Clauzon *et al. Molecular Cancer* (2026) 25:181
https://doi.org/10.1186/s12943-026-02690-x

Molecular Cancer

REVIEW

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Hallmarks of epithelial-mesenchymal plasticity in cancer

Laurie Clauzon[1†], Francesco Verona[2†], Kimiya Shams[1†], Ornella Roberta Brancato[1], Sebastiano Di Bella[1], Noemi Di Miceli[1], Giorgio Stassi[1,3] and Simone Di Franco[1*]

Abstract

Cancer stem cells (CSCs) drive tumour initiation, progression, metastasis, and therapy resistance through their remarkable plasticity, enabling dynamic transitions between stem-like and differentiated states. A pivotal mechanism underlying this plasticity is epithelial-mesenchymal plasticity (EMP), which encompasses epithelial-mesenchymal transition (EMT), partial or hybrid EMT (E/M) states, and mesenchymal-epithelial transition (MET), allowing cancer cells to acquire invasive, stem-like properties while maintaining proliferative potential. Unlike the traditional binary view of EMT, recent evidence reveals a spectrum of intermediate E/M phenotypes that exhibit increased tumorigenicity, metastatic potential, and therapy resistance. This plasticity is orchestrated by intricate regulatory networks involving EMT-inducing transcription factors, signalling pathways, and non-coding RNAs. The tumour microenvironment (TME), with its cellular and non-cellular components, provides critical extrinsic cues that stabilize E/M states. Notably, metabolic reprogramming cooperates with EMP. Indeed, E/M flexibly shifts between glycolysis, oxidative phosphorylation, and lipid metabolism alterations to fuel invasion, buffer oxidative stress, and evade ferroptosis. Advanced and recently developed in vitro and in vivo models have illuminated these dynamics: dual-fluorescent reporters, microfluidic tumour-on-a-chip, genetically engineered mouse models, bioluminescence imaging, and intravital microscopy enable real-time tracking of EMP during progression and therapy response. On the other side, in silico tools, single-cell/spatial transcriptomics, network inference, machine learning, and agent-based modelling, map hybrid states, predict trajectories, and help identify biomarkers, revealing EMP's role in evolutionary fitness. Therapeutically, targeting EMP holds promise to target resistant cancer cells and prevent relapse, though challenges arise from redundancy and plasticity. Strategies include pathway inhibitors, metabolic disruptors, epigenetic agents, TME modulators, and differentiation inducers. Combination therapies, guided by EMP biomarkers and rational models, act in combination with standard treatments to lock cells in epithelial states, disrupt hybrid phenotypes, and overcome resistance. This review highlights EMP as the main driver of tumour evolution, offering a unified framework for understanding tumour heterogeneity and heterogeneity-driven failures in therapy. By elucidating molecular mechanisms and vulnerabilities, it paves the way for precision interventions that could transform outcomes in aggressive malignancies, ultimately restraining metastasis and recurrence.

†Laurie Clauzon, Francesco Verona and Kimiya Shams contributed equally to this work.

*Correspondence:
Simone Di Franco
simone.difranco@unipa.it

[1]Department of Precision Medicine in Medical, Surgical and Critical Care, University of Palermo, Palermo 90127, Italy
[2]Department of Health Promotion Sciences, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo 90127, Italy
[3]IRCCS SYNLAB SDN, Naples 80143, Italy

Introduction

In this review, we deepen the EMP from its molecular foundations to its therapeutic implications. We first explore the intimate connection between CSCs and EMP, highlighting how dynamic transitions between stem-like and differentiated states, spanning classical EMT, its reverse MET, and intermediate E/M states, drive tumorigenicity, stemness, and therapy resistance. We then examine EMP's role in the metastatic cascade, with particular focus on the E/M phenotype as the configuration most competent for systemic dissemination. Metabolic reprogramming is discussed as an integral dimension of EMP, covering dynamic shifts between glycolysis, oxidative phosphorylation, and lipid metabolism that fuel invasion and immune evasion. The TME is subsequently addressed as a critical extrinsic regulator of E/M states, through paracrine signals, biomechanical forces, and metabolic cues delivered by cancer-associated fibroblasts (CAFs), immune, endothelial, and neural cells. Experimental approaches, including dual-fluorescent reporters, organoids, genetically engineered mouse models, and spatial transcriptomics, are then surveyed alongside in silico methodologies. Finally, therapeutic strategies targeting EMP through signalling inhibition, epigenetic modulation, metabolic disruption, and TME reprogramming are critically discussed, emphasizing combination approaches and emerging targets for true EMP modulation.

CSCs and EMP

CSCs, which were first identified in 1997 [1], constitute a minority but pivotal subpopulation within tumours that possess the unique ability to self-renew, differentiate into heterogeneous cancer cell types, and initiate tumour growth. Their discovery revolutionized the understanding of tumour biology by providing a hierarchical model, akin to normal tissue stem cells, in which CSCs drive tumour initiation, maintenance, progression, and metastasis [2]. CSCs often possess a dysregulated metabolic profile [3], and are endowed with resistance to conventional therapies such as chemotherapy and radiation [4], which preferentially kill the bulk of differentiated tumour cells but fail to eradicate the CSC fraction. This therapy resistance contributes to tumour relapse and poor patient prognosis. Plasticity is a hallmark of CSCs, referring to their ability to transition between stem-like and non-stem states, as well as among various intermediate states. This dynamic/plastic state enables tumours to adapt rapidly to changing TME conditions and therapeutic interventions, resulting in increased heterogeneity and resilience. The plastic nature of CSCs is influenced by intrinsic factors such as transcriptional regulators (OCT4, NANOG, SOX2, KLF4), epigenetic modifications, and non-coding RNAs, as well as extrinsic signals from the TME [5]. Indeed, TME plays a crucial role in driving the expansion or selection of specific CSC subsets during the different phases of tumour progression. The TME components, including CAFs, immune cells, extracellular matrix (ECM), hypoxia, and secreted cytokines, play essential roles in maintaining CSC phenotypes and plasticity by providing niche signals that modulate cellular states [6, 7]. For instance, hypoxia-inducible factors drive the expression of stemness and EMT-related genes in hypoxic tumour regions, promoting CSC maintenance and metastasis [8, 9]. The plasticity model merges the traditional hierarchical CSC model with the clonal evolution model by recognizing bidirectional transitions and cellular state interconversions as key drivers of intratumoral heterogeneity [2]. One major molecular program involved in CSC plasticity is the EMT and its reverse, MET. The EMT is a fundamental biological process essential for embryogenesis [10], wound healing, and tissue remodelling, and its partial and reversible activation in carcinoma cells has been linked to cancer progression and metastatic dissemination [11]. EMT is a dynamic and reversible biological process where epithelial cells can acquire mesenchymal features, thus adopting new characteristics regarding motility, immune evasion, and resistance to therapeutic agents [12]. Epithelial cells, from which EMT originates, are characterized by stable cell–cell junctions, apical–basal polarity, and interactions with the underlying basement membrane. In the context of EMT regulation, several key signalling pathways are activated at the plasma membrane and are influenced by lipid metabolism. Mutations in oncogenic pathways such as AMPK, PI3K, LKB1, RAS, WNT, TGF-B, P53, and mTOR continuously alter cellular metabolism and drive the induction of EMT [13, 14]. Extensive research over the past decade has shifted this paradigm, revealing that EMT is better conceptualized as a continuum of states, rather than a strict epithelial versus mesenchymal binary status. To challenge this binary vision, since 2020, the term EMP has been preferred to encompass the diversity of the cellular states that can arise [15–17]. EMT endows epithelial tumour cells with mesenchymal properties, including motility and invasiveness, which are indispensable for metastasis. Importantly, EMT is tightly linked with the acquisition of stem-like traits, enabling disseminated tumour cells to initiate secondary tumours at distant sites [18]. However, full EMT is not always necessary or sufficient for stemness; rather, cells in E/M states often display the highest plasticity, tumorigenic potential, and resistance to therapies [19–21]. A recent study highlighted how cells can gain/retain mesenchymal properties, but not stemness, a mechanism regulated by the cell division process, particularly by epithelial splicing regulatory protein 1 (ESRP1). The overexpression of ESRP1 prevents the acquisition of stem-like traits, without

impairing the mesenchymal phenotype. In this study, den Hollander and colleagues have shown that all cancer cells endowed with stemness exhibit mesenchymal traits, but not all mesenchymal cells are characterized by stemness [22]. Technological advances, such as single-cell RNA sequencing (scRNA-seq), have recently demonstrated that EMT does not operate as a binary process but rather spans a spectrum of intermediate or hybrid states, each characterized by distinct properties that fuel tumour heterogeneity, metastatic potential, and treatment resistance. Within this spectrum, E/M states coexist with fully epithelial and fully mesenchymal cells and display mixed marker profiles, endowing cancer cells with plasticity and diverse functional behaviours during tumour evolution and in response to therapy [23]. The relationship between CSC's identity and EMP has been fundamentally revised by evidence demonstrating that stemness is not a property of fully mesenchymal cells but rather emerges preferentially within intermediate E/M states. As elegantly framed by Lambert and Weinberg, activation of an EMT programme generates a heterogeneous spectrum of quasi-mesenchymal phenotypes. It is specifically within these intermediate states, rather than at the fully mesenchymal pole, that cells acquire the highest tumour-initiating potential, a concept encapsulated in the notion of a tuneable "stemness window" along the EMT axis [24]. This association is mechanistically underpinned by the coupled regulatory circuitry of the miR-200/ZEB and LIN28/let-7 feedback loops, which collectively permit only E/M cells to achieve the intermediate levels of OCT4 and stemness gene expression that define a functional CSC state, as mathematically predicted by Jolly and colleagues and subsequently validated in multiple cancer models [25]. In this context, Canciello and co-authors further extended this framework by demonstrating that the E/M state represents a peak in both plasticity and stemness gene expression along the EMT continuum, with fully mesenchymal cells paradoxically losing the self-renewal and colonization capacity that defines metastatic competence [19]. To note, while the convergence of evidence linking E/M states to CSC properties has been compelling, it is important to acknowledge that these two cellular categories, despite sharing a remarkably overlapping molecular and functional signature, should not be regarded as fully coincident or interchangeable populations [26, 27]. Both E/M cells and CSCs could be inherently heterogeneous entities: the E/M compartment itself encompasses a spectrum of distinct intermediate phenotypes, differing in the relative balance of epithelial and mesenchymal marker expression, in their gene regulatory network configurations, and in their degrees of phenotypic stability, while the CSC compartment is equally heterogeneous, harbouring coexisting epithelial-like and mesenchymal-like stem cell subsets that differ in their proliferative activity, quiescence, and tumour-initiating capacity [28]. Demonstrating that these two populations are fully overlapping remains technically and conceptually challenging, as the markers currently used to isolate E/M cells and CSCs are not identical, are context- and tumour-type-dependent, and may capture distinct, although partially overlapping, subsets of cells within the same tumour [29]. Furthermore, the dynamic and reversible nature of both EMP and CSC state transitions means that the degree of overlap between these populations is likely to fluctuate over time and in response to microenvironmental cues, rendering any static characterization inherently incomplete [30]. Taken together, while E/M states and the CSC phenotype are deeply interrelated and likely mutually reinforcing, caution should be exercised in equating them, and future studies employing single-cell multi-omic approaches combined with functional lineage tracing will be essential to precisely delineate the boundaries and the true extent of the overlap between these two biologically distinct yet intertwined cellular identities.

Thus, a better understanding of the molecular and microenvironmental mechanisms governing cancer cell plasticity, particularly the interplay with EMT programs, holds promise for developing targeted therapies designed to overcome intratumoral heterogeneity and improve patient outcomes.

EMP: from primary tumour to metastases

EMT is a pivotal biological process in tumour progression whereby cancer cells lose their epithelial characteristics, such as cell-cell adhesion and polarity, and acquire a mesenchymal phenotype, conferring enhanced migratory, invasive, and metastatic capabilities that drive disease advancement [31–33]. Besides its effect on tumour cells, EMT also affects TME thanks to the secretion of soluble factors, direct cell contact, release of exosomes and enzymes, as well as metabolic reprogramming [34, 35]. Between the two extremes lie E/M phenotypes, also called partial EMT states, in which cancer cells co-express epithelial and mesenchymal markers [25] (Fig. 1).

Cells in these hybrid states retain cell-cell junctions and some degree of epithelial morphology while acquiring mesenchymal traits such as motility and stress resistance. This plastic, intermediate phenotype confers several advantages during metastatic progression [36]. Notably, E/M cells demonstrate enhanced collective migration capabilities, allowing groups or clusters of tumour cells to invade and disseminate together while maintaining intercellular communication, which is critical for survival in the bloodstream and metastatic colonization [23, 31, 37]. Collective migration and cancer stemness represent two deeply intertwined facets of tumour progression, and recent research has begun to illuminate the

