oxidative stress[115]. It has been proposed that E-cadherin acts as a survival factor to overcome the stress response and facilitate metastasis occurrence[15]. In MMTV-PyMT tumour cells, although the loss of E-cadherin increases invasion and dissemination in vitro, it reduces the potential to metastasize and form colonies in vivo. Similarly, E-cadherin knockdown decreases metastasis in orthotopic breast cancer xenografts[116]. Interestingly, loss of E-cadherin induces apoptotic signalling and cell death in tumour cells, and this defect can be rescued by applying antioxidants[15], suggesting the involvement of E-cadherin in limiting ROS-mediated apoptosis.

Altogether, these findings suggest that acquiring the invasive property is not the sole determinant of efficient metastasis. Possessing both epithelial and mesenchymal features provides the hybrid cells with more plasticity to adapt better to the changing environment, resulting in the highest metastatic potential.

In vivo functional characterization of EMT states with lineage tracing

Genetic lineage tracing has been instrumental in unravelling stem cell fate, renewal, lineage commitment, cell identity and plasticity across different tissues within their native in vivo microenvironment during development, homeostasis and disease[117]. Cancer biologists have now used such genetic approaches to assess the role of distinct EMT populations in metastasis formation and resistance to therapy. Most of the functional studies to date on EMT states in vivo were achieved through the perturbation of EMT regulators or transplantation experiments that in theory could expand the cell fate potential or restrict tumour states or their plasticity, while in fact, the execution of the EMT programme is very dynamic and plastic. It is therefore important to develop new tools to temporally record the fate of different tumour cell populations during EMT to obtain a faithful readout of the EMT dynamic during primary tumour growth and metastasis in vivo.

The initial attempt to develop an EMT lineage tracing system was performed in the MMTV-PyMT mouse model, in which *Fsp1-cre* or *Vim-creER* was used to track cells that underwent EMT[39]. The *Fsp1*-expressing or *Vim*-expressing cells are rarely observed in primary tumours and are not observed in lung metastases. However, *Fsp1*-expressing cells have reduced susceptibility to chemotherapy and grow out in the metastatic lungs after chemotherapy. On the basis of these observations, the authors concluded that EMT is not required for metastasis but contributes to chemotherapy resistance[39]. However, this conclusion had been challenged by the demonstration that *Fsp1-cre* might not be as reliable and faithful a marker to monitor EMT as initially thought[17,42]. By crossing the same mammary tumour model with a knockin mouse reporting E-cadherin expression (E-cadherin-mCFP)[118], it has been shown that E-cadherin^{LO} cells, which express low levels of E-cadherin, upregulate vimentin expression, suggesting that they undergo p-EMT. By combining lineage tracing with the *Fsp1*-cre and E-cadherin-mCFP mouse model, it was found that not all E-cadherin^{LO} mesenchymal cells are marked by FSP1 activation[17]. Instead, E-cadherin^{LO} cells are found in CTCs and can form E-cadherin^{+} epithelial metastases upon intravenous injection, supporting the notion that EMT cells contribute to metastasis. The E-cadherin^{LO} cells may represent the hybrid EMT state, whereas the *Fsp1*-expressing cells may represent the fully mesenchymal state. These studies emphasize the importance of choosing the appropriate marker to lineage trace specific EMT states.

Through a combination of two orthogonal recombination systems, Li et al.[21] developed an EMT lineage tracing system in the MMTV-PyMT mouse model, which allows the monitoring of cells even after transient gene expression. By tracing cells expressing the EMT marker genes, vimentin and N-cadherin, the authors demonstrated that although both vimentin-expressing and N-cadherin-expressing cells contribute to primary tumour growth, only N-cadherin-expressing cells, but not vimentin-expressing cells, mark metastasis-initiating cells. In addition, deletion of N-cadherin strongly reduced the occurrence of lung metastases, suggesting the importance of N-cadherin expression for the formation of metastases. Although both vimentin and N-cadherin are considered to be EMT markers, N-cadherin may label a more hybrid EMT state, whereas vimentin labels a late mesenchymal state, demonstrating the different metastasis-initiating capacities of hybrid and full EMT tumour cells. Similar results were also observed by Lüönd et al.[12], in which they used tenascin C (*Tnc*) expression to trace cells that underwent p-EMT, and N-cadherin (*Cdh2*) to trace cells that underwent late EMT in the MMTV-PyMT mouse model. Interestingly, *Tnc*-labelled p-EMT cells can revert to the epithelial phenotype and form colonies after long-term tracing, whereas *Cdh2*-labelled cells remain in the mesenchymal phenotype. *Tnc*-labelled p-EMT cells also invade and migrate collectively and contribute to metastasis and chemotherapy resistance. Although the reasons for the discrepancy in the results of *Cdh2* lineage tracing between the two studies described earlier remain unclear, these studies do demonstrate that hybrid EMT cells possess the highest plasticity and metastatic potential, whereas the fully mesenchymal tumour cells are less metastatic and provide a new platform for monitoring EMT states and following their fates in vivo.

With the identification of specific markers for different EMT states, we anticipate more EMT lineage tracing systems to be developed in the future. Different intermediate EMT states may exhibit diverse marker expression in different tumour systems and be regulated by different factors. Therefore, careful consideration needs to be taken when interpreting the results comparing different tumour EMT models, even with the same lineage tracing system.

Intrinsic and extrinsic regulators of EMT

EMT can be triggered by both intrinsic oncogenic mutations and extrinsic environmental cues (Fig. 3). In the following section, we will discuss different regulators of EMT.