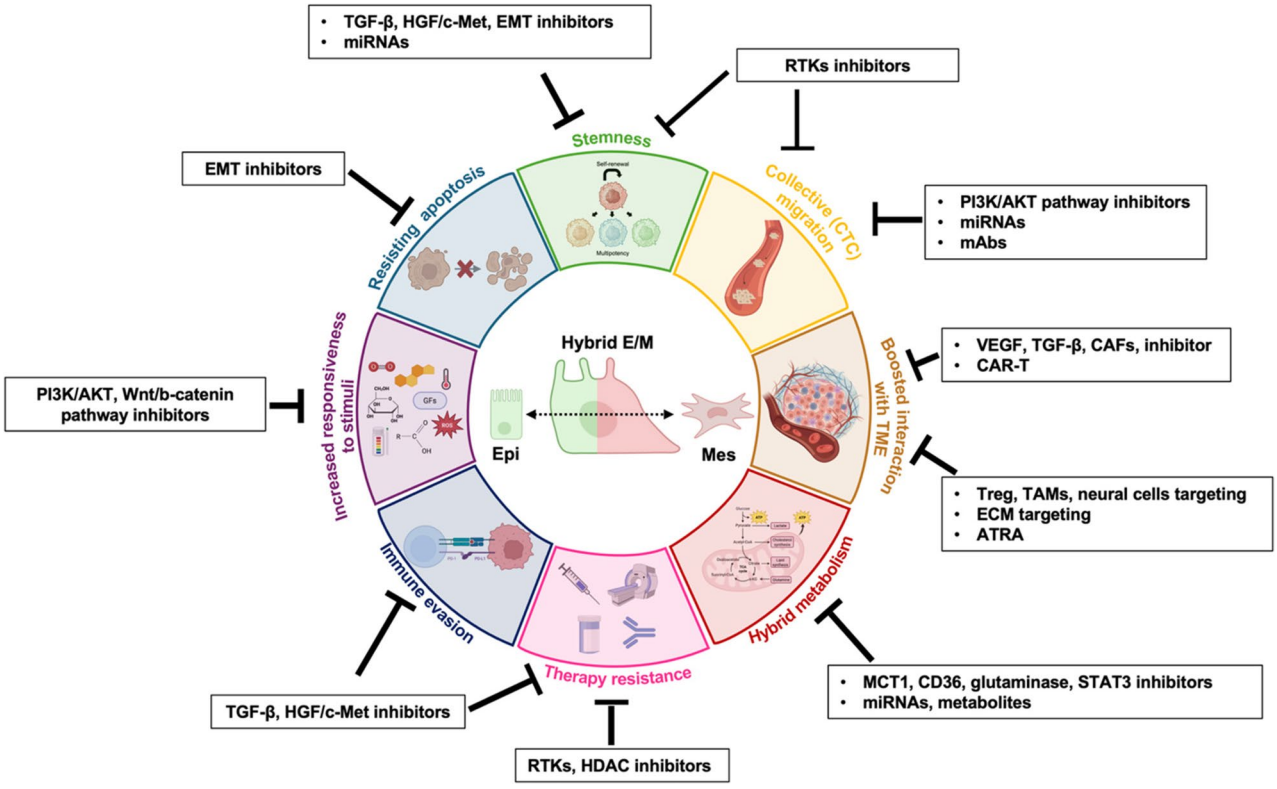

Fig. 1 Hallmarks of EMP and target therapies. This figure represents a schematic diagram of the hallmarks of cancer cells endowed with EMP (coloured spokes and rounded boxes), and the novel therapeutic strategies to target them (black coloured squared boxes)

molecular mechanisms that coordinate these processes at both the intracellular and intercellular levels [38]. Different studies shed light on distinct but converging regulatory axes that govern cancer cell plasticity, self-renewal, and invasive behaviour. Vilchez Mercedes and colleagues demonstrated that NRF2 acts as a phenotypic stability factor for E/M states during collective cancer migration. Using experimental and computational approaches, they showed that NRF2 prevents complete EMT and stabilizes an intermediate E/M phenotype immediately behind the leading edge, where it coordinates NOTCH-DLL4-JAG1 signalling to govern leader-follower cell asymmetry [39]. In this context, Lüönd et al. employed sophisticated dual recombinase lineage tracing in a breast cancer mouse model to demonstrate that E/M cells, marked by TENASCIN C expression, dynamically cycle between hybrid and epithelial states, acting as leader cells during collective invasion [40]. These cells were significantly enriched in lung metastases, and their selective ablation dramatically reduced metastatic burden, confirming their functional necessity. Full EMT cells, by contrast, remained stationary in perivascular niches and failed to colonize distant organs. Interestingly, both partial EMT (p-EMT) and full EMT populations contributed to chemotherapy resistance.

Indeed, these E/M states are implicated in tumour-initiating ability, immune evasion, and therapeutic resistance [41–46]. Unlike fully mesenchymal cells, which often show reduced proliferative capacity, E/M cells balance motility with growth potential, making them especially effective in establishing metastatic lesions. In different cancer models, circulating tumour cell (CTC) clusters displaying E/M markers have been shown to possess significantly higher metastatic capacity compared to solitary mesenchymal or epithelial CTCs [40, 47–49]. At the transcriptional level, E/M cells can be recognized by the concomitant expression of epithelial genes such as *CDH1*, *EPCAM*, or *CLAUDIN (CLDN)* family members together with mesenchymal genes including *VIM*, *ZEB1/2*, *SNAI1/2*, *TWIST1*, or *CDH2*-related programs, although the exact marker set is context-dependent and can vary by tumour type and assay platform. At the phenotypic level, these cells may display intermediate morphology, partial loss of cell-cell adhesion, retained epithelial organization, and acquisition of motility or invasive competence without full conversion to a mesenchymal phenotype. Functionally, E/M cells are often distinguished by a combination of collective migration, increased plasticity, stemness-associated traits, metastatic competence, and the capacity to dynamically revert toward either epithelial or mesenchymal states [50]. Several biomarkers

have been proposed to identify hybrid EMT populations, but no single marker is sufficient on its own. Among transcript-based approaches, the VIM: CDH1 axis, and specifically the VIM: CDH1/CLDN7-associated framework, has been used to assign cells to epithelial, hybrid, or mesenchymal categories [51], while scRNA-seq can better resolve mixed marker expression within individual tumour cells. Protein-level markers that support a hybrid state include co-expression of epithelial markers such as EPCAM, E-CADHERIN, and cytokeratins with mesenchymal markers such as Vimentin, N-CADHERIN, and EMT-associated transcription factors, especially when these signals are detected in the same cell rather than in different cells within the sample. Additional support can come from functional readouts, including enhanced motility, invasion, spheroid/cluster dissemination, and stem-like behaviour, which are frequently associated with hybrid states. Importantly, an E/M state should be distinguished from a mixed population of separate epithelial and mesenchymal cells. In a true E/M state, both epithelial and mesenchymal markers are co-expressed in the same cell, whereas in a mixed population, the epithelial markers are confined to one subset and mesenchymal markers to another. This distinction requires single-cell or spatially resolved methods, such as scRNA-seq, multiplex immunofluorescence, imaging cytometry, or in situ hybridization, because bulk assays can incorrectly interpret admixture of distinct populations as hybridity. Recent advances have identified phenotypic stability factors, including GRHL2 and OVOL2, which modulate standard EMT regulators to maintain the E/M state [52–55]. However, it remains unclear if the hybrid EMT state constitutes a fixed cellular phenotype or merely captures a transient phase of fluctuating EMP in individual tumour cells.

To note, although EMT has long been considered a key driver of metastasis, accumulating evidence indicates that certain tumours can form distant lesions through EMT-independent routes. In these settings, cancer cells disseminate while largely maintaining epithelial features, using alternative mechanisms such as collective migration, passive shedding into circulation, or cooperation with stromal and immune components. Indeed, while EMP is widely implicated in promoting tumour dissemination, recent lineage-tracing studies in genetically engineered mouse models have challenged its necessity for metastatic colonization. In breast cancer models using mesenchymal-specific reporters (e.g., Fsp1-Cre or VIMENTIN-CreER driving a fluorescent switch from RFP to GFP upon EMT), lung macro metastases were predominantly composed of non-EMT-marked epithelial cells (RFP^+), despite EMT events (GFP^+) being detectable in 1–2% of primary tumour cells and enriched in circulating tumour cells (CTCs) [56, 57]. These findings indicate that EMP facilitates early steps such as invasion and chemoresistance but is dispensable for seeding and outgrowth of secondary tumours, as epithelial cells can directly form metastases [56, 58]. However, limitations of single-marker reporters, such as insensitivity to E/M or dynamic reversions, may underestimate EMP's role, prompting calls for multi-marker, single-cell-resolved lineage-tracing [28, 59, 60]. Alternative mechanisms, including phenotypic cooperativity between EMT and non-EMT tumour subpopulations, heterotypic clustering, or stromal support, could enable epithelial cells to exploit mesenchymal advantages without undergoing full transition. Such findings challenge the traditional view that EMT is an obligate prerequisite for metastasis and underscore the remarkable plasticity of tumour cells in adapting to different microenvironmental and mechanical constraints.

The ability of cancer cells to reversibly transit via E/M states facilitates dissemination and seeding while permitting re-establishment of epithelial traits through MET, necessary for colonization and outgrowth of macro metastases [61]. The regulation of E/M involves complex signalling networks integrating diverse inputs from the TME and intracellular signalling pathways. EMT-inducing transcription factors (EMT-TFs) such as SNAIL, SLUG, TWIST, and Zinc finger E-box-binding homeobox (ZEB) families are extensively studied regulators of this plasticity [62]. These EMT-TFs can activate or repress epithelial and mesenchymal gene programs through direct binding to promoter regions or by recruiting epigenetic modifiers that remodel chromatin accessibility. EMT-TFs such as SNAIL and TWIST not only mediate phenotypic transitions but also contribute to the emergence of a subpopulation of cancer cells with self- renewal [63]. Similarly, expression of pluripotency factors like NANOG and OCT4 reinforces mesenchymal plasticity in oesophageal adenocarcinoma [64]. Overexpression of stemness markers such as NANOG, OCT4, and EMT-key regulator such as SNAI2 has been associated with poor prognosis across multiple tumour types, including ovarian, colorectal, oesophageal, and lung [65, 66]. A study by Mani et al. demonstrated a causal link between EMT and the CSC phenotype. They observed the emergence of cells expressing CSC markers and enhanced sphere-forming ability in mammary epithelial cells after EMT induction [63]. EMP is governed by a complex interplay of signalling pathways, including TGF-β/SMAD, WNT/ β-CATENIN, NOTCH, and HEDGEHOG, all of which are implicated in maintaining stemness and promoting EMT [67]. In colorectal cancer (CRC), disheveled3 (DVL3), involved in TGF-β1-induced EMT, was also shown to promote stemness and EMT-phenotype through the WNT/β-CATENIN/c-MYC/SOX2 axis [68]. A recent study performed on pancreatic

ductal adenocarcinoma (PDAC) has shown that JAG1, one of the major cell surface ligands that activates the Notch signalling pathway, regulates the E/M state and identifies pancreatic CSCs [69]. From a preclinical perspective, the depletion of the E/M cell population led to a gradual collapse of organoids and inhibition of their tumorigenic capacity. Contrarily, the overexpression of JAG1 boosted the tumour-initiating cell self-renewal, confirming that JAG1-mediated regulation of the Notch signalling pathway controls the E/M cell phenotype in PDAC [69].

The EMP onset and CSC traits acquisition are orchestrated by multifactorial mechanisms, including epigenetic modifications and regulatory non-coding RNAs. These latter can modulate gene expression without altering the DNA sequence, thereby enabling dynamic and reversible changes in cellular phenotype [70]. A key example of epigenetic regulation is illustrated by DNA-methyltransferase 1 (DNTM1), an enzyme responsible for gene silencing through CpG island methylation. In prostate cancer cells, an increase of protein kinase C alpha (PKCα) and reduced DNA- methyltransferase 1 (DNMT1) expression by 5-azacitidine promotes the suppression of H3K9me3 and H3K27me3 on the promoter of EMT and stemness-related genes ZEB2 and KLF4, involved in somatic reprogramming. This therefore increases EMT, metastatic potential, emergence of CSC, and sphere formation in vitro [71]. PKCα is also reported to be involved in CSC-enriched populations of breast cancer [72]. Long non-coding RNAs (lncRNAs) play a pivotal role in modulating EMT and CSC programs by regulating transcriptional and post-transcriptional networks [73]. For instance, lncRNA MALAT1 and HOTAIR have been shown to promote EMT, stemness, and drug resistance in several cancer types through epigenetic remodelling and interaction with chromatin-modifying complexes [74–76]. The tumour microenvironment exerts a profound influence over EMP and CSC dynamics. In CRC, the 3D soft fibrin microenvironment may be responsible for the emergence of a hybrid phenotype resistant to ferroptosis via glutathione peroxidases/ferritin signalling. This resistant phenotype is thought to be the result of concomitant action of histone acetylation and the Wnt/β-catenin pathway [77]. It was also shown that microenvironment cues sustain expression of EMT and CSC markers and enhance mesenchymal potential of cells in a zebrafish xenograft model of human prostate cancer [78]. Similarly, the crosstalk between stromal and cancer cells can lead to both EMP and CSC phenotypes. For example, in the highly aggressive triple-negative breast cancer (TNBC), tumour-associated macrophages (TAMs) activate AKT signalling by CCL2 secretion, which increases β-catenin expression and leads to its nuclear localization, where it interacts with TCF/LEF transcription factors. This, therefore, promotes EMT and enriches the cancer cell population in $CD24^{-}/CD44^{+}$ and ALDH1 expressing cells [79]. In oesophageal adenocarcinoma, IL-6 secretion by fibroblasts favours cancer stem-like cells expansion by increasing mRNA and protein levels of CSC transcription factors like SOX2, OCT4, and NANOG, leading to EMP by NANOG [64]. Recent studies have demonstrated that cellular metabolism, mechanical stresses, and microenvironmental heterogeneity also orchestrate E/M states. For example, hypoxic niches induce HIF-1α, which cooperates with EMT-TFs to sustain partial EMT, while metabolic reprogramming towards glycolysis or oxidative phosphorylation supports cellular energy requirements during transition states [8, 80, 81]. Furthermore, extracellular matrix stiffness and cell-cell adhesion forces influence EMT progression by modulating intracellular signalling cascades and cytoskeletal reorganization. The E/M paradigm challenges traditional drug development strategies focused solely on eliminating purely epithelial or mesenchymal states. Instead, it highlights the importance of targeting intermediate phenotypes, which are arguably the most metastatic and therapy resistant. A better understanding of how E/M states arise, are maintained, and their crosstalk with CSC properties will guide novel interventions aimed at preventing dissemination, overcoming drug resistance, and curbing tumour recurrence. Increasing evidence suggests that EMP is a central driver of cancer stemness and tumour- initiating potential. In skin squamous cell carcinoma, deletion of the tumour suppressor FAT Atypical Cadherin 1 (FAT1) activates a dual signalling cascade. On one hand, phosphorylated Ca2+/calmodulin-dependent protein kinase II (CAMK2) activates the CD44-SRC pathway, promoting nuclear translocation of YAP, leading to ZEB1 expression, which induces a mesenchymal state. Simultaneously, FAT1 loss also inactivates EZH2, enabling SOX2 expression with the emergence of epithelial traits. The activation of these pathways results in a hybrid phenotype with enhanced tumour-initiating capacity and malignant progression [82]. EMP also contributes to disease progression and recurrence by enabling normal cancer cells to acquire CSC-like features. It has been demonstrated that stem-like cells arising from EMP resemble stem cells isolated from normal and neoplastic populations [63]. In TNBC, it was reported that intermediate E/M cells are enriched in CSCs [83]. The stable E/M phenotype driven by the SNAIL EMT-transcription factor and canonical Wnt signalling, identified by integrin β4 $(CD104)^{+}/CD44^{high}$ expression, a marker combination for CSCs, was also associated with enhanced tumorigenicity [84, 85]. EMP facilitates metastatic colonization through MET, which supports the implantation of CTCs in distant tissues [44]. In this scenario, recent studies demonstrated that E/M

cancer cells act as leaders in collective invasion [86]. In PDAC, E/M favours CTC cluster formation and enables collective migration of cells, in contrast to the complete EMT program, associated with single-cell dissemination [38]. EMP may also promote intravasation of CTC and dissemination by activating angiogenesis-related genes, thereby facilitating escape from the primary tumour microenvironment [87]. Distinct EMT states play different roles in cancer progression [88]. Lineage tracing in a mouse model of breast cancer revealed that hybrid cells, but not fully mesenchymal cells, are required for lung metastasis. Although both partial and complete EMT cells contribute to chemoresistance, the latter contribute to a larger extent to it [40]. Further research by Youssef et al. helps distinguish two distinct EMP programs: one associated with invasion and the generation of hybrid stem-like cells, and another linked to inflammatory signalling in fully transitioned cells [89]. The dynamic nature of E/M states provides tumours with the flexibility to adapt and survive under therapeutic pressure. Future therapeutic strategies should aim to target the regulatory networks inducing both EMP and CSC phenotypes. Since EMP and CSC programs are often co-activated, targeting shared molecular nodes, such as VIMENTIN phosphorylation, could simultaneously suppress stemness and plasticity [90].