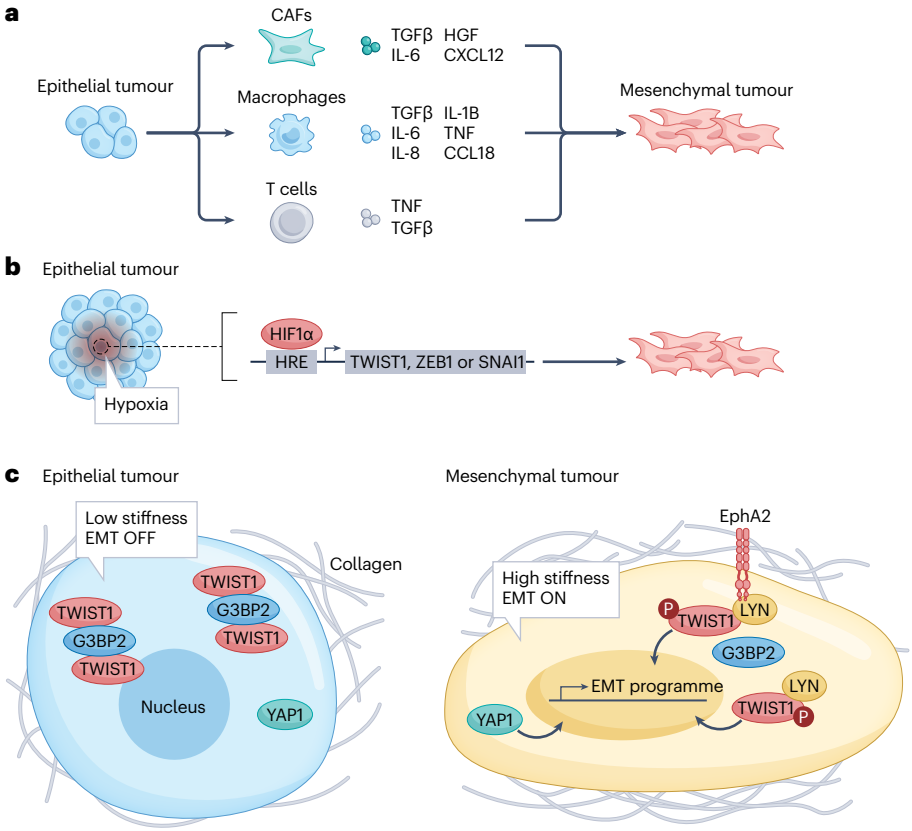


Fig. 3 | Microenvironmental regulation of EMT states. a, Tumour cells are interacting with the other stromal cell types within the tumour microenvironment. Cancer-associated fibroblasts (CAFs), macrophages and T cells secrete different factors such as transforming growth factor-β (TGFβ), tumour necrosis factor (TNF), IL-6 and IL-8 to promote tumour epithelial-to-mesenchymal transition (EMT). **b**, Hypoxia-inducible factor 1α (HIF1α) directly promotes EMT through binding to the hypoxia response element (HRE) in the proximal promoter of TWIST1, ZEB1 or SNAI1 to promote their expression. **c**, In low-stiffness environments, TWIST1 is sequestered by GAP SH3 domain-binding protein 2 (G3BP2) in the cytoplasm. Upon high extracellular matrix stiffness, TWIST1 is released from G3BP2. High stiffness also promotes ephrin type A receptor 2 (EphA2) signalling to activate LYN kinase, which further phosphorylates TWIST1 to allow its nuclear translocation for EMT activation. Yes-associated protein 1 (YAP1) is also translocated into the nucleus upon high matrix stiffness to activate the EMT programme. CCL18, CC-chemokine ligand 18; CXCL12, CXC-chemokine ligand 12; HGF, hepatocyte growth factor.

Oncogenic mutations and cellular origin of EMT

RAS oncogenes are one of the most frequently mutated oncogenes in tumours[119]. However, the activation of mutated RAS genes alone does not always give rise to invasive carcinoma[120,121]. For example, the expression of *Kras*^{G12D} in HFSCs using keratin 15 (*Krt15*) or *Krt19* inducible Cre induces benign skin tumour formation[120,121]. However, deletion of the *Trp53* tumour suppressor gene in combination with *Kras* mutation leads to invasive carcinoma, often presenting with mesenchymal features with the expression of vimentin[120,121], suggesting the necessity for multiple 'genetic hits' in mediating EMT tumours. Other tumour suppressor genes such as isocitrate dehydrogenase 1 (IDH1), IDH2 and RB1 have also been reported to contribute to EMT[122–124]. Activating mutations of RAS family members together with *TP53* deletion are found in human cancers[125], and a combination of oncogene expression with tumour suppressor gene deletion or mutation is widely used in generating cancer mouse models with tumours exhibiting an EMT phenotype[6,10,13,40,52,126–128].

Interestingly, even with the same oncogenic mutations, tumours derived from different cells of origin display different degrees of EMT[58,120,121,127]. In skin SCCs induced by *Kras* mutation and *Trp53* deletion, HFSC-derived tumours frequently undergo EMT, whereas interfollicular epidermis-derived tumours show a well-differentiated phenotype[58]. Similarly, in mouse breast tumours induced by *Pik3ca* mutation and *Trp53* deletion, luminal cell-derived tumours consist of aggressive carcinomas with EMT features, whereas basal cell-derived tumours consist mostly of luminal-like benign tumours[127]. All these reports show that different tumour cells of origin possess different potentials to undergo EMT, suggesting that the intrinsic priming of the cells of origin of cancer is important in promoting EMT.

Transcriptional and epigenetic regulation of EMT

The heterogeneity of identified EMT subpopulations has led to further investigation into the transcriptional and epigenetic landscapes of different EMT states. In HFSC-derived mouse skin SCC, the expression of epithelial markers gradually decreases from the epithelial state to the hybrid EMT state and is not expressed in the full EMT state[6]. Furthermore, EMT-TFs *Twist1*, *Twist2*, *Zeb1* and *Zeb2* are already highly expressed in the hybrid EMT state and maintain their expression until the full EMT state, whereas other EMT markers such as fibronectin 1 (*Fn1*), *Prrx1* and *Col3a1* exhibit a gradual increase from the hybrid to the full EMT state, indicating a hierarchical regulation during EMT.