Implications of E/M and metabolic reprogramming in cancer cells

Metabolic reprogramming in neoplastic cells has been investigated since the identification of the Warburg effect in the 1920s [91]. Cancer cells exhibiting E/M possess the ability to switch between distinct metabolic states, such as glycolysis and oxidative phosphorylation (OXPHOS), depending on microenvironmental cues, nutrient availability, and energy demand. Metabolic reprogramming associated with E/M involves complex modifications in glucose metabolism, mitochondrial function, and lipid metabolism [92] (Fig. 2).

This metabolic adaptability confers upon CSCs the capacity to adapt to endogenous and exogenous microenvironmental stimuli, primarily through their own plasticity and considerable versatility in nutrient uptake and utilization [93]. Significant focus has been directed towards the metabolic perturbations intrinsically linked to pivotal stages of cancer dissemination, including but not limited to the initiation of metastasis, systemic circulation, subsequent colonization, and the development of chemoresistance [94, 95]. Here, although molecular mechanisms are not fully understood, we reported recent findings about the existing link between the metabolic reprogramming and the co-existence of the E/M phenotype in the CSC subpopulation.

The role of glycolytic and OXPHOS metabolism in shaping the EMT plasticity spectrum

EMT drives cancer stemness through a spectrum of plastic cell states, and this phenotypic flexibility is fundamentally sustained by continuous shifts in metabolic flux, linking cellular identity transitions directly to metabolic reprogramming. Consequently, the interplay between EMT plasticity and the shifting metabolic landscape defines the adaptive potential of CSCs within the evolving TME. Interestingly, the acquisition of stemness during the EMT is driven by a sophisticated coupling of phenotypic plasticity and metabolic reprogramming [96]. While differentiated epithelial cancer cells primarily utilize OXPHOS [97, 98], the transition into an E/M CSC state necessitates a concomitant upregulation of glycolysis, resulting in a highly proliferative, bi-energetic phenotype (OXPHOS^{high}/glycolysis^{high}) [99–101]. These E/M cells may subsequently shift toward a mesenchymal-like

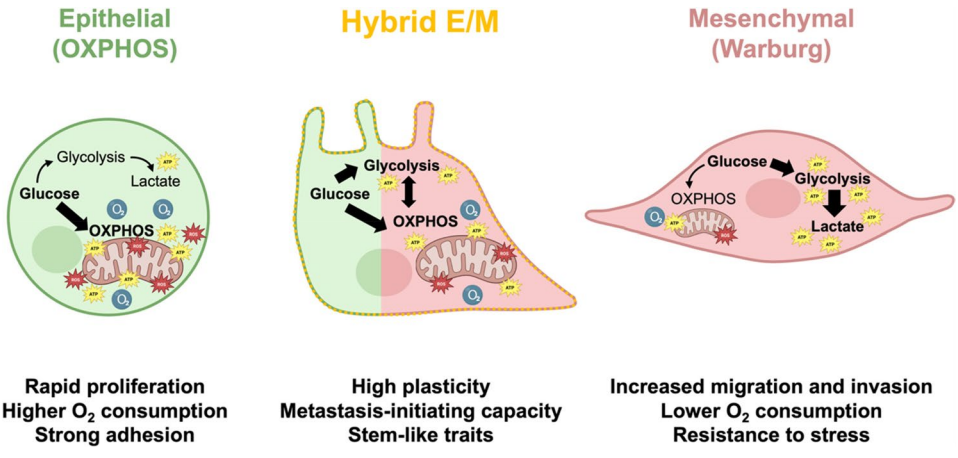

Fig. 2 Metabolic rewiring associated with EMP. This figure shows the metabolic reprogramming of cancer cells from an epithelial phenotype (green colour), which mostly relies on OXPHOS metabolism, to a mesenchymal one (red colour) that is associated with enhanced glycolysis, passing through a hybrid phenotype that maintains both characteristics (yellow colour)

state, characterized by metabolic quiescence and a reduction in OXPHOS and an increase in glycolysis (OXPHOS^{low}/glycolysis^{high}) [102, 103], or lose their stemness by downregulating glycolysis to differentiate into mesenchymal cancer cells (OXPHOS^{high}) [103]. Accordingly, Ren et al. have discovered that stem-like and E/M breast cancer cells are able to drive tumour progression and metastasis formation via a metabolic reprogramming driven by the GPX2 /HIF1A/P63 axis. In particular, within the primary tumour, the epithelial cells start acquiring an upregulation of mesenchymal genes, leading to the emergence of E/M subpopulations, supported by both OXPHOS and glycolysis. Later during tumour progression, disseminated cells in metastatic lesions regain epithelial traits, thus becoming mainly dependent on OXPHOS. This precise regulatory framework is governed by p63, which appears to orchestrate partial EMT or MET transitions in both primary and metastatic sites. Mechanistically, GPx2 knockdown initiates redox signalling by activating HIF1α, which regulates p63, leading to the onset of partial EMT and MET [104]. Similarly, a mouse breast cancer stem-like model, represented by 4T1 cells, exhibits an increased level of OXPHOS and glycolysis underlying the acquisition of a mixed metabolic and E/M phenotype (CK8^{+}/αSMA^{+}). Specifically, this metabolic signature appears to be sustained by PGC-1α, a transcription factor that drives the expression of metabolic signatures (including oxidative phosphorylation, lipid biogenesis, and mitochondrial biogenesis) and EMT-related genes, thereby driving the acquisition of an invasive phenotype [105]. In line with these findings, breast CSCs have been shown to comprise two EMT subpopulations. Firstly, the CD44^{high}/CD24^{low}, which are more-mesenchymal-like breast CSCs, and the aldehyde dehydrogenase (ALDH^{+}) E/M cells. Interestingly, this latter is characterized by a mixed metabolic phenotype showing both OXPHOS and glycolysis, which drives metastatization [103, 106]. Moreover, another ALDH family member, ALDH1A3, drives the acquisition of E/M in CD44^{+}/CD24^{-} TNBC stem cells. acc This finely regulated metabolic balance determines the acquisition of an E/M state in TN-BCSCs [107].

Finally, focusing only on glycolytic metabolism, Lactate dehydrogenase A (LDHA), which catalyses the conversion of pyruvate to lactate, the final step of anaerobic glycolysis, can promote EMT by upregulating the transcription factor ZEB2 [108]. Knockdown of LDHA has been shown to increase E-CADHERIN expression while decreasing the expression of focal adhesion kinase (FAK), matrix metalloproteinase 2 (MMP2), and vascular endothelial growth factor (VEGF), all of which are key regulators of EMT [109, 110]. Moreover, lactate produced by cancer cells contributes to EMT by lowering extracellular pH, which activates latent extracellular TGF-β [111]. Simultaneously, the efflux of lactate elevates intracellular pH, stimulating Wnt signalling and further promoting EMT [112]. NRF2, a key transcription factor of cellular metabolism, has been shown to induce and maintain an E/M phenotype. Specifically, NRF2 is positively regulated at the transcriptional level by AHR, ARNT, NF-κB, JUN, and MYC, while being negatively controlled at the post-translational level by CRLKeap1, SCFβ-TrCP, CRIF1, SIAH2, and RNF4 [113, 114]. From a metabolic perspective, emerging evidence suggests that NRF2 exerts a profound influence on cellular metabolism by enhancing fatty acid oxidation and repressing lipogenesis [115, 116]. The pivotal role of NRF2 in lipid metabolism is linked to the suppression of gluconeogenesis [117]. Taken together, these findings suggest that such a plastic and versatile phenotype cannot be confined to rigid metabolic categories. Instead, the acquisition of the EMT spectrum must be contextualized based on the specific cancer model, tumour stage, TME involvement, and driver mutations.

Lipid metabolism and EMP

In this scenario of high heterogeneity and metabolic plasticity, lipid metabolism has recently been recognized as a key regulator of the EMT in cancer cells, and it is also linked to the balance between proliferative and invasive cellular states. Lipid metabolism has been recently defined as a main regulator of the EMT state of cancer cells, being also associated with different proliferative/invasive states and with the ability to adapt in highly nutrient and growth factor-deprived tumour microenvironments. Alterations in fatty acid synthesis and oxidation pathways help meet the increased energy demands and provide essential building blocks for cell membrane remodelling and signalling molecule production. Indeed, lipid metabolism is significantly altered in various cancers, during tumour initiation and progression. E/M cells have been shown to exhibit increased lipid uptake along with enhanced accumulation of lipid droplets, which serve as reservoirs to buffer oxidative stress, to supply energy during the metastatic cascade, and to resist therapy [13, 107, 118]. Dysregulated lipid metabolism can trigger EMT and stimulate multiple oncogenic pathways linked to this transition by modulating key signalling cascades, such as EGFR, RAS, MAPK, JAK2, STAT3, PI3K, AKT, and mTOR. Within EMT phenotypes, several critical signalling pathways are initiated at the plasma membrane and are influenced by lipid metabolic processes. Mammalian cells acquire lipids through two main mechanisms: de novo lipogenesis and lipid uptake. De novo lipogenesis is an intricate metabolic process whereby fatty acids are produced from non-lipid precursors, predominantly carbohydrates, leading to the production of long-chain fatty acids stored as triglycerides [13, 119, 120]. The enzyme primarily involved in this process is

ATP citrate lyase (ACLY), which converts citrate into acetyl-CoA and plays a rate-limiting role in lipid synthesis. The produced acetyl-CoA is then carboxylated to form malonyl-CoA, marking the initial step in fatty acid biosynthesis. In addition, acetyl-CoA is a crucial substrate in the mevalonate pathway, where two molecules of acetyl-CoA combine to form acetoacetyl-CoA, ultimately leading to cholesterol production [121]. The phosphoinositide 3-kinase (PI3K)/AKT signalling pathway plays a central role in promoting cell survival across many types of cancer. In tumour cells, AKT directly regulates ACLY by inducing its phosphorylation and activation. Additionally, AKT enhances *ACLY* gene expression by activating the sterol regulatory element-binding protein 1 (SREBP-1), a transcription factor that controls genes involved in cholesterol and fatty acid biosynthesis. However, evidence suggests that the PI3K/AKT pathway primarily increases ACLY activity through post-translational modification, specifically phosphorylation, rather than through transcriptional upregulation. This phosphorylation also helps stabilize the ACLY protein. Beyond AKT, ACLY can be phosphorylated at multiple sites by other kinases, including nucleoside diphosphate kinase and cyclic AMP-dependent protein kinase [122]. ACLY serves as a central enzyme in de novo lipogenesis and acts as a metabolic link connecting lipogenesis with the Krebs cycle and gluconeogenesis [122]. Besides its specific role in regulating lipid metabolism, ACLY can act as a bridge, promoting EMT phenotypes through β-catenin signalling [92, 121]. Inhibition of ACLY suppresses tumour cell proliferation, growth, and stemness, with knockdown studies demonstrating reversal of EMT and inhibition of tumour growth via modulation of the PI3K/AKT pathway [123]. Acetyl-CoA carboxylase (ACC) catalyses the carboxylation of acetyl-CoA to malonyl-CoA, resulting in the synthesis of palmitate (16:0), a critical step in fatty acid metabolism [124, 125]. In different studies, ACC has been widely identified as a potential therapeutic target for suppressing tumour growth by modulating the metabolic alterations characteristic of cancer cells [126]. ACC is activated within the mitochondria in response to elevated citrate levels generated by citrate synthase. Two isoforms have been identified: ACC1, which is cytoplasmic, and its function is to synthesize malonyl-CoA used for *de novo* synthesis of fatty acids [127], and ACC2, which is associated with the mitochondrial membrane. ACC2 regulates fatty acid oxidation by catalysing the carboxylation of acetyl-CoA to malonyl-CoA, thereby inhibiting carnitine palmitoyl transferase I (CPT-I), the rate-limiting step in fatty acid uptake and oxidation [127]. These isoforms regulate de novo lipogenesis and fatty acid oxidation, respectively. Silencing ACC2 results in reduced β-oxidation and increased lipid accumulation, ultimately promoting the expression of genes associated with EMT, particularly an increase in TGF-β expression [127]. ACC1 regulates EMT through non-canonical pathways; in particular, TGF-β and leptin suppress ACC1 activity via AMPK-mediated phosphorylation at Ser79, thereby promoting EMT [128]. This effect is driven by the accumulation of acetyl-CoA following ACC1 inhibition, which enhances the acetylation of SMAD2, a key mediator of TGF-β-induced EMT [128]. In addition, ACC supplies fundamental substrates for de novo lipogenesis and functions in coordination with fatty acid synthase (FASN), converting acetyl-CoA into malonyl-CoA to support fatty acid chain elongation [124]. Fatty acid synthesis by FASN occurs in three main stages: (1) initiation, where acetyl-CoA and malonyl-CoA undergo condensation; (2) elongation, involving repeated cycles of reduction and dehydration that extend the fatty acid chain by two carbons each round; and (3) termination, in which palmitate is cleaved from the acyl carrier protein (ACP) [129]. In cancer cells, fatty acid synthase (FASN) expression is controlled by the transcription factor SREBP-1c [129]. Fatty acid synthase (FASN) catalyses the last step of de novo lipogenesis in different cancer types and is strongly associated with tumour progression [125, 129, 130] and is widely recognized as a driver of EMT [124, 129]. Evidence from patient-derived samples highlights a functional association between FASN, EMT, and major signalling pathways, including mTOR and EGFR. These pathways not only enhance lipogenic flux but also converge on transcriptional programs that promote EMT, including the upregulation of factors like SNAIL, TWIST, and ZEB family proteins. In this context, FASN-derived lipid products contribute to membrane remodelling and the formation of lipid rafts, thereby facilitating receptor-mediated signalling and amplifying downstream pro-EMT pathways [131]. In CSCs, FASN signalling plays a pivotal role in regulating the EMT phenotype. Its activity is tightly regulated at the post-transcriptional level, where specific microRNAs suppress FASN expression with a significant inhibition of EMT. Furthermore, TGF-β upregulates FASN expression, establishing a positive feedback loop in which enhanced lipogenesis promotes EMT [132, 133]. Stearoyl-CoA desaturase (SCD) introduces a double bond at the C9 position of long-chain saturated fatty acids, primarily converting stearoyl-CoA into oleoyl-CoA, a monounsaturated fatty acid, and is regulated by sterol regulatory element binding transcription factor 1c (SREBP1c) [134]. This modification markedly alters lipid properties and functions. Two isoforms of SCD are found in human tissues: SCD1 and SCD5. Both SCD1 and SCD5 isoforms are overexpressed and highly active in cancer cells, where they contribute to tumour progression. Increased SCD expression activates the Akt pathway, leading to phosphorylation and inhibition of

GSK3β, which stabilizes β-catenin and enhances EMT signalling [135]. Stearoyl-CoA desaturase (SCD) is frequently co-expressed with EMT markers, where it downregulates the epithelial marker E-CADHERIN while upregulating the mesenchymal marker vimentin [136]. Acyl-CoA synthetases (ACSLs) are key enzymes in fatty acid metabolism that catalyse the conversion of long-chain fatty acids to acyl-CoA, a crucial step for phospholipid and triglyceride synthesis. Several isoforms, including ACSL1, ACSL3, and ACSL4, are frequently upregulated in cancer [92]. These isoforms display substrate specificity: ACSL1 preferentially activates oleate and linoleate, ACSL3 metabolizes myristate, palmitate, arachidonate, and eicosapentaenoic acid, whereas ACSL4 primarily utilizes arachidonate [137]. Notably, co-overexpression of ACSL and SCD has been associated with enhanced EMT regulation, marked by increased expression of SNAI2 and N-CADHERIN, as well as metastatic phenotypes. This synergistic effect likely arises from their combined roles in providing metabolic energy and promoting EMT-associated gene expression, thereby facilitating cancer cell invasiveness and progression [136, 138]. Lipolysis is a metabolic pathway that, under basal conditions, catalyses triacylglycerols into three fatty acids and one glycerol molecule through the action of specific enzymes: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL). Inhibition of lipolysis enhances expression of EMT-associated genes and reduces endothelial cell differentiation capacity by suppressing E-CADHERIN transcription. In contrast, blocking glycolysis diminishes the expression of key EMT markers, including VIMENTIN, ZEB1, and SNAIL [139–141]. Furthermore, a strong association exists between EMT and fatty acid β-oxidation (FAO), largely driven by mitochondrial ROS signalling pathways, underscoring the importance of ROS in the biology of CSCs. FAO is regulated by transcriptional programs involving peroxisome proliferator-activated receptors (PPARs), particularly PPARα, which drive the expression of enzymes required for lipid catabolism. PI3K/AKT/mTOR is a key regulatory pathway for FAO, and dysregulation of this pathway in tumour cells is closely associated with abnormal FAO metabolism [142]. Carnitine palmitoyl transferase 1 (CPT1) is the key rate-limiting enzyme of FAO, responsible for catalysing the transfer of long-chain acyl groups from coenzyme A to carnitine, enabling the transport of long-chain fatty acids into the mitochondrial matrix for oxidation [124]. Increased FAO has been suggested to sustain the self-renewal capacity of CSCs by controlling lipid and membrane biosynthesis, reducing oxidative stress through NADPH generation, and enhancing resistance to chemotherapy. CPT1A-driven FAO sustains cancer cell survival during detachment by maintaining intracellular NADPH levels and redox balance, thereby promoting anoikis resistance [143]. Conversely, a metabolic shift toward lipogenesis favours the generation of monounsaturated fatty acids, which are less prone to lipid peroxidation, thereby limiting ROS-induced damage in CSCs. Excess free fatty acids can also be converted into triglycerides and stored in lipid droplets to prevent lipotoxicity, an accumulation commonly observed in CSCs. Notably, this lipogenic shift further enhances glycolytic reprogramming, as fatty acid synthesis regenerates NAD+, which is essential for sustaining glycolysis [119]. Moreover, upstream mechanisms of lipid acquisition significantly contribute to the dependency on FAO observed in CSCs. Among these mechanisms, the fatty acid translocase CD36 has emerged as a key regulator of lipid uptake in both aggressive tumours and CSC populations. CD36-mediated fatty acid import fuels mitochondrial FAO, thereby meeting the heightened energetic and biosynthetic requirements characteristic of highly plastic cancer cells. Furthermore, CD36 signalling promotes EMT through activation of TGF-β pathways, characterized by reduced E-CADHERIN and increased vimentin expression [144, 145]. This contributes to the maintenance of stemness and enhanced metastatic potential, positioning CD36 at the crossroads of metabolic reprogramming and phenotypic plasticity. Upstream mechanisms of lipid acquisition significantly contribute to the dependency on FAO observed in CSCs. Notably, enhanced lipid uptake via CD36 has been demonstrated to support CSC survival and confer resistance to various stress conditions, including anchorage-independent growth. In mesenchymal-like CSC subsets, CD36-driven FAO is particularly crucial for survival under detachment conditions. By generating ATP and NADPH, FAO mitigates oxidative stress and facilitates prolonged persistence in the circulatory system, thereby promoting resistance to anoikis and enabling successful metastatic colonization [146]. The signalling networks that orchestrate EMT involve multiple growth factor pathways, such as mitogen-activated protein kinase (MAPK), Hedgehog, epidermal growth factor (EGF), NOTCH, and TGF-β, along with related downstream effectors like extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K)/AKT, and nuclear factor kappa-B (NF-κB) [124]. These pathways are activated in response to metabolic stress, hypoxia, and inflammation, which collectively stimulate EMT-associated transcription factors, including ZEB1/2, SNAIL, and TWIST [32, 119]. In addition, EGF receptor (EGFR) contributes to the regulation of cancer cell metabolism by sustaining intracellular lipid and glucose homeostasis through mechanisms involving protein stability rather than kinase activity. When cellular energy levels are low, AMP and ADP binding activate AMPK, which stimulates FAO and promotes glucose uptake. Additionally, AMPK

activates peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), supporting oxidative metabolism and enhancing mitochondrial biogenesis [109, 147]. Notably, PGC-1α-driven metabolic reprogramming toward OXPHOS collaborates with EMT activation, further promoting cancer cell plasticity and progression [105]. Another example is represented by TGF-β, which is a potent inducer of EMT in various cancer types and is strongly associated with pro-oncogenic responses [148]. TGF-β signalling activates the TGF-β type I receptor (TGF-βRI), which phosphorylates the receptor-regulated Smad proteins, Smad2 and Smad3. Once phosphorylated, Smad2 and Smad3 form a complex with the common mediator Smad4 and translocate into the nucleus. At the nuclear level, they function as transcription factors, promoting the expression of SMAD2 and SMAD3 themselves and inducing genes associated with EMT (SNAIL and ZEB) and increasing the expression of mesenchymal markers [149]. Notably, blocking TGF-β signalling can reverse mesenchymal features and restore epithelial characteristics in cancer cells. Inhibition of FASN has been shown to disrupt EMT by downregulating mesenchymal markers, partly by interrupting the positive feedback loop between FASN and TGF-β1. Moreover, TGF-β-driven EMT is linked to increased ATP production and enhanced oxygen consumption, supporting the energy demands of transitioning cells. TGF-β1 has also been found to elevate ROS levels, which further drive cancer cell proliferation and EMT progression [150, 151].

While recent research has shed light on specific metabolic pathways implicated in this transition, the precise molecular mechanisms orchestrating these changes remain elusive. Further investigation is critically needed to unravel how alterations in metabolic pathways contribute to the plasticity and stability of the E/M state. Understanding these unresolved complexities is paramount for developing targeted therapeutic strategies. From a therapeutic perspective, targeting the metabolic vulnerabilities unique to E/M CSCs presents a promising avenue to disrupt metastasis and overcome drug resistance. However, the inherent metabolic plasticity of E/M cells poses challenges, as these cells can compensate for the inhibition of one pathway by shifting toward another metabolic route. Therefore, combination therapies and the development of biomarkers to predict and monitor metabolic states are critical for effective targeting. In conclusion, the interconnection between E/M and metabolic reprogramming endows cancer cells, especially CSCs, with phenotypic plasticity and metabolic flexibility that are essential for tumour progression, metastasis, and resistance to therapy. Investigating the molecular mechanisms underlying this coupling and developing strategies to disrupt these pathways may reveal novel and effective therapeutic modalities aimed at eradicating the most aggressive cancer cell populations.

The bidirectional crosstalk between EMT and cancer metabolism

The molecular interplay between EMT and metabolic reprogramming remains a subject of intense debate and is not yet fully understood. Despite this uncertainty, emerging evidence suggests a bidirectional relationship: EMT can drive metabolic alterations and vice versa [152].

EMT induces metabolic reprogramming

EMT involves extensive metabolic reconfiguration, driven by specific transcription factors, to meet the bioenergetic demands of enhanced motility and survival within adverse microenvironments. For example, SNAI1 expression was negatively associated with fructose-1,6-bisphosphatase 1 (FBP1), an enzyme involved in the glycolytic pathway [153]. In an NSCLC model, it was found that ZEB1 activates the expression of glucose transporter 3 (GLUT3) [154]. Similarly, overexpression of caveolin-1 (CAV1) in colorectal cancer models has been shown to enhance aerobic glycolysis by promoting HMGA1-induced GLUT3 transcription [155]. Furthermore, in NSCLC, TGF-β induces a metabolic shift from glycolysis toward OXPHOS. Specifically, TGF-β suppresses glycolytic flux while enhancing glutamate utilization by increasing carbon entry into the tricarboxylic acid (TCA) cycle. This metabolic reprogramming is mediated by the downregulation of pyruvate dehydrogenase kinase 4 (PDK4), an enzyme associated with EMT [156]. Similarly, in colorectal cancer, TGF-β drives EMT and promotes the nuclear translocation of pyruvate kinase M2 (PKM2), a key glycolytic enzyme responsible for converting phosphoenolpyruvate (PEP) to pyruvate [157]. In a glioblastoma model, TGF-β showed a crucial role in the upregulation of glucose transporter (GLUT) 1, hexokinase 2 (HK2), and lactate dehydrogenase A (LDHA) in glioblastoma [158]. It has been reported that TGF-β exhibits increased expression of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB-3) in PANC-1 cells, which is linked to increased glucose flux within the cells and, consequently, lactate production [159]. Hexokinase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and ENO, three glycolytic enzymes, were found positively enriched in both early and late EMT stages, upon TGF-β induction [160]. Analogously, ZEB1 enhances glucose uptake by upregulating the expression of glucose transporter 3 (GLUT3) [154]. Furthermore, during EMT, SNAIL plays a crucial role in the metabolic reprogramming from glucose flux between aerobic glycolysis and the pentose phosphate pathway (PPP) by repressing phosphofructokinase platelet (PFKP) [161]. Regarding lipid metabolism in prostate cancer, both

TNF-α and TGF-β have been implicated in the increasing levels of unsaturated triacylglycerols [162]. Furthermore, SNAI1 overexpression in A549 NSCLC cells inhibits the transcriptional regulator of lipogenesis, carbohydrate-responsive element-binding protein (ChREBP), thereby downregulating both fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC) [151]. Taken together, these studies demonstrate that the EMT-driven metabolic landscape is highly context-dependent, varying between the promotion of glycolysis, the enhancement of oxidative process, and the modulation of lipid metabolism. By orchestrating the expression of key enzymes and transporters, the EMT program ensures that migrating cells can adapt their bioenergetic strategies to diverse microenvironments. Understanding this complex interplay could offer critical insights into identifying new therapeutic vulnerabilities in metastatic cancer.

Metabolic reprogramming induces EMT changes

A growing body of evidence suggests that metabolic reprogramming plays a pivotal role in driving EMT-related changes. Recent findings indicate that mesenchymal cancer cells exhibit heightened metabolic requirements compared to their epithelial counterparts. This phenomenon may be attributed to the increased energy demands associated with the superior motility and invasive capacity typical of the mesenchymal phenotype. Consistently, Shaul et al. utilized public in silico datasets to demonstrate a robust correlation between the expression of 44 metabolic genes and of 1000 tumours characterized by high mesenchymal marker expression. Among these metabolic genes, they found Dihydropyridine dehydrogenase (DPYD), which encodes for an enzyme driving pyrimidine catabolism [163]. For example, from a lipid metabolism point of view, some lipid enzymes such as acetyl-CoA synthases (ACSL1 and ACSL4), SCD, support the EMT program in colorectal cancer [136]. Accordingly, hepatic cancer cells activate the EMT program, via Wnt and TGF-β, upon free fatty acids (FFAs) uptake driven by CD36 [144]. Emerging evidence links glycolytic metabolism and EMT alterations. The overexpression of the glycolytic enzyme phosphoglucose isomerase (PGI), which is implicated in the conversion of glucose-6P to fructose-6P, induces, via NF-κB, ZEB1 and ZEB2 activation and miR-200s repression [164]. In addition to glycolytic enzymes, fructose-bisphosphate aldolase A (ALDOA), an enzyme implicated in the conversion of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and hydroxy-acetone, induces EMT via mesenchymal markers upregulation in lung squamous carcinoma [165]. Similarly, GAPDH supports EMT through SNAI1 expression in colon cancer [166]. The lactate dehydrogenase (LDHA/B), the enzyme that converts pyruvate into lactate during anaerobic glycolysis, showed a positive correlation with EMT markers in muscle-invasive bladder cancer [167]. Some evidence showed that high glucose plays a crucial role in cancer. For example, glucose drives, in CRC, a reduction of E-CADHERIN and upregulation of VIMENTIN [168]. In addition, high glucose levels promote EMT by increasing YAP1 and TAZ in breast and bladder cancer models [169, 170]. Metabolic mitochondrial dysfunctions are also linked to EMT reprogramming in several cancer histotypes. Fumarate hydratase (FH) is a mitochondrial enzyme that is involved in fumarate to malate conversion. In renal cancer, FH induces EMT signature via miR-200b/200a/429 [171, 172]. Conversely, lymphoid-specific helicase (LSH), after recruiting the epigenetic silencer factor G9a, represses FH. Downregulation of FH level leads to an abrogation of succinate, fumarate, and malate production, which in turn downregulates E-CADHERIN and ZO-1 expression and overexpresses VIMENTIN in nasopharyngeal carcinoma [173, 174]. Succinate dehydrogenase (SDH) converts succinate to fumarate. SDH downregulation promotes cell migration and invasion via TGF-β/SNAI1 in colon and ovarian cancer [175, 176]. Furthermore, Succinate Dehydrogenase Complex Assembly Factor 2 (SDHAF2) via glycogen-synthase kinase (GSK-3β)-β-catenin axis induces EMT in lung cancer cells [177]. Isocitrate dehydrogenases (IDHs) are enzymes that catalyse the conversion of isocitrate to alpha-ketoglutarate (α-KG). Mutations in IDH genes are involved in the activation of the EMT pathway via the miR200-ZEB1 axis [178]. Amino acids represent an alternative metabolic source useful for cancer metabolism [179]. It has been shown that the distal-less homeobox-2 (Dlx-2)/glutaminase 1 (GLS1)/glutamine (Gln) metabolism axis drives TGF-β/Wnt/SNAIL-induced EMT and the glycolytic switch. Mechanistically, DLX-2 enhances SNAIL mRNA stability by inducing GLS1/Gln metabolism [180]. Glutaminase 2 (GLS2) has a contradictory role in inducing or suppressing the EMT process [181, 182]. However, it has been demonstrated that GLS2 induces EMT by increasing ERK phosphorylation and, consequently, both ZEB1 and VIMENTIN levels [182]. Similarly, another enzyme involved in the amino acid metabolism/EMT duality is glutamate dehydrogenase (GDH), which is implicated in α-KG production from glutamate. GDH upregulation drives the tyrosine phosphorylation of STAT3, mediating EMT acquisition in CRC models [183]. Collectively, this evidence highlights the existence of a regulatory axis from cellular metabolism to EMP. The modulation of these metabolic pathways underscores how intermediate metabolites act as signalling molecules to determine the fate of specific EMT states. Deciphering these complex interactions provides a framework for identifying metabolic vulnerabilities that could be exploited in anti-cancer therapies.