The induction of EMT with TGFβ in the breast cell line MCF10A has revealed simultaneous activation of multiple signalling pathways at different time points[66]. During early time points of TGFβ treatment, the expression of *CDH1*, *EPCAM* and several keratins is downregulated, whereas the expression of *CDH2*, *FN1*, *VIM*, *ITGB1* and *ITGB5* is upregulated. Pathways related to EMT-associated stem cell features such as bone morphogenetic protein (BMP), Yes-associated protein (YAP)–transcriptional co-activator with PDZ-binding motif (TAZ), Hippo, Notch and WNT are enriched during the late EMT stage. Furthermore, profiling of EMT tumour cell lines of lung, prostate, breast and ovarian origin using multiplexed scRNA-seq at different time points following EMT induction with different ligands (TGFβ, epidermal growth factor (EGF) and tumour necrosis factor (TNF)) revealed that the EMT transcriptional response is context-dependent[129]. Although all tumour cell

lines lose expression of epithelial markers and acquire EMT features, the top variable genes are distinct. Some canonical EMT genes such as *CDH2* and *SNAI2* are not always enriched upon EMT induction. Similarly, in human tumour samples in which only the hybrid but not the full EMT state is observed, the increased expression of canonical EMT-TFs is not always detected[16,25]. All these studies suggest that EMT cells of different origins respond differently to EMT stimuli, indicating diverse transcriptional networks across different tumour types.

An EMT-priming effect on epigenetic landscapes can be observed in different cancer cells of origin[58]. In skin SCC originating from HFSCs which undergoes spontaneous EMT, the chromatin regions of some EMT genes are already accessible in untransformed HFSCs, which primes these cells to undergo EMT upon oncogenic mutations. By contrast, the chromatin of EMT genes is less accessible in the interfollicular epidermis, which does not give rise to EMT tumours upon the same oncogenic mutations and is primed to undergo squamous differentiation[58]. Different waves of chromatin remodelling are observed during the different stages of EMT. The chromatin accessibility of enhancers controlling the expression of canonical epithelial markers, such as *Epcam* and *Cdh1*, is immediately lost in the first stage of EMT, and the enhancers controlling the expression of mesenchymal genes such as *Vim* and *Zeb1* already become more accessible in the hybrid EMT state in HFSC-derived skin SCC[6]. The chromatin of regulatory regions associated with some cytokeratin genes such as *Krt14* and *Krt17* are still accessible although at a reduced level in the hybrid EMT state, corresponding with their sustained expression in the hybrid state[6]. Transcription factor motif analysis in the differentially accessible chromatin regions during EMT shows that EMT is regulated by a common core set of transcription factors composed of AP1, ETS, TEAD and RUNX transcription factors that act together with tumour state-specific TFs. For example, in addition to the common core TFs, epithelial and early hybrid EMT states show an enrichment of motifs for TP63 and GRHL2 (ref. 6). Supporting this observation, sustained expression of ΔNp63, an isoform arising from *TP63*, increases the proportion of cells in the hybrid EMT state in skin SCC[6], whereas loss of ΔNp63 leads to a shift from quasi-mesenchymal to fully mesenchymal state in breast cancer cells[36], which is accompanied by a decreased metastatic ability. This defect in metastatic colonization is associated with reduced expression of epidermal growth factor receptor (EGFR) ligands in breast tumour cells[36], suggesting that ΔNp63-mediated EGFR stimulation can promote the metastatic potential of the quasi-mesenchymal EMT state. This correlation between ΔNp63 expression and EGFR ligand levels is also observed in patients with breast cancer[36]. Through sharing the same binding motif as ΔNp63, p73 is also involved in maintaining the quasi-mesenchymal EMT state[36]. By contrast, the SMAD motif is enriched in later stages of EMT[6], suggesting a TGFβ-mediated late regulation of EMT. SMAD transcription factors have been reported to interact with the MAPK-activated RAS effector Ras-responsive element-binding protein 1 (RREB1) to induce the expression of EMT-TFs[130,131]. RREB1 preoccupies the enhancers of SMAD transcription factors and facilitates the activation of SMAD target genes[132]. A recent study identified key cofactors for RREB1, DExH-box helicase 9 (DHX9) and the chromatin-remodelling ATPase INO80, which bind with SMAD3 and SMAD4, respectively, for activating the expression of EMT-TFs and fibrogenic factors[132]. Upon TGFβ induction, SMAD4-mediated INO80 recruitment is essential for opening the chromatin at RREB1-primed enhancers, suggesting the involvement of chromatin remodelling events in executing the late EMT programme.

Combined transcriptomic and chromatin accessibility data have also revealed core-binding factor subunit β (CBFβ) as a stabilizer of the hybrid EMT state[133]. Knockout of CBFβ using CRISPR–Cas9 in human breast cancer cells leads to the downregulation of *SNAI1* and *VIM*, indicating a shift to a more epithelial state. Consistent with this, the injection of CBFβ-knockout tumour cells into immunodeficient mice resulted in tumours with proliferative features and reduced incidence of lung metastasis. Additional transcription factors responsible for maintaining the hybrid EMT state have also been identified through mathematical models. NRF2 and SNAI2 are predicted to function as maintainers of the hybrid EMT state by preventing transition from a hybrid EMT state to a fully mesenchymal state[73,74,134]. Supporting this prediction, the overexpression of NRF2 in RT4 bladder cancer cells increases both epithelial marker E-cadherin and mesenchymal marker ZEB1 expression[74]. These studies highlight the power of using bioinformatic approaches to uncover novel, EMT state-specific regulators. They also show that maintenance of the hybrid EMT state requires active signals that prevent reversion into the epithelial state, as well as signals preventing further progression into the fully mesenchymal state, indicative of a complex hierarchical regulatory network during EMT. However, combinational analysis of transcriptional and epigenetic landscapes across different EMT states at single-cell resolution will provide a better understanding of the underlying gene networks that control the different states of EMT.

Epigenetic regulators have also been identified to modulate the hybrid EMT state[81,135–139]. Expression of the forkhead box protein Q1 (FOXQ1) is increased upon TGFβ-induced EMT[140]. A recent study found that FOXQ1 promotes EMT through binding to the RBBP5 subunit of the histone methyltransferase complex KMT2 (also known as MLL), which mediates the deposition of the active mark H3K4me3 at the promoters of EMT-associated genes including the EMT-TFs TWIST1 and ZEB1 (ref. 136). Forced expression of FOXQ1 in human mammary epithelial HMLE cells induces a full EMT state, with upregulation of vimentin and N-cadherin expression and loss of E-cadherin expression. Disrupting the binding ability of FOXQ1 to the KMT2 core complex leads to a hybrid EMT state, with cells maintaining an epithelial morphology, accompanied with increased E-cadherin expression and reduced vimentin and N-cadherin expression. Consistently, knockdown of KMT2A (also known as MLL1) in MDA-MB-231 breast cancer cells impairs cell migration and invasion in vitro[136]. Interestingly, loss of another histone methyltransferase, KMT2C (also known as MLL3), in mesenchymal breast tumour cells leads to the induction of a hybrid EMT state as a result of an increased histone H3 lysine 27 acetylation (H3K27ac) signal at the enhancers of interferon γ response genes[135]. Other chromatin modifiers such as PRC2 (refs. 81,137,138) and KMT2D–COMPASS[81], as discussed earlier, have also been identified to regulate EMT in distinct manners. Additional regulatory layers such as post-transcriptional, post-translational and RNA regulators (reviewed elsewhere[141]) appear to have important roles in controlling EMT, adding further complexity to the regulatory networks of EMT.

Microenvironmental regulation of EMT

Tumour cells can communicate with the different stromal cells that comprise their niche through paracrine signalling pathways[142] (Fig. 3a). Co-culture systems or conditioned media exposure is widely used to assess the impact of distinct niche components on tumour EMT. Through these approaches, TGFβ was identified to be secreted by the TME including CAFs[143–145], macrophages[146–148], regulatory T cells[149] and myeloid-derived suppressor cells[150] to induce EMT in tumour cells. Other signals such as IL-6 (refs. 148,151–153), TNF[148] and CXC-chemokine

ligand 12 (CXCL12)[154] are also secreted by activated T cells, CAFs and macrophages to promote EMT. Some of the inflammatory signals can cooperate to regulate EMT. For example, TNF stabilizes SNAI1 in a nuclear factor-κB (NF-κB)-dependent manner, suggesting crosstalk among different inflammatory signals[155]. However, what is missing in these in vitro studies is the spatial correlation between signal-sending cells and the tumour cells. Within a tumour, different cells are exposed to multiple cytokines with different gradients of intensity, depending on the distance from the ligand sources. To mimic the distance effect, an integrated computational–experimental analysis created a multicell lattice setup that allows TGFβ to diffuse only from one end to the other to study its crosstalk with the IL-6-induced Notch–Jagged pathway during EMT[156]. This setup revealed a spatial pattern of different EMT states according to the distance from the TGFβ source. Although the Notch–Jagged pathway triggered an overall hybrid E/M phenotype, more invasive mesenchymal-like cells were observed at the front end of the TGFβ source, highlighting the importance of incorporating spatial information and concentration gradients to understand the microenvironmental regulation of different EMT states.

Equally, tumour cells can also interact with their proximal environment in positive feedback loops. For example, EMT cells can also secrete TGFβ to activate SNAI1 expression in CAFs and to facilitate tumour invasion[157]. Through the secretion of granulocyte–macrophage CSF, mesenchymal-like breast cancer cells polarize macrophages to an M2-like phenotype, which in turn leads to the secretion of CCL18 from these macrophages to promote EMT and metastasis[158]. Tumour cells also communicate with other cellular compartments through direct binding. The EMT programme upregulates the expression of CD90 in breast tumour cells, which serves as an anchor to adhere monocytes and macrophages through a juxtacrine signalling pathway[159]. Specifically, this interaction between tumour cells and monocytes and macrophages allows the ephrin-A ligand expressed by tumour cells to engage with ephrin type A receptor 4 (EPHA4) expressed by inflammatory cells and induce the production of cytokines including IL-6 and IL-8 to further activate the EMT programme. In the PDAC model, EMT cells interact with CAFs through a homophilic sodium/potassium-transporting ATPase subunit α1 (ATP1A1) interaction, which triggers calcium oscillations and induces activin A secretion by CAFs to stimulate EMT in tumour cells and a myofibroblast phenotype in CAFs to facilitate tumour invasion[160]. During dissemination in the circulation, tumour cells are in direct contact with platelets, red blood cells and immune cells[161–163]. CTCs express high levels of collagen genes to facilitate platelet binding and form cell clusters during dissemination[162]. Platelets in turn secrete TGFβ to maintain the mesenchymal identity of CTCs to promote metastasis[163].

Hypoxia is a common phenomenon in most solid tumours owing to imperfect tumour neo-angiogenesis[164]. It is reported that hypoxia-inducible factor 1α (HIF1α) overexpression represses E-cadherin expression and promotes tumour invasion[165,166]. Further studies have also shown that HIF1α directly binds to the hypoxia response element in the proximal promoters of TWIST1 (ref. 167), ZEB1 (ref. 168) and SNAI1 (ref. 169) to promote their expression and EMT induction (Fig. 3b). Hypoxia can also stabilize the SNAI1 protein through inhibiting glycogen synthase kinase-3β (GSK3β)-induced degradation[170] or SNAI1 de-ubiquitination[171,172]. Furthermore, hypoxia induces histone deacetylase 3 (HDAC3) expression, which in turn recruits WD repeat-containing protein 5 (WDR5) and the histone methyltransferase complex to deposit active histone marks on vimentin and N-cadherin to promote their expression[173]. Recently, the HIF pathway has been proposed to mediate hypoxia-induced EMT by regulating the TGFβ signalling pathway[174,175]. In addition, hypoxia induces the expression of cytokines such as CCL8 and CCL20 in EMT cells[176,177], which facilitates macrophage recruitment and the subsequent polarization of such recruited macrophages to an immunosuppressive phenotype, suggestive of a hypoxia-mediated co-evolution of tumour cells and immune populations within the TME.