Disentangling the causal directionality of the EMT–metabolism axis requires orthogonal deconvolution strategies that collectively resolve both the molecular hierarchy and the spatiotemporal dynamics of this crosstalk. First, temporal single-cell multi-omics, integrating scRNA-seq with single-cell metabolic gene signatures and pseudo time trajectory inference, could enable the mapping of EMP states onto discrete metabolic programs, identifying whether glycolytic, oxidative, or lipid-anabolic shifts precede or follow transcriptional EMT commitment; this approach is particularly powerful for resolving hybrid E/M intermediates, which bulk transcriptomics systematically obscures. Second, stable isotope tracing with ^{13}C-labeled glucose or glutamine in isogenic CRISPR-edited models, where individual EMT-TFs are selectively knocked out or activated, would allow direct quantification of carbon flux through glycolysis, the pentose phosphate pathway, and the TCA cycle under defined genetic perturbations, causally assigning each metabolic rewiring to a specific transcriptional driver rather than inferring it from correlative data. Third, in vivo genetic lineage tracing, combining Cre-lox systems driven by EMT-specific promoters with fluorescent metabolic biosensors, would enable dynamic tracking of metabolic state transitions within cells undergoing EMT in living tumour models, capturing the spatially resolved heterogeneity between invasive fronts and tumour cores that static in vitro assays cannot recapitulate. Each of these strategies, discussed in detail in the following sections, addresses a distinct layer of the EMT–metabolism relationship and, when applied in concert, offers a framework to determine whether metabolic reprogramming is a driver, an enabler, or a consequence of specific EMP states, with direct implications for identifying context-dependent therapeutic vulnerabilities.

Tumour microenvironment and EMP

EMP in cancer cells is dynamically regulated by the multifaceted TME, encompassing cellular components, CAFs, immune cells (i.e., tumour-associated macrophages TAMs, myeloid-derived suppressor cells (MDSCs), T cells, endothelial cells (ECs), pericytes, and non-cellular elements like the ECM, all of which deliver paracrine signals, biomechanical forces, and metabolic cues to drive E/M states promoting invasion, stemness, metastasis, and therapy resistance [184–186].

CAFs, endothelial, and immune cells

CAFs, the predominant stromal cells originating from resident fibroblasts, epithelial/endothelial-mesenchymal transitions, or bone marrow precursors, secrete TGF-β, IL-6, PDGF, and HGF to activate SMAD2/3, STAT3, and PI3K/AKT pathways, upregulating EMT transcription factors (SNAIL, SLUG, TWIST, ZEB1/2) while repressing E-CADHERIN [187, 188]. Endothelial cells contribute via VEGF/Notch signalling, inducing endothelial-mesenchymal transition-like responses in tumour cells [189], and secreting TGF-β/Wnt ligands that stabilize β-catenin and NICD to reinforce partial EMP [190, 191], facilitating vascular mimicry and intravasation [192, 193]. Pericytes stabilize aberrant vessels but, when activated, release PDGF-BB to crosstalk with CAFs [194, 195], amplifying ECM remodelling and stiffness [196]. Immune cells profoundly shape EMP: TAMs (M2-polarized) produce TGF-β, TNF-α, IL-6/10, and CCL2 to trigger STAT3/SMAD-mediated Snail/ZEB expression and immune evasion via PD-L1 upregulation in mesenchymal states [197–199]; MDSCs suppress anti-tumour immunity while secreting ARG1/IL-6 to sustain plasticity [200, 201]; regulatory T cells contribute TGF-β/ID1 signals for hybrid phenotypes [202–204]. On the other hand, E/M cancer cells can be crucial during the metastatic process, thanks to their crosstalk with immune cells, as highlighted by their capacity to express high levels of inhibitory signals that protect them from NK cell-mediated clearance [45], or to express high levels of PD-L1, thus generating an immune-evasive phenotype, without requiring the need to undergo a transition toward a full mesenchymal phenotype [46]. These interactions create immunosuppressive loops, with mesenchymal cells recruiting more protumour immune infiltrates [205].

Neural cells

Neural cells within the TME, including neurons, Schwann cells, and neural crest-derived glia, actively contribute to cancer progression by releasing neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurturin (NRTN), thereby fostering tumour-neural crosstalk that drives malignancy. These peritumoral nerves innervate tumours and promote plasticity through synaptic-like connections, where neurotransmitter release and neurotrophins signalling reprogram cancer cells toward stemness and invasiveness [206, 207]. Neurotrophic factors play a pivotal role in cancer biology by modulating tumour cell plasticity, particularly through the regulation of EMT [206]. These factors bind to high-affinity Tropomyosin receptor kinase (Trk) receptors (e.g., TRKA for NGF, TRKB for BDNF) and the low-affinity p75NTR receptor, activating downstream pathways such as PI3K/AKT, MAPK/ERK, and PLCγ, which converge to reprogram cellular states [208]. In CSCs, BDNF/TrkB signalling upregulates EMT transcription factors like SLUG, SNAIL, and TWIST1, driving the acquisition of mesenchymal traits, such as increased N-CADHERIN, VIMENTIN, and ZEB1 expression, while suppressing epithelial markers like E-CADHERIN [206, 209, 210]. This shift not only enriches CSC pools with self-renewal capacity (e.g.,

via sphere formation and $CD44^{high}/CD24^{low}$ phenotypes in breast and lung cancers) but also confers resistance to therapies by enabling adaptive plasticity in response to stress. In specific contexts, NGF induces SLUG via p75NTR in breast cancer cells, promoting invasion and stemness, while NRTN enhances ZEB1/N-CADHERIN in colorectal and pancreatic tumours to boost motility and angiogenesis [211]. BDNF further supports glioma progression through synaptic plasticity mechanisms, including AMPA receptor trafficking and CAMK2 activation, linking neural-tumour interactions to metastatic potential [207]. Collectively, these neurotrophin-driven processes underscore their contribution to tumour heterogeneity, progression, therapeutic evasion, and plasticity, positioning their targeting as promising agents to disrupt plasticity.

ECM

The ECM, comprising collagens (I/IV), fibronectin, hyaluronic acid, and laminins, undergoes CAF/MMP-driven remodelling, increasing stiffness that engages integrin-α5β1/αvβ3-FAK/Src/RhoA/YAP/TAZ mechanosignalling to lock EMP, disrupt basement membranes, and promote invadopodia formation [212–215]. Moreover, ECM proteins like fibronectin cooperate with TGF-β for ERK/MAPK activation [216–218]. However, recent studies have highlighted that matrix stiffness can regulate invadopodia in a context-dependent manner. In 3D basement membrane-like matrices, Chang et al. showed that increasing stiffness reduced the probability of invadopodia extension, shortened invadopodia length and lifetime, and impaired cell migration, indicating that a stiffer and less permissive environment can physically restrict protrusive invasion. Importantly, these findings contrast with earlier 2D gelatine- or collagen-based assays, in which increased stiffness was associated with greater invadopodia-mediated degradation, suggesting that stiffness does not exert a uniform effect across experimental systems. This apparent discrepancy likely reflects fundamental differences in dimensionality, matrix composition, and readout. In 2D assays, invadopodia are scored mainly by proteolytic degradation on rigid, ligand-coated substrates, whereas in 3D basement membrane-like matrices, invadopodia must extend through a confined, mechanically plastic environment and support directional migration. Thus, stiffness may enhance degradative activity under planar conditions while simultaneously limiting protrusion extension in 3D by increasing confinement and reducing matrix plasticity. Taken together, the available evidence suggests that invadopodia respond not only to stiffness per se, but also to the mechanical architecture of the surrounding matrix, interpreting stiffness-dependent effects as highly context dependent [219]. Collectively, these TME elements act in synergy, with CAF-ECM stiffening that amplifies EC/TAM-derived TGF-β/Notch signalling, yielding reversible hybrid states resistant to therapies. Thus, the targeting of multi-axis using specific inhibitors (TGF-β traps, anti-FAK, CAF-depleters) could disrupt EMP networks.

Models to monitor the E/M state of cancer cells

In vitro models

Recent advances in in vitro modelling have dramatically expanded our capacity to monitor and dissect EMP in cancer cells at unprecedented molecular and temporal resolution (Table 1).

Dual-fluorescent reporter systems, which utilize promoter regions of epithelial (e.g., E-CADHERIN) and mesenchymal (e.g., VIMENTIN) markers to drive spectrally distinct fluorophores (such as RFP and GFP), have emerged as powerful tools to track EMT dynamics in real time across colorectal, lung, and breast cancer models [220]. These dual-reporter constructs enable the identification and isolation of distinct epithelial (E), mesenchymal (M), and E/M subpopulations via flow cytometry and live-cell imaging, revealing that intermediate phenotypes represent a balanced stemness and invasive capacity,

Table 1 Summary table showing in vitro and in vivo models to investigate the EMT status of cancer cells

Approach	Applications	Representative Studies/Tools (PMID)	Strenghts	Limitations
Network inference & GRN modeling	Identify EMT drivers, reconstruct regulatory circuits	SpidermiR [173], Network analysis [171]	Reveals key regulators, integrates multi-omics	Dependent on data quality, may miss context-specific effects
Single-cell & spatial transcriptomics	Map EMT/hybrid states, analyze heterogeneity	Spatial transcriptomics [34, 168], scRNA-seq [179]	High resolution, spatial context, captures plasticity	Costly, complex analysis, limited by tissue sampling
Machine learning & deep learning	Predict EMT states, drug response, biomarker discovery	UNAGI [169], ML review [170], ML organelles [175], ML morphology [176].	Handles high-dimensional data, enables *in silico*screening	Requires large datasets, interpretability challenges
Agent-based & dynamic modeling	Simulate EMT transitions, study plasticity & evolution	Agent-based [177], >ODE/Boolean [178]	Captures dynamics, models cell-cell interactions	Model assumptions, computationally intensive
Multi-omics integration	Uncover novel signatures, link molecular layers	SpidermiR [171],UNAGI [169], ML review [170]	Holistic view, identifies cross-talk between pathways	Data integration challenges, batch effects

compared to pure epithelial or mesenchymal states, thus making it the ideal target for designing novel anti-tumour therapies. Additional reporter strategies incorporate microRNA-based sensors, such as miR-200 fused to fluorescent proteins, to robustly detect EMT and MET events, thereby capturing bidirectional plasticity and distinguishing cancer stem cell-like populations within heterogeneous tumours [221]. Complementing these plasmid-based systems, CRISPR/Cas9-mediated endogenous tagging of EMT-related genes (*SNAI1*, *ZEB1*, *CDH2*) with fluorescent proteins provides physiologically relevant models that preserve native regulatory elements, subcellular localization, and post-translational modifications, thus avoiding overexpression artifacts [222, 223]. CRISPR-based knockout/knockdown and overexpression approaches dysregulating phenotypic stability factors (e.g., ESRP1, GRHL2, and OVOL2) further permit dissection of the molecular circuitry governing E/M state stabilization and reveal how loss of these factors drives cells toward complete EMT [224–227]. In parallel, sophisticated three-dimensional (3D) culture platforms, including patient-derived organoids (PDOs), tumour spheroids embedded in ECM, and microfluidic tumour-on-chip devices, recapitulate critical TME features such as tissue architecture, hypoxia, matrix stiffness, and biochemical gradients [228–232]. TGF-β-induced EMT in breast cancer PDOs, for instance, triggers morphological changes, downregulation of E-CADHERIN, cytoskeletal reorganization, and enhanced organoid adhesion to culture substrates, reflecting migration and invasion through the ECM [233]. Advanced imaging modalities, combined with deep learning algorithms, enable the quantitative, high-throughput assessment of EMT susceptibility, inter- and intra-organoid heterogeneity, and dynamic cellular responses across patient-derived lines. Microfluidic platforms further enhance these capabilities by providing spatiotemporal control over nutrient delivery, drug exposure, and mechanical cues, while permitting real-time visualization of collective cell invasion, single-cell EMT dynamics, and phenotypic switching under defined conditions [234, 235].