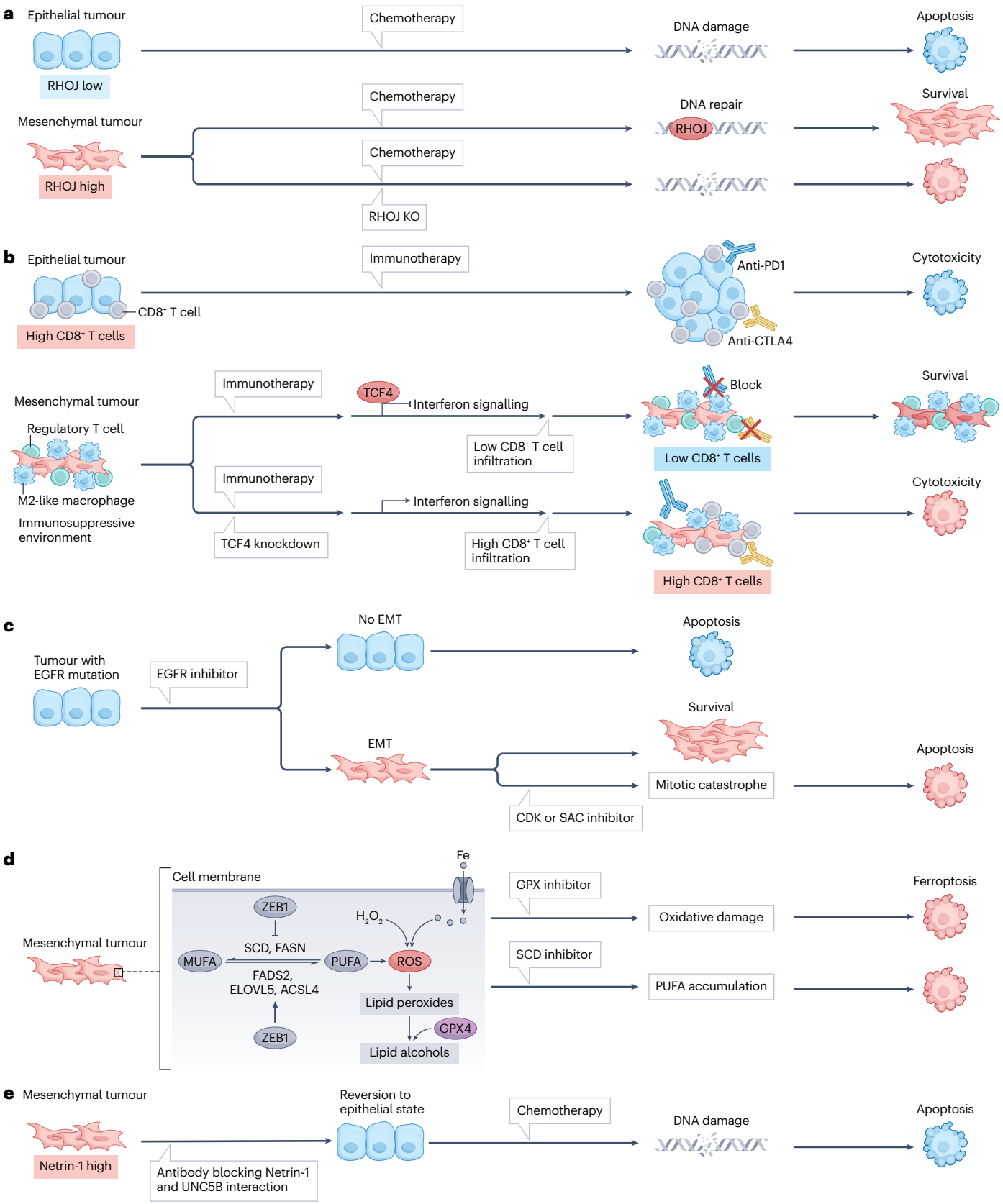
In addition to biochemical signals, mechanical properties of the surrounding stroma also regulate EMT[178]. As tumour cells proliferate, they exhibit increased pressure from their surrounding tissue architecture. The increased stiffness is associated with EMT progression[84–87]. YAP1, a key transcriptional activator in mediating mechano-responses, translocates into the nucleus upon increased stiffness[84] and induces the expression of mesenchymal genes including *VIM*, *FN1*, and *SNAI2* (ref. 179). In an in vitro 3D hydrogel culture system with different rigidities, breast tumour cells were shown to acquire a hybrid EMT feature in the high matrix stiffness environment[84]. At low stiffness, TWIST1 becomes sequestered in the cytoplasm through binding to GAP SH3 domain-binding protein 2 (G3BP2). Upon increased matrix stiffness, LYN kinase phosphorylates TWIST1, leading to its nuclear translocation to activate the EMT programme[84,86] (Fig. 3c). Implanting breast tumour PDXs embedded in collagen gels with different stiffnesses into mice also revealed increased expression of EMT-associated genes in the stiffened collagen gel, accompanied by increased tumour outgrowth and larger metastatic lesions[85]. Similarly, activating integrin mechanosignalling through the expression of a mutant human integrin-β1 (V737N) in MMTV-PyMT mice resulted in densely packed tumours with an aggressive phenotype and higher expression of mesenchymal genes including *Snail1*, *Snail2*, *Fn1* and *Vim*[87].

Targeting EMT to overcome therapy resistance

It is widely acknowledged that EMT contributes to therapy resistance[39,40,107,180–182]. The acquisition of mesenchymal features is often detected in tumour biopsy samples after anticancer treatments[5]. Moreover, decreasing *Zeb1* and *Zeb2* expression in mouse breast cancer[39] by the overexpression of microRNA miR-200 (refs. 183,184), or genetic deletion of *Snail1* or *Twist1* in mouse PDAC[40], renders tumour cells more susceptible to chemotherapy. Multiple lineage tracing systems with specific marker labelling of different EMT states have also revealed that tumour cells that undergo EMT are more resistant to therapy treatment[12,17,39]. In addition, tumours can undergo therapy-induced EMT, which leads to poor clinical outcomes in patients with cancer[185], including in prostate cancers[186], breast cancers[187,188] and melanoma[189]. Therefore, pharmacological strategies targeting EMT have been developed based on preventing the induction of EMT, reverting EMT or targeting EMT cells that have acquired resistance (Fig. 4). Most of the current clinical trials are focusing on drugs that indirectly target EMT cells, such as targeting the upstream signalling pathways[190], which does not specifically target EMT cells, and the associated clinical trials do not examine the effect on EMT itself after the treatment. Nevertheless, a few clinical trials attempting to directly target EMT cells and examine the effects on EMT have begun to emerge recently (Table 1).

Targeting chemotherapy-resistant EMT cells

Cisplatin and 5-fluorouracil are standard chemotherapy treatments used to treat patients with metastatic SCCs[191] and act by interfering with DNA replication and repair mechanisms, causing DNA damage and eventually leading to the apoptosis of cancer cells[192,193]. A recent study has demonstrated that mouse skin SCC EMT cells show profound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RHOJ low
Chemotherapy
DNA damage
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RHOJ high
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H2O2
Lipid peroxides
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Lipid alcohols
ZEB1
GPX inhibitor
Oxidative damage
Ferroptosis
SCD inhibitor
PUFA accumulation
e
Mesenchymal tumour
Netrin-1 high
Antibody blocking Netrin-1 and UNC5B interaction
Reversion to epithelial state
Chemotherapy
DNA damage
Apoptosis

Fig. 4 | Therapeutic strategies targeting EMT states. a, Chemotherapy eliminates tumour cells through inducing DNA damage. RHOJ is highly expressed in epithelial-to-mesenchymal transition (EMT) tumour cells and helps to overcome chemotherapy-induced DNA damage by promoting DNA repair. RHOJ knockout (KO) overcomes this resistance and sensitizes mesenchymal cells to chemotherapy. **b**, Epithelial tumours have higher $CD8^+$ T cell infiltration and respond to immune checkpoint blockade such as PD1 and anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA4) therapy. Mesenchymal cells often reside in an immunosuppressive environment with M2-like macrophages and regulatory T cells to escape immunosurveillance. Mesenchymal cells express high levels of transcription factor 4 (TCF4) and inhibit interferon signalling to prevent $CD8^+$ T cell infiltration. Knockdown of TCF4 can release the inhibition of interferon signalling, allowing $CD8^+$ T cell infiltration and sensitizing mesenchymal cells to immune checkpoint blockade. **c**, Tumour cells acquiring resistance after epidermal growth factor receptor (EGFR) inhibitor treatment sometimes gain mesenchymal features through activating the EMT programme. EGFR inhibitor-resistant mesenchymal cells have a high expression of genes encoding spindle assembly checkpoint (SAC) factors to avoid mitotic catastrophe. Targeting the resistant mesenchymal cells with cyclin-dependent kinase (CDK) or SAC inhibitors can improve EGFR inhibitor treatment efficiency. **d**, EMT tumour cells are very sensitive to ferroptosis inducers owing to the high level of glutathione peroxidase 4 (GPX4) expression that promotes iron-induced lipid peroxidation. EMT tumour cells modulate the ratio of polyunsaturated fatty acids (PUFAs) to monounsaturated fatty acids (MUFAs) in a ZEB1-dependent manner to generate more pro-ferroptotic PUFAs. Pharmacological inhibition of the lipogenic enzyme stearoyl-CoA desaturase (SCD) favours the transition of MUFAs to PUFAs, increasing ferroptosis sensitivity in EMT tumour cells. **e**, Netrin-1 is highly expressed in mesenchymal tumours. A monoclonal antibody blocking the netrin-1–UNC5B interaction promotes EMT tumour cell transition towards a more epithelial state and therefore sensitizes tumour cells to chemotherapy. ACSL4, long-chain acyl-CoA synthetase 4; ELOVL5, elongation of very long-chain fatty acid 5; FADS2, fatty acid desaturase 2; FASN, fatty acid synthase; Fe, iron; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species.

resistance to a wide range of genotoxic drugs and identified the small GTPase, RHOJ, as being strongly upregulated in these cells, contributing to EMT-associated chemotherapy resistance through the inhibition of apoptosis[107]. Specifically, in the context of p53-deficient skin SCC, RHOJ overcomes chemotherapy-induced DNA damage and replicative stress by promoting nuclear actin polymerization[107], which was previously shown to be important for DNA repair[194–196]. Following chemotherapy, RHOJ promotes the recruitment of dormant origin of DNA replication in EMT tumour cells[107], allowing tumour cells to repair DNA damage by homologous recombination faster and therefore decreasing chemotherapy-induced cell death[192,193] (Fig. 4a). RHOJ has also been reported to modulate the response to chemotherapy in melanoma through regulating p53-mediated cell death[197].

Recently, another chemotherapeutic agent, eribulin, which functions through inhibiting microtubule dynamics, has been reported to induce MET in patients with TNBC[198]. Unlike other microtubule inhibitors, eribulin reduces ZEB1 binding to its target enhancers that regulate the EMT programme and thereby sensitizes tumours to other chemotherapies. Interestingly, hybrid EMT cells are more sensitive to eribulin-induced MET than full EMT cells[198], possibly due to their higher plasticity, suggesting that hybrid cells may be a better target for strategies aiming to revert EMT. Finally, combining chemotherapy with simvastatin, a statin that can downregulate vimentin to inhibit EMT[199], is currently in a phase II clinical trial in patients with TNBC (NCT05550415)[200].

Targeting immunotherapy-resistant EMT cells

Cancer immunotherapy can enhance the cytotoxic potential of T cells to enable them to eliminate tumour cells[201,202]. Immune checkpoint blockade (ICB) with antibodies targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA4), PD1 and PDL1 has revolutionized cancer immunotherapy. However, some patients acquire resistance to immunotherapy[203,204]. Several reports now provide evidence that this acquired resistance can be associated with the induction of EMT in tumour cells. EMT cells establish an immunosuppressive environment with M2-like macrophages and regulatory T cells in NSCLC[205,206], breast cancer[207,208] and melanoma[209,210] to escape from immunosurveillance. Consequently, the knockout of *Zeb1* in melanoma cells favours $CD8^+$ T cell infiltration and improves response to ICB therapy in mice[210]. Interestingly, inducing EMT through *Zeb1* or *Snail* overexpression in PDAC cells did not change the immunosuppressive environment after orthotopic transplantation into mice but repressed the expression of interferon regulatory factor 6 (*Irf6*) to block T cell-derived TNF-mediated apoptosis[211], suggesting the role of EMT in immunotherapy resistance is context-dependent. A recent study has revealed that the expression of immune checkpoint ligands shifts between epithelial and mesenchymal mouse and human cSCC tumour cells, with epithelial cells expressing more PDL1 and CD112, whereas hybrid and full EMT cells express more CD80 and CD155, respective ligands for CTLA4 and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT)[212]. Furthermore, resistance to anti-PD1 or anti-PDL1 therapies in patients with cSCC, HNSCC and melanoma is associated with a higher frequency of hybrid and mesenchymal EMT cells[212], suggesting that the assessment of EMT could help to identify patients likely to respond to ICB therapy and that ICB combination approaches targeting both epithelial and mesenchymal populations may need to be considered when treating patients with tumours with hybrid or full EMT states. Longitudinal single-cell profiling of metastatic melanoma before and shortly after ICB therapy revealed an enrichment for a mesenchymal-like state in non-responding patient samples[213]. Moreover, an EMT inducer, transcription factor 4 (TCF4), was further identified to be specifically expressed in the non-responding mesenchymal-like cells (Fig. 4b). Inhibiting the bromodomain and extra-terminal domain (BET) protein bromodomain-containing protein 4 (BRD4), which is required for TCF4 transcription, with the BET-degrader ARV-771 decreases TCF4 expression and can therefore restore tumour cell sensitivity to MAPK inhibitors[214], suggesting BET inhibition may help to restore tumour cell sensitivity to ICB therapy.