In vivo models

In vivo monitoring of EMP in mouse models has been revolutionized by bioluminescence imaging (BLI) and fluorescent protein-based reporter systems, enabling real-time, non-invasive tracking of EMT dynamics during tumour progression and metastasis. Luciferase-based reporters provide the most sensitive and quantitative approach for longitudinal in vivo tracking, as bioluminescent signals from firefly or Renilla luciferase-expressing cancer cells can be detected throughout the body, even in deep tissues, with photon emission correlating linearly with tumour burden. Dual luciferase reporter systems that combine constitutive expression of one luciferase isoform (e.g., firefly luciferase for tumour burden), with EMT-responsive expression of a second isoform (e.g., Renilla luciferase driven by ZEB1 or SNAI1 promoters) enable ratiometric quantification of EMT status in xenograft and orthotopic models [236]. For instance, Renilla luciferase fused to the 3′-UTR of ZEB1 mRNA serves as a biosensor for miR-200 family activity, with bioluminescence signals being strongly repressed in epithelial cells but activated in mesenchymal or TGF-β-treated cells undergoing EMT. Orthotopic xenograft models implanted with luciferase-tagged cancer cells into anatomically relevant sites (e.g., colon, pancreas, lung) recapitulate the natural tumour microenvironment and metastatic dissemination patterns observed in patients, with serial BLI measurements enabling detection of primary tumour growth and distant metastases without sacrificing animals. In colorectal cancer models, subcutaneous or orthotopic injection of CT-26-Luc cells results in lung metastases detectable by BLI within 1–2 weeks, with bioluminescence intensity showing strong correlation with tumour volume measured by micro-computed tomography (micro-CT) imaging. Genetically engineered mouse models (GEMMs) carrying tissue-specific, inducible EMT reporters represent the most physiologically relevant systems for studying spontaneous EMT during *de novo* tumorigenesis. Dual reporter GEMMs of PDAC engineered with fluorescent proteins driven by EMT markers, such as α-smooth muscle actin (αSMA) and fibroblast-specific protein 1 (FSP1), enable lineage tracing of EMP in spontaneously arising tumours within an intact immune microenvironment [237]. These GEMM-based systems revealed that EMT programs are dynamically regulated during PDAC progression, with αSMA-positive mesenchymal cells exhibiting enhanced invasiveness and contributing to tumour heterogeneity. Intravital microscopy (IVM) combined with fluorescent protein reporters provides the highest spatiotemporal resolution for visualizing EMT dynamics in living mice. Multi-photon IVM through surgically implanted imaging windows (e.g., mammary, cranial, dorsal skin, or lung windows) allows repeated imaging of the same tumour region over days to weeks, revealing that motile, invasive tumour cells express mesenchymal markers (e.g., VIMENTIN-GFP) while proliferative cells at the tumour core retain epithelial characteristics (E-CADHERIN-RFP) [238, 239]. Recent IVM studies using CSC biosensors, such as the SORE6 > GFP reporter activated by SOX2/OCT4 binding, demonstrate that CSCs exhibiting EMT-like properties are enriched at tumour-microenvironment of metastasis doorways, specialized microanatomical sites where cancer cells interact with perivascular macrophages to facilitate intravasation [240, 241]. Furthermore, IVM with FRET-based biosensors for SRC,

Rho GTPases, or caspase-3 activity enables real-time visualization of signalling events driving EMT, migration, and resistance to apoptosis at single-cell resolution within the native TME [242–244]. Collectively, luciferase-based BLI for whole-body metastasis tracking, fluorescent protein reporters in GEMMs for lineage tracing, and IVM for subcellular dynamics constitute a comprehensive in vivo toolkit for dissecting EMP across multiple scales, from molecular signalling to tissue-level tumour architecture, during cancer progression and therapeutic response. Collectively, these integrated in vitro and in vivo models (Fig. 3), spanning genetically encoded reporters, CRISPR-engineered cell lines, 3D organoid systems, microfluidic devices, and GEMMs, constitute robust and versatile toolkits for interrogating the molecular mechanisms, regulatory networks, and therapeutic vulnerabilities underlying epithelial-mesenchymal plasticity in cancer.

In conclusion, single- and dual-reporter systems offer distinct advantages and caveats in studying EMP. Single reporters, such as luciferase- or fluorescence-based constructs driven by EMT-associated promoters, provide sensitive, quantitative, and often non-invasive tracking of EMT dynamics in vitro and in vivo, yet they capture only unidimensional aspects of phenotypic transitions. In contrast, dual-reporter systems linking epithelial and mesenchymal promoters to spectrally distinct fluorophores enable simultaneous visualization of bidirectional transitions, intermediate hybrid states, and cell-state heterogeneity, thus offering higher spatiotemporal resolution and functional insight into dynamic EMP trajectories. However, these approaches may suffer from promoter leakiness, artificial overexpression, or limited physiological relevance, particularly in plasmid-based models that lack endogenous regulatory context. CRISPR/Cas9-mediated knock-in reporters and GEMMs partially overcome these limitations by preserving native gene regulation and allowing lineage tracing within the tumour microenvironment. Overall, integrating single and dual reporters across complementary platforms, from 3D organoids to intravital microscopy, provides a more comprehensive and mechanistically faithful framework to dissect EMP regulation during cancer progression and therapeutic response.

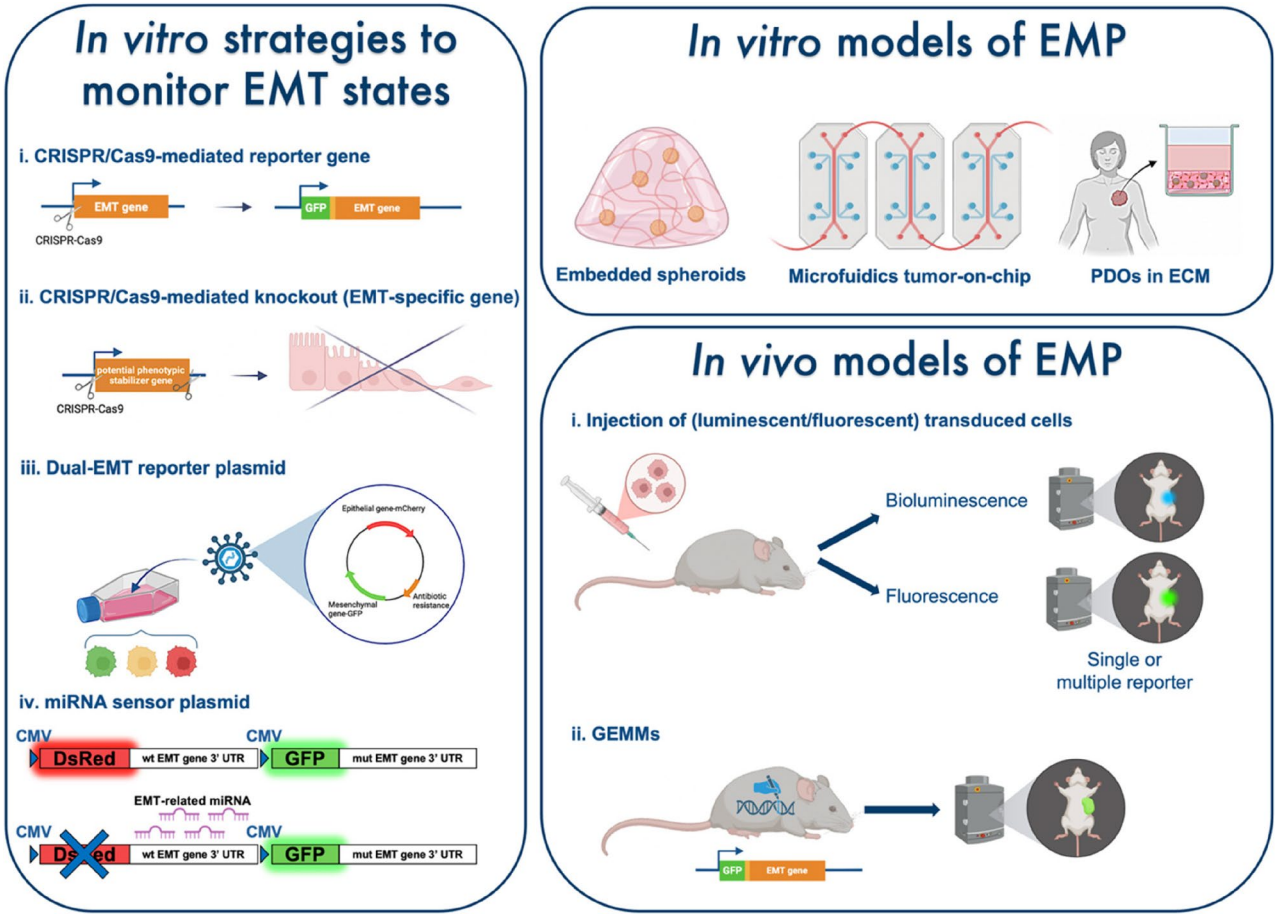

Fig. 3 In vitro and in vivo models to study EMP. Schematic overview of in vitro and in vivo strategies for monitoring EMT states and studying EMP in preclinical models

In silico methodology to study the EMT process

The identification and quantification of E/M states remain challenging due to the lack of reliable biomarkers and the inherent fluidity of the process. However, scRNA-seq and spatial transcriptomics have enabled the mapping of these populations within tumours, revealing that E/M cells are often enriched at the invasive front and are associated with increased metastatic potential and therapy resistance [23, 44, 245]. In this context, in silico methodologies have emerged as indispensable tools, enabling the integration and analysis of high-dimensional omics data, the modelling of complex regulatory networks, and the simulation of dynamic cellular processes [246–248]. For example, spatial transcriptomics has been used to localize E/M populations within the tumour microenvironment, demonstrating their spatial association with invasive niches and their dynamic response to microenvironmental cues [245]. GEMMs employing lineage tracing strategies, such as vimentin- and E-CADHERIN-driven GFP and RFP reporters [220], have provided direct evidence that hybrid and mesenchymal lineages are crucial for tumour progression and metastasis. Ablation of EMT-proficient cells in these models dramatically impairs both primary tumour growth and metastatic dissemination, while spatial profiling and whole-genome sequencing have revealed that EMT-derived cells are associated with increased chromosomal instability and chromothripsis, directly linking EMP to tumour evolution and the emergence of high-fitness malignant clones [249, 250]. In this scenario, network-based approaches have been instrumental in dissecting the regulatory logic underlying EMT and its hybrid states. Tools such as SpidermiR [251] enable the integration of gene expression, copy number variation, and miRNA data to reconstruct gene regulatory networks (GRNs) in cancer. In prostate cancer, SpidermiR was used to identify miR-145-5p as a central regulator of an aggressive gene network, highlighting how network inference can pinpoint non-canonical drivers of EMT and hybrid phenotypes. Similarly, network topology analysis has been applied to identify master regulators of EMT in various tumour types, revealing that E/M states are often governed by complex, multi-layered regulatory circuits [248].

Single-cell RNA-seq and spatial transcriptomics have revolutionized the study of EMT dynamics by enabling the high-resolution mapping of cellular states within tumours [252]. These technologies have been pivotal in identifying and characterizing E/M populations. For instance, spatial transcriptomics was used to map E/M cells at the invasive front of tumours, demonstrating their spatial heterogeneity and association with metastatic potential [245]. Single-cell analysis has further revealed that E/M states are not rare intermediates but constitute a significant fraction of tumour cell populations, with distinct transcriptional programs and functional properties [23, 44]. A landmark study using scRNA-seq in head and neck squamous cell carcinoma identified a specific E/M signature that was spatially localized at the leading edge of primary tumours; notably, this E/M program was found to be a better predictor of nodal metastasis than canonical EMT markers, highlighting the clinical relevance of hybrid states [253–255]. These approaches have also uncovered the dynamic plasticity of E/M cells, including their ability to revert to epithelial or progress to fully mesenchymal states in response to environmental cues. In parallel with single-cell and spatial approaches, several transcriptomics-based scoring metrics have been developed to quantitatively position patient samples along the epithelial–hybrid–mesenchymal (E–H–M) spectrum. A seminal study introduced an E/M gene expression scoring metric capable of stratifying tumours based on their hybrid phenotype and predicting survival outcomes across multiple cancer types, demonstrating that E/M states are often associated with poor prognosis and increased metastatic potential [51]. Subsequent comparative analyses systematically evaluated different EMT scoring frameworks, highlighting their relative strengths, limitations, and consistency in capturing the E-H-M continuum [256]. These studies emphasized that EMT is not a binary switch but a continuous and multidimensional spectrum requiring quantitative tools for robust clinical interpretation. Beyond prognostic stratification, EMT scoring approaches have been integrated with stemness and immune-related signatures, revealing that EMP is tightly linked to CSC traits and the modulation of the tumour immune microenvironment [257]. Such integrative scoring systems provide a systems-level framework to correlate E/M states with therapeutic resistance, immune evasion, and disease progression. From a theoretical perspective, the existence of stable hybrid states predicted by transcriptomic scoring metrics is supported by computational studies of multistable GRNs. Recent in silico studies have further refined this dynamical systems perspective by demonstrating that the enhanced plasticity of E/M states can be explained by specific topological features of EMT regulatory networks. In particular, network analyses have revealed the existence of mutually reinforcing "teams" of nodes corresponding to epithelial and mesenchymal programs, each characterized by dense intra-team positive feedback and inter-team antagonism [258, 259]. Fully epithelial or mesenchymal states are stabilized when one team dominates the regulatory landscape; in contrast, hybrid E/M states arise in configurations where neither team achieves complete dominance. This intermediate topological balance results in reduced structural reinforcement, rendering hybrid states more responsive to perturbations and

therefore more plastic. Such findings provide a mechanistic explanation for the experimentally observed adaptability of E/M cells and link network topology directly to phenotypic stability and transition potential. Theoretical and computational frameworks describing multistability, bifurcations, and attractor landscapes have demonstrated how feedback loops and nonlinear regulatory interactions can generate multiple coexisting stable phenotypes, including epithelial, mesenchymal, and hybrid attractor states [260]. These approaches provide a rigorous dynamical systems foundation for understanding EMP, linking molecular regulatory circuits to phenotypic stability and transitions. Together, EMT scoring metrics and multistability-based modelling frameworks bridge quantitative transcriptomic data with dynamical systems theory, enabling a more precise and predictive characterization of EMP in patient samples.