Targeting EGFR tyrosine kinase inhibitor-resistant EMT cells

In patients with NSCLC, which often carries EGFR activating mutations, treatment using targeted EGFR tyrosine kinase inhibitors (TKIs) can lead to the emergence of EMT-mediated therapeutic resistance[54,215,216]. Transcriptomic examination of EGFR TKI-resistant cell lines uncovered repression of the epithelial marker *CDH1* and increased expression of the mesenchymal markers *VIM* and *AXL*, as well as the EMT-TFs *ZEB1* and *ZEB2* (ref. 180). Interestingly, these EGFR TKI-resistant cells showed resistance to a diverse range of serine/threonine kinase inhibitors and non-EGFR TKIs, while remaining sensitive to drugs targeting cyclin-dependent kinases (CDKs) and the spindle assembly checkpoint (SAC) (Fig. 4c). Upon EGFR TKI-triggered EMT, YAP1 exhibits nuclear

translocation and *trans*-activates FOXM1 expression, which further promotes the expression of genes encoding SAC factors to avoid mitotic catastrophe, and therefore conferring vulnerability to SAC inhibitors. Furthermore, a screen of targetable cell surface markers on EGFR TKI-resistant cells uncovered that CD70 is highly expressed in these cells and induced by EMT[217]. Consequently, targeting CD70 through an antibody–drug conjugate and chimeric antigen receptor T cells and NK cells demonstrated antitumour activity against EGFR TKI-resistant tumours in mice[217]. Interestingly, combining the EGFR TKI lapatinib with an HDAC inhibitor vorinostat has been reported to suppress EMT[218,219], and is currently in a phase II clinical trial in patients with human epidermal growth factor receptor 2 $(HER2)^+$ metastatic breast cancer (NCT01118975)[220]. *FAT1*, encoding a protein belonging to the cadherin superfamily, is one of the most frequently mutated genes in human cancers[221–225]. Knockout of *Fat1* in mouse skin SCC promotes tumour malignancy and metastasis through promoting a hybrid EMT state and shows resistance to the commonly used treatment of EGFR and MEK inhibitors[8]. It has been reported that SRC family kinases show increased activity in resistant cells following treatment with the EGFR inhibitor cetuximab[226]. Similarly, a phosphoproteomic analysis revealed the increased phosphorylation of tyrosine kinases, YES1 and SRC, in *FAT1*-deficient human SCC tumours, suggesting that targeting SRC might be able to overcome EGFR inhibitor resistance in FAT1-mutated tumours[8]. In line with this, *FAT1*-deficient hybrid EMT cells are more sensitive to inhibition of SRC or its upstream phosphatase Ca^{2+}/calmodulin-dependent protein kinase II (CAMK2), providing a potential new approach to target hybrid EMT tumours with *FAT1* mutations.

Direct targeting of EMT cells

Pharmacological strategies directly targeting EMT cells before treatment have been developed to overcome therapy resistance. Netrin-1, a secreted glycoprotein, and its receptor UNC5B are highly expressed in mouse skin SCC EMT cells[68] and human endometrial adenocarcinomas[227]. Netrin-1 is also highly expressed in a wide range of cancer types[228,229] and blocking the netrin-1–UNC5B interaction using genetic manipulation or NP137, a humanized anti-netrin-1 monoclonal blocking antibody, decreases tumour growth in multiple mouse tumour models[68,227,229,230]. Targeting netrin-1 with NP137 inhibits primary tumour formation, prevents the appearance of the full EMT state and metastasis as well as sensitizes tumour cells to chemotherapy in mouse models of skin SCC and endometrial cancer[68,227] (Fig. 4e). NP137 administration also decreases EMT in a mouse model of endometrial adenocarcinoma[227], and xenografts using an endometrial adenocarcinoma cell line[68] and an NSCLC cell line transplanted following TGFβ treatment[68]. Furthermore, a phase I clinical trial (NCT02977195)[231] shows that NP137 treatment decreases EMT in patients with endometrial cancers, demonstrating that EMT can be pharmacologically targeted in human cancers[227].

Targeting the secreted form of clusterin, an EMT inducer[232], with a humanized monoclonal antibody, AB-16B5, has completed a phase I clinical trial in patients with advanced solid tumours (NCT02412462)[233], which monitors EMT marker expression in both patient CTCs and paired tumour biopsies after AB-16B5 administration. Biomarker analysis in paired tumour biopsies showed evidence of EMT inhibition with increased E-cadherin expression after treatment in some patients[234]. A phase II clinical trial combining AB-16B5 with chemotherapy is now currently ongoing for patients with colorectal cancer metastasis (NCT06225843)[235].

A selective drug targeting screen using epithelial and mesenchymal lung cancer cell lines revealed that AKT is responsible for epithelial cell survival, whereas AXL is responsible for mesenchymal cell survival[236]. A subsequent combined drug treatment targeting both MEK and AXL in mice after subcutaneous tumour transplantation showed a notable reduction in tumour growth and metastasis compared with a single targeting strategy, indicating that the hybrid EMT cells largely contribute to the resistance to single drug treatments. Similarly, combined inhibition of AXL and Janus kinase 1 (JAK1) in human lung cancer cell lines also showed an increased efficiency in tumour cell elimination in vitro[237], further highlighting how the targeting of tumour heterogeneity with combined treatment may be a promising therapeutic approach to targeting EMT.