The complexity and volume of multi-omics data often necessitate the use of machine learning (ML) and artificial intelligence (AI) to extract meaningful patterns and make predictions. Deep generative models, such as the UNAGI framework [246], power time-series single-cell data to model disease progression and simulate the effects of pharmacological perturbations. UNAGI learns disease-informed cell embeddings that capture the continuous transcriptomic shifts during EMT, enabling the identification of intermediate (hybrid) states and dynamic marker genes. In practical terms, UNAGI has been used to simulate in silico drug screening, predicting which compounds can revert hybrid or mesenchymal states toward a more epithelial phenotype, and prioritizing candidates for experimental validation. Complementing transcriptomics-based EMT scoring, a recent morphology-driven machine learning framework leveraging high-content Cell Painting imaging and organelle feature extraction (e.g., endoplasmic reticulum/mitochondrial texture metrics and cell-shape solidity) enables continuous, annotation-independent quantification of epithelial–mesenchymal plasticity, capturing EMT kinetics, hybrid states, and MET across different inducers and cancer types, with clear scalability for high-throughput phenotypic drug screening [261]. Beyond transcriptomics and molecular marker-based readouts, deep learning pipelines for morphology-driven phenotyping, benchmarked on brightfield intestinal organoid images, demonstrate that CNN architectures and Vision Transformers can accurately classify organoid developmental stages and morphological transitions reflective of epithelial plasticity, offering a scalable, automated imaging workflow applicable to EMT-like morphological changes across diverse organoid and cancer models [262].

Agent-based models and dynamic simulations provide a powerful framework for studying the temporal and spatial evolution of EMT and its hybrid states. For example, agent-based modelling has been used to simulate the transition between epithelial, hybrid, and mesenchymal states, demonstrating how cellular plasticity influences metastatic dissemination and therapeutic response [263]. Dynamic modelling approaches, including ordinary differential equations (ODEs) and Boolean networks, have been applied to simulate the regulatory circuits controlling EMT, predicting the existence of stable hybrid states and identifying key feedback loops that stabilize these phenotypes [264]. These models have revealed that E/M states can act as attractors in the regulatory landscape, indicating that hybrid phenotypes are not merely transient intermediates but can represent dynamically stable configurations determined by the structure of the underlying gene regulatory network, conferring robustness and adaptability to tumour cell populations. Integrative analysis of genomics, transcriptomics, proteomics, and metabolomics data provides a holistic view of EMT and its hybrid states. For example, SpidermiR [251] and UNAGI [246] both exemplify how multi-omics integration can uncover novel regulatory interactions and therapeutic targets. By combining data from multiple molecular layers, these approaches have revealed that E/M states are associated with unique gene signatures and regulatory networks that are not apparent from single-omic analyses [247].

In silico approaches have significantly advanced the identification of novel EMT drivers, moving beyond canonical transcription factors to uncover complex regulatory networks involving microRNAs and other molecular effectors. Computational predictions are increasingly validated using sophisticated experimental models, such as GEMMs, PDOs, and patient-derived xenografts, which preserve tumour heterogeneity and microenvironmental complexity [44]. SpidermiR-based identification of miR-145-5p as a key regulator in prostate cancer was followed by experimental validation in cell lines and animal models, confirming its role in driving aggressive phenotypes [251]. Similarly, AI-driven models like UNAGI have been used to predict the response of tumour cells to anti-EMT therapies, guiding the prioritization of candidate compounds for preclinical testing [246]. The study of cellular plasticity, particularly the identification and characterization of E/M states, has been greatly facilitated by single-cell and spatial transcriptomics, which reveal the heterogeneity and dynamic nature of tumour cell populations. These insights are critical for the development of therapies targeting the supportive niches that sustain tumour progression. Despite their transformative potential, in silico models face significant challenges. The validation of computational predictions remains a bottleneck, often constrained by the resource-intensive nature of experimental work and the need for robust, scalable validation pipelines. The predictive accuracy of

Table 2 Summary of main in silico approaches for EMT research, their applications, representative studies/tools, strengths, and limitations

Approach	Key Question Answered	Applications	Representative Studies/Tools (PMID)	Strengths	Limitations
Network inference & GRN modelling	Which molecular circuits and master regulators drive EMT/hybrid states?	Identify EMT drivers, reconstruct regulatory circuits	SpidermiR [252], Network analysis [249]	Reveals key regulators, integrates multi-omics	Dependent on data quality, may miss context-specific effects
Single cell & spatial transcriptomics	Where are hybrid cells located and how does the microenvironment influence them?	Map EMT/hybrid states, analyse heterogeneity	Spatial transcriptomics [246], scRNA-seq [254–256]	High resolution, spatial context, captures plasticity	Costly, complex analysis, limited by tissue sampling
Machine learning & deep learning	Can we predict drug responses or identify new biomarkers from complex data?	Predict EMT states, drug response, biomarker discovery	UNAGI [247], ML review [248], ML organelles [262], ML morphology [263].	Handles high-dimensional data, enables in silico screening	Requires large datasets, interpretability challenges
Agent-based & dynamic modelling	What are the temporal trajectories and stability of EMT/MET transitions?	Simulate EMT transitions, study plasticity & evolution	Agent-based [264], E/M states [265]	Captures dynamics, models cell-cell interactions	Model assumptions, computationally intensive
Multi-omics integration	How do different molecular layers (DNA, RNA, protein) crosstalk during EMT?	Uncover novel signatures, link molecular layers	SpidermiR [252], UNAGI [247], ML review [248]	Holistic view, identifies crosstalk between pathways	Data integration challenges, batch effects

these models is fundamentally dependent on the quality and diversity of training data, with issues of data standardization and interoperability persisting as major obstacles [247]. Future progress will hinge on the integration of emerging technologies, including AI, single-cell multi-omics, and spatial transcriptomics, to build more realistic and comprehensive models of disease. The harmonization of high-resolution data with advanced analytics promises to enhance diagnostics, identify novel therapeutic targets, and enable the development of personalized therapies [246]. In silico methodologies have revolutionized the study of EMT, complementing traditional experimental approaches and enabling the integration and interpretation of complex, high-dimensional data. These computational tools facilitate the modelling of dynamic cellular processes, the identification of regulatory networks and biomarkers, and the simulation of therapeutic interventions. Their application holds significant promise for improving diagnosis, prognosis, and the development of targeted therapies, ultimately advancing the realization of personalized medicine in oncology.

Despite their transformative potential, each of these in silico approaches faces specific limitations that must be addressed to ensure clinical translatability (Table 2). For instance, while transcriptomics-based scoring metrics provide a quantitative framework, they often lack cross-platform consistency; a sample might be classified differently depending on the specific gene set or algorithm used, complicating the establishment of universal thresholds for 'hybrid' states. Furthermore, single-cell and spatial transcriptomics, while high-resolution, are frequently hampered by technical noise, 'dropout' events in gene detection, and the high costs associated with large-scale patient cohorts. Network-based and dynamic models (such as ODEs and Boolean networks) are inherently limited by the 'curse of dimensionality' and the scarcity of kinetic parameters for most regulatory interactions, often requiring simplifying assumptions that may overlook the stochastic nature of cellular transitions. Similarly, while AI and deep learning frameworks like UNAGI offer powerful predictive capabilities, they often operate as 'black boxes', making it difficult to extract the underlying biological logic behind a prediction. Moreover, the predictive accuracy of these models is fundamentally dependent on the quality and diversity of training data; issues of data standardization and interoperability persist as major obstacles, particularly when integrating multi-omics datasets from different laboratories. Finally, the validation of computational predictions remains a significant bottleneck, as the resource-intensive nature of experimental work with GEMMs or organoids cannot yet match the throughput of in silico screenings, necessitating more robust and scalable automated validation pipelines.

Therapeutic strategies to target EMP in cancer

Despite significant advances in tumour treatment, the acquisition of EMP features remains a key driver of cancer recurrence and chemoresistance. Targeting the EMT plasticity represents a promising approach in cancer therapy, given its critical role in tumour progression, metastasis, and resistance to conventional treatments [265]. However, the inherent plasticity and dynamic nature of EMT, particularly the existence of heterogeneous populations with completely epithelial or mesenchymal phenotypes and E/M states that confer both stemness and invasive capabilities, pose significant challenges to developing effective therapies [266]. EMP is

regulated by complex signalling networks and transcriptional programs, including pathways such as TGF-β and PI3K/AKT, and transcription factors like SNAIL, SLUG, and ZEB1 [258]. Supporting this, Verstappe et al., using a Krt14CreTg/$^{+}$-Trp53Fl/Fl mice, responsible for the cutaneous squamous cell carcinomas development, in association with the expression of ZEB2, a transcriptional factor modulating EMP, highlighted that the expression of ZEB2 induces the generation of heterogenic cancer cells population with a plastic phenotype [267]. Similarly, in a hybrid cellular model of NSCLC, characterized by a dynamic equilibrium between E-CADHERIN and SNAI2 expression, TGF-β1 treatment induced the acquisition of an EMP state and increased CD133 expression [268], a marker associated with highly metastatic lung CSCs [269]. These findings collectively indicate that EMP contributes to tumour progression and increased malignancy [268]. Considering this, the possibility of preventing or reversing the mesenchymal-like phenotype represents a critical approach to effective cancer therapy. Current therapeutic strategies targeting EMP focus on inhibiting upstream pathways involved in EMT, modulating metabolic reprogramming, epigenetic regulation, and co-targeting both the TME and intracellular mechanisms that sustain tumour growth and progression.

Intracellular signalling pathways

The inhibition of intracellular pathways is based on the use of inhibitors directed against key intracellular signalling pathways, including TGF-β, c-MET, NF-κB, EGFR, WNT, and Notch, as well as the use of ligand-neutralizing antibodies. The TGF-β pathway is well-known for its involvement in several cellular processes, such as proliferation, differentiation, and immunosurveillance [270]. Recently, its role in regulating EMP through the expression of EMT transcriptional factors has also emerged. In this context, bone morphogenetic protein-7 (BMP-7), a member of the TGF-β superfamily, plays a key role in the inhibition of EMT in melanoma, breast, and colon cancer [271–273]. In melanoma cells, treatment with BMP-7 induces MET, characterized by a reduction in the expression levels of TWIST and SNAIL [272]. In a recent study, Rastgar-Pouyani and colleagues have shown that the combinatorial treatment based on pirfenidone (PFD), which is an inhibitor of TGF-β, and paclitaxel (PTX), possesses a synergistic effect on the downregulation of breast cancer cell viability, colony-forming, and invasive capacity [274]. Importantly, the PFD + PTX combinatorial approach was able to reduce the expression of pluripotency transcription factors, and it mitigated the tumorigenic and metastatic ability of cancer cells. The HGF-c-MET axis is a potential therapeutic target primarily involved in cell proliferation. Additionally, it plays a significant role in regulating the EMT mechanism, ultimately enhancing the invasiveness of cancer cells [275]. Indeed, in vitro and in vivo studies have shown that HGF stimulation has been shown to upregulate the expression of Snail and Vimentin, key markers of the mesenchymal phenotype. Furthermore, activation of the HGF/c-MET signalling pathway in vitro induces chemoresistance. The treatment with crizotinib, a Met inhibitor, restores the chemotherapy sensitivity of small cell lung cancer cells, and alongside this reduces the expression of mesenchymal markers [276]. Despite its critical role, targeting the TGF-β pathway remains challenging due to its involvement in regulating stromal and immune cell behaviour [277, 278]. Nonetheless, inhibitors of this pathway are currently being evaluated in both preclinical and clinical settings. AXL, a receptor tyrosine kinase, is another receptor implicated in the regulation of EMT markers. Analysis of matched pairs of primary tumours and metastatic sites has shown that AXL expression is higher in metastases compared to primary tumours, suggesting that AXL is associated with poor prognosis in breast cancer patients and is crucial for the metastatic process. Silencing AXL in mesenchymal-like breast cancer cell lines has been shown to reduce both in vitro and in vivo tumour initiation and metastasis formation [279]. In addition, Koorstra and colleagues demonstrated that in pancreatic adenocarcinoma cells, AXL sustains the mesenchymal phenotype, with its silencing leading to the downregulation of key EMT transcription factors, such as SNAIL, TWIST, and SLUG [280]. In NSCLC, the upregulation of AXL and its ligand GAS6 is associated with EMT signature and resistance to EGFR and PI3K inhibitors. Notably, combined treatment with EGFR inhibitor erlotinib and the AXL inhibitor SGI-7079 counteracted drug resistance in mesenchymal-like NSCLC in a mouse xenograft model. These findings highlight AXL's role in driving the EMT phenotype and CSC-like characteristics, suggesting it is a promising therapeutic target for eliminating CSCs that have undergone EMT [281]. The PI3K/AKT signalling pathway plays a crucial role in the induction of EMT through a SMAD-independent mechanism, leading to the activation of EMT-related transcription factors. Mechanistically, PI3K/AKT signalling inhibits the activity of GSK-3β, preventing the double phosphorylation of Snail at distinct serine residues, thereby blocking its cytoplasmic translocation (Ser 97, Ser 101) and proteasomal degradation (Ser108, Ser112, Ser116, and Ser120), and enhancing its transcriptional activity [282]. In lung cancer cells under hypoxic stress, overexpression of NETRIN-1, a laminin-related glycoprotein, activates the PI3K/AKT pathway, which in turn promotes cell proliferation, migration, and stimulates the EMT process [283]. Furthermore, Cassier et al. showed that in endometrial adenocarcinoma, the treatment with NP137, an anti-Netrin-1 antibody, not only induces tumour cell

death but also effectively inhibits the expression of mesenchymal genes in preclinical models and patients with endometrial adenocarcinoma, highlighting its potential as a therapeutic agent [284]. In line with the key role of the PI3K pathway in the regulation of EMT, a recent study showed that the knockdown of the polycomb group factor 1 (PCGF1) led to reduced colorectal cancer cell proliferation, with the concomitant downregulation of EMT biomarkers, cancer cell invasive and migratory capacity, through the downregulation of Wnt/β-Catenin and PI3K/AKT/mTOR pathways. Another intracellular regulator implicated in EMP is the EGFR. In a tyrosine-kinase inhibitor (TKI) resistant model of NSCLC, the treatment with metformin, an anti-diabetic drug, has been shown to overcome this resistance and inhibit EMT [285]. In conclusion, targeting intracellular signalling pathways involved in EMP represents a promising therapeutic strategy to counteract tumour progression, metastasis, and drug resistance. Key pathways such as TGF-β, HGF/c-MET, AXL, EGFR, and PI3K/AKT play central roles in regulating EMT and associated cancer stem cell–like properties. The inhibition of these pathways, either alone or in combination with conventional therapies, has demonstrated significant potential in reversing EMT, restoring drug sensitivity, and reducing tumour aggressiveness in preclinical and clinical settings.