Ferroptosis is a non-apoptotic cell death pathway that is related to iron-dependent ROS production and phospholipid peroxidation[238–240]. A correlation analysis linking cancer cell sensitivity to drugs and the expression of resistance markers has revealed that mesenchymal tumour cells have a high sensitivity to ferroptosis-inducing drugs[241]. Specifically, the compounds that were able to target the mesenchymal state function through inhibiting glutathione peroxidase 4 (GPX4) activity, a key regulator of ferroptosis, and this dependency on GPX4 are more pronounced in cancer cells adopting a therapy-resistant EMT state. Interestingly, in EMT states induced by forced expression of EMT-TFs, only ZEB1 expression, but not SNAI1 or TWIST1 expression, is highly correlated with sensitivity to GPX inhibition[241], suggesting ZEB1 is required for the dependency of the mesenchymal state on GPX4. A further mechanistic study has revealed that ZEB1 promotes the expression of lipogenic enzymes (such as long-chain acyl-CoA synthetase 4 (ACSL4), fatty acid desaturase 2 (FADS2) and elongation of very long-chain fatty acid 5 (ELOVL5)) for the production of pro-ferroptotic polyunsaturated fatty acids and represses the expression of lipogenic enzymes (such as stearoyl-CoA desaturase (SCD) and fatty acid synthase) involved in the production of monounsaturated fatty acids, which are resistant to phospholipid peroxidation[242]. As a result, pharmacological inhibition of SCD increases ferroptosis sensitivity in both therapy-resistant and TGFβ-induced human lung carcinoma mesenchymal models, providing a new avenue for targeting EMT cells (Fig. 4d).

Table 1 | Clinical trials targeting EMT

Target	Drug	Phase	Tumour type	Clinical Trial identifier
sCLU	AB-16B5[a]	I	Solid tumour	NCT02412462 (ref. 233)
		II	Colorectal cancer	NCT06225843 (ref. 235)
HMG-CoA reductase	Simvastatin[b]	II	Breast cancer	NCT05550415 (ref. 200)
HDAC	Vorinostat[c]	II	Breast cancer	NCT01118975 (ref. 220)
Netrin-1	NP137[d]	I	Solid tumours	NCT02977195 (ref. 231)

EMT, epithelial-to-mesenchymal transition; HDAC, histone deacetylase; HMG-CoA, 3-hydroxy-3-methyl glutaryl-coenzyme A; sCLU, secreted form of clusterin. [a]AB-16B5 is a humanized monoclonal antibody targeting sCLU, a potent EMT inducer in tumour cells[232]. [b]Simvastatin is a statin targeting HMG-CoA reductase, which can downregulate vimentin to inhibit EMT[199]. [c]Vorinostat is a HDAC inhibitor and has been reported to suppress EMT in combination with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) lapatinib[218,219]. [d]NP137 is a humanized monoclonal antibody targeting a secreted glycoprotein Netrin-1, which, in association with its receptor UNC5B, is highly expressed in mesenchymal tumours[68,227].

Targeting cellular communication in EMT-mediated therapy resistance

As EMT cells reside in distinct niches and different niche components contribute to EMT differently, targeting the interactions between

Glossary

Cell plasticity
The ability of a cell to switch to a different cell fate in response to intrinsic or extrinsic factors.

Juxtacrine signalling pathway
A type of cell–cell communication that relies on direct contact between neighbouring cells.

Metastasis-initiating cells
A subset of tumour cells within a primary tumour that possess increased capacity to give rise to metastasis.

Myeloid-derived suppressor cells
A heterogeneous population of immature myeloid cells that can suppress immune responses.

Replicative stress
When DNA replication is slowed or stalled, this can result in genomic instability, which is a hallmark of cancer.

Spindle assembly checkpoint
A cell cycle checkpoint during metaphase of mitosis that delays the cell cycle to guarantee accurate chromosome segregation ensuring genome stability.

Tumour dormancy
A tumour state in which tumour cells undergo growth arrest for an extended period and can be reawaken upon external stimuli to re-enter the cell cycle and mediate tumour relapse.

EMT cells and their niche has emerged as a potential strategy to overcome EMT-mediated therapy resistance. For example, IL-6 secreted by the tumour stroma induces EMT in tumour cells[148,151–153], and IL-6 can be therapeutically inhibited with IL-6R blockade, which has been shown to enhance the efficiency of chemotherapy treatment in mouse PDAC tumours[243]. However, the detailed mechanisms of how modulation of tumour–stroma interactions would promote therapy sensitivity in the context of EMT remain largely to be explored.

Conclusions and perspectives

Our understanding of EMT and its different states has accelerated considerably in the past decade, with the identification and functional characterization of distinct intermediate EMT states. However, outstanding questions including how EMT is initiated in tumour cells and how the transition across different intermediate states is controlled by intrinsic and extrinsic mechanisms will provide further mechanistic insights that will be important for developing better therapies targeting EMT. Uncovering new and more potent pharmacological approaches to target EMT and sensitize tumour cells to anticancer therapies is warranted. To date, studies investigating the EMT intermediate states have been mostly performed using mouse tumour models. Yet, understanding how EMT occurs in human cancers and how it differs from that observed in mouse models will provide further insights for translating this knowledge into tangible therapeutic applications.

The tumour is an evolving ecosystem in which interactions between different cellular compartments of the TME take place in a spatially and dynamically controlled manner to facilitate tumour survival and invasion. The exact nature of the dialogue between these different tumour states, and the variety of stromal cells that compose the TME and how these cell–cell communications promote EMT, initiate metastasis and contribute to therapy resistance is only just beginning to be solved. Yet, targeting the cellular interactions within the TME also represents a potential therapeutic avenue to overcome EMT-mediated therapy resistance.

Considering the different functional characteristics of hybrid and full EMT states, therapeutic strategies should be developed to specifically target the different EMT states. For example, the hybrid state can be reverted to an epithelial state more easily than the full EMT state, whereas the full EMT state should probably be directly targeted as it is less plastic than the hybrid state and thus might be more difficult to revert to an epithelial state. In addition, any given tumour may contain multiple EMT states, and so this intratumoural heterogeneity also needs to be considered and suggests that combined therapeutic approaches might be necessary to target the different states to improve outcomes for patients with cancer.

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