Redifferentiation strategy

An effective strategy to target the EMT process is to promote the re-differentiation of mesenchymal cells into an epithelial phenotype, regulating the EMT marker expression, including E-CADHERIN, N-CADHERIN, and Vimentin. Fang et al. demonstrated in an in vitro study that lung cancer stem cells exhibit upregulation of BTBD7, a protein-protein interaction motif related to epithelial tissue remodelling and formation of branched organs [286, 287]. The upregulation of this protein induces a decrease in E-CADHERIN expression, contributing to the development of chemoresistance and facilitating the EMT. Importantly, silencing BTBD7 leads to a reduction in mesenchymal markers, specifically targeting cancer stem cells that are undergoing EMT [287]. In a recent work, Yan and colleagues showed that treatment with ß-elemene, a natural sesquiterpenic compound, mainly extracted from Curcuma wenyujin, reduced cell viability of breast cancer cells by inducing anoikis, downregulation of EMT markers such as MMPs, VIMENTIN, N-CADHERIN, TWIST1, and IGF, leading to a reversion of the EMT state in cancer cells [288]. Another example of EMT suppression and differentiation therapeutic approach is represented by Taxifolin (a natural, plant-derived flavonoid with powerful antioxidant, anti-inflammatory, and anti-tumour properties), which has been shown to inhibit proliferative and self-renewal of glioblastoma stem cells, and induce apoptosis, thus supporting its potential as a differentiation-based adjuvant therapy [289]. Interestingly, this work demonstrated that Taxifolin suppresses tumour growth, without toxicity, and boosts the therapeutic effect of temozolomide.

Targeting EMP-associated metabolism

In recent years, the interplay between EMP and the metabolic reprogramming in cancer cells has attracted significant interest as a key factor in cancer cell adaptation during the metastatic process. One example of this connection is the increased uptake of FFAs via the fatty acid translocase (CD36), which promotes Wnt/β-Catenin-mediated EMT in hepatocellular carcinoma. This is accompanied by elevated expression of mesenchymal markers, such as SNAIL, TWIST, ZEB, VIMENTIN, in HepG2 cells [144]. Importantly, chemical inhibition of CD36 has been shown to reduce the expression of these mesenchymal markers, further supporting the functional role of CD36-mediated FFAs uptake in driving EMT. A well-characterized aspect of this metabolic-EMT interplay is the dependence of cancer cells on glutamine metabolism to fuel the tricarboxylic acid (TCA) cycle [290], a process known to regulate EMT. In particular, in lung cancer, the suppression of glutaminolysis, the conversion of glutamine to glutamate mediated by glutaminase GLS1, has been shown to suppress the expression of the EMT-inducing transcription factor Snail, thereby reducing metastatic dissemination [291]. These findings highlight the potential of targeting lipid metabolism as a therapeutic strategy to inhibit EMT and suppress metastasis.

Consistent with this, emerging therapeutic strategies exploit the dynamic metabolic rewiring that sustains E/M states, invasion, stemness, and resistance. EMT shifts metabolism from glycolysis toward oxidative phosphorylation (OXPHOS), fatty acid oxidation, and oncometabolite accumulation like succinate, which stabilizes mesenchymal traits via HIF-1α stabilization, SUCNR1/GPR91 signalling, or ROS-mediated pathways, while extracellular vesicles (EVs) from cancer-associated fibroblasts transfer mtDNA or miRNAs to further reprogram mitochondrial function in tumour cells. In this scenario, repurposed metabolic inhibitors offer promise: MCT1 inhibitors (e.g., AZD3965, phase I/II trials) block lactate efflux critical for EMT migration; glutaminase inhibitors (CB-839) disrupt glutamine-fuelled anaplerosis in mesenchymal cells; and STAT3 inhibitors (e.g., napabucasin) revert partial EMT by normalizing OXPHOS. Emerging approaches include EV-engineered delivery of mitochondrial-targeted miRNAs or doxorubicin to OXPHOS-addicted cells, citrate supplementation to override hypoxic adaptation in low-glycolytic hepatocellular carcinomas (reducing HIF1α), and succinate pathway

antagonists like SUCNR1 blockers or TRAP1 inhibitors to curb metastasis [292, 293]. Along similar lines, treatment with simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase, in bladder cancer cells reduces the expression of the mesenchymal marker vimentin while increasing the expression of the epithelial marker E-CADHERIN. Additionally, this treatment decreases the proliferative, invasive, and migratory capacities of these cells [294]. In NSCLC, activation of the lipid metabolism enzyme fatty acid synthase (FASN) enhances the EMT induced by TGF-β, and the treatment with TVB-2640, a FASN inhibitor, reduces the EMT-associated metastatic potential in NSCLC cells resistant to cisplatin [131]. Another potential therapeutic target is glucose metabolism. In colorectal cancer cells, activation of the TGF-β/JNK/ATF2 signalling pathway increases GLUT-3 expression, thereby promoting EMT [295]. In this context, targeting this transporter could reduce the EMT-associated metastatic potential of cancer cells. Altogether, integrating metabolic targeting with conventional therapies may provide a more effective strategy to suppress EMP-driven tumour plasticity and improve clinical outcomes.

Targeting the epigenetic and post-transcriptional regulation of EMP

Epigenetic therapies have gained attention as an alternative strategy to reverse chromatin modifications that maintain EMT and stemness traits. Histone deacetylase inhibitors (HDACis) and DNMT inhibitors (DNMTis) can restore the expression of epithelial markers like E-CADHERIN and attenuate mesenchymal characteristics. For example, HDACis such as vorinostat have been shown to sensitize tumours to chemotherapy by modulating EMT plasticity, thereby overcoming resistance mechanisms [296, 297]. Moreover, the reduction of ZEB1 expression induced by HDACis or DNMTis is able to restore the sensitivity to chemotherapy in pancreatic cancer and osteosarcoma models. In the same way, the inhibition of ZEB1 resensitizes ovarian cancer cells to paclitaxel treatment [298]. In parallel, the use of monoclonal antibodies specific for crucial mediators of EMP is emerging as a novel and efficient therapeutic approach. Simanovich et al. have recently demonstrated that a monoclonal antibody specific for EMMPRIN (hMR18-mAb), a membrane glycoprotein implicated in cell–cell interactions, proliferation, angiogenesis, and EMT, loaded on U937 monocytic-like cells, inhibits the EMT process in breast and oral squamous cancer cells. This effect is mediated by a reduced release of EMT-inducer cytokines by macrophages, an increased E-CADHERIN, and reduced vimentin expression. Moreover, the treatment with hMR18-mAb was sufficient to reduce the onset of dormant phenotypes, which are often associated with the metastatic potential of cancer cells [299]. Recently, the role of miRNA, a single-stranded non-coding RNA, has emerged in the context of EMT transition [300]. As evidenced by the miR-200 family, microRNAs can suppress EMT by targeting EMT-regulating transcription factors such as ZEB1 and ZEB2 [62]. Ma et al. reported that in gemcitabine-resistant pancreatic cancer cells, high expression of this miRNA induces a mesenchymal state, whereas inhibition of miR-223 activates MET and reduces cell motility and invasion capacity [301].

TME reprogramming

A complementary strategy focuses on targeting the TME, which plays a crucial role in sustaining EMP and promoting tumour progression. By modulating both cellular and non-cellular components of the TME, these strategies aim to interfere with the external cues that sustain mesenchymal-like phenotypes, ultimately enhancing the efficacy of conventional and targeted anticancer treatments [302, 303]. Therapies targeting CAFs include fibroblast activation protein (FAP) inhibitors or CAR-T cells for direct depletion [304, 305], TGF-β pathway blockers (e.g., Galunisertib, AVID200) to prevent activation and EMT induction, and reprogramming agents like vitamin D analogues or all-trans retinoic acid (ATRA) to revert myCAFs to quiescent states, thereby reducing cytokine secretion and ECM remodelling that sustain EMP [306]. Losartan, an angiotensin II receptor antagonist, curbs collagen deposition and CAF contractility, improving drug penetration in preclinical models [307]. Anti-angiogenic agents such as bevacizumab (anti-VEGF) or sunitinib normalize tumour vasculature, alleviating hypoxia-driven HIF-1α/EMP signalling from ECs [308]. Interestingly, vascular normalization therapies enhance the efficacy of immunotherapy by improving T-cell infiltration. Several pieces of evidence have demonstrated that the acquisition of EMP phenotype modulated the TME toward an immunosuppressive state. Additionally, in different tumours, it was demonstrated that the EMP induces an enhancement of immune checkpoint expression, such as PD-1 and PD-L1 [309, 310]. In this scenario, phase I/II trials demonstrate safety and synergy with PD-1 inhibitors by restoring TME immunosurveillance [211, 311–314]. In a mouse model, the use of Galunisertib (LY2157299), an inhibitor of TGF-β, induces the reduction of tumour growth and metastasis formation, enhancing the immune cells' response following anti-PD-1/PD-L1 treatment [315]. Regarding the immune cell compartment, TAMs can be depleted or reprogrammed to M1 antitumor phenotypes using CSF1R inhibitors (e.g., pexidartinib) or CD40 or TLR agonists [316, 317]. This treatment reduces TGF-β/IL-6 output, fuelling STAT3/Snail-mediated EMP. On the other hand, MDSCs succumb to low-dose gemcitabine/5-FU or ATRA to block recruitment/differentiation via

CCL2/ARG1 inhibition, while Tregs are depleted by anti-CD25 antibodies or targeted via PI3Kδ/γ inhibitors, alleviating immunosuppression and partial EMT stabilization [318]. Targeting neurotrophic factors and neural cells in tumours offers promising strategies to disrupt tumour plasticity, EMT, and progression driven by tumour-neural crosstalk. Small-molecule tyrosine kinase inhibitors like Larotrectinib and entrectinib potently block TrkA/B/C signalling, reducing BDNF/NGF-mediated CSC enrichment and EMT in cancers such as lung, breast, and neuroblastoma. These FDA-approved agents (NTRK fusion-positive tumours) show clinical efficacy in shrinking Trk-driven lesions while overcoming plasticity-induced resistance; ongoing trials combine them with chemotherapy to target perineural invasion [210, 319]. Differently, monoclonal antibodies or decoy receptors against p75NTR can be used to interrupt low-affinity neurotrophins binding, suppressing SLUG induction and invasion in breast/glioma models [320–322]. Anti-BDNF or anti-NGF antibodies (e.g., tanezumab analogues repurposed for oncology) neutralize circulating ligands, curbing tumour angiogenesis and motility [323]. Surgical or pharmacological denervation (e.g., botulinum neurotoxin) severs peritumoral nerves, halting the release of neurotransmitters and neurotrophins that fuel tumour plasticity and EMT [324, 325]. Finally, the combinatorial therapy based on the use of neurotrophin receptor inhibitors and EMT reversers (e.g., TGF-β blockers), or metabolic disruptors, could exploit synthetic lethality in E/M states. Future strategies may pair these with CAR-T cells engineered against neural antigens to eliminate innervating glia/Schwann cells. Finally, ECM stiffness is mitigated by losartan or relaxin to decrease collagen crosslinking/HA accumulation, inhibiting integrin-FAK-YAP/TAZ mechanotransduction that locks E/M states; LOXL2 inhibitors (e.g., simtuzumab) prevent fibrosis [326, 327]. These approaches enhance chemotherapy/radiotherapy penetration and cooperate with immunotherapies like ICB by easing physical barriers and T-cell exhaustion. Targeting the TME offers a synergistic approach to limit EMP and its associated pro-tumorigenic effects, representing a strategy to overcome resistance, limit tumour plasticity, and improve overall clinical outcomes.

In conclusion, while the therapeutic strategies described above underscore the breadth of potential targets within EMP-associated signalling, metabolism, epigenetics, and the TME, a fundamental challenge remains: the very plasticity that renders EMP clinically dangerous could potentially allow cancer cells to circumvent therapeutic pressure. Unlike static oncogenic drivers, EMP is inherently dynamic. Cancer cells do not simply occupy a fixed epithelial or mesenchymal state but continuously shift along a phenotypic spectrum in response to microenvironmental cues, including those inadvertently generated by treatment itself. Therefore, targeting a single EMT state or a specific molecular mediator may be insufficient, as surviving cells can readily transition toward alternative E/M states, effectively escaping therapeutic intervention. This adaptive phenotypic switching, rather than classical genetic resistance, represents one of the most formidable obstacles in anti-EMP therapy, demanding strategies that account not only for the identity of the target, but for the fluidity of the system being targeted.

Unsolved questions and future directions

Despite significant advances, the study of EMP continues to raise fundamental questions regarding its regulation and functional relevance in tumour progression. Clarifying how EMP operates across distinct tumour types, microenvironmental contexts, and disease stages remains a central challenge, as does defining the contribution of transient or hybrid phenotypic states to metastatic dissemination and therapeutic resistance. The development of more sophisticated lineage-tracing systems, spatial transcriptomic approaches, and integrative multi-omic analyses will be critical to dissect these dynamic processes in vivo. Moreover, integrating EMP research with studies of immune modulation, stromal crosstalk, and metabolic adaptation may illuminate how these interconnected layers collectively shape tumour evolution. A mechanistic and context-dependent understanding of EMP may ultimately reconcile current discrepancies in the field and provide the basis for novel therapeutic strategies designed to constrain malignant plasticity, enhance treatment sensitivity, and limit disease recurrence.

Conclusion

Based on accumulating evidence, this review establishes EMP as the main driver of CSC self-renewal, intratumor heterogeneity, metastatic dissemination, and resistance to therapies across diverse malignancies. EMP states, enriched in CSCs, confer higher tumorigenicity through balanced epithelial-mesenchymal traits, dynamic metabolic shifts (glycolysis/OXPHOS/lipid rewiring), and TME synergies involving CAFs, TAMs, endothelial cells, immune cells, neural cells, and ECM stiffness. Sophisticated tools, from dual-fluorescent reporters and organoids to spatial transcriptomics, intravital microscopy, and AI-driven models, have recently helped in mapping EMP trajectories, validating its evolutionary fitness and biomarker potential. However, enduring gaps continue to hinder translation. Despite these current challenges, therapeutic optimism remains: single-pathway inhibitors (e.g., targeting TGF-β or Notch) often fail because redundancy and cellular plasticity enable compensatory adaptations, and metabolic disruptors can cause off-target toxicity unless they achieve true CSC specificity. Future advances demand multi-omics integration,

patient-derived avatars for combinatorial regimens (e.g., EMP-blockers + immunotherapy), and robust biomarkers to stratify hybrid-enriched subsets, ultimately dismantling this adaptive vulnerability to avert relapse. Given the redundancy and complexity of EMT regulatory networks, combination therapies targeting multiple facets of EMT alongside conventional chemotherapies, targeted therapies, or immunotherapies are likely necessary to prevent treatment resistance and metastatic relapse [328]. Rational design guided by biomarkers capable of identifying EMT and CSC states will improve patient stratification and therapeutic outcomes. In summary, therapeutic strategies targeting EMT and particularly E/M states in cancer encompass inhibition of signalling pathways, epigenetic modulation, metabolic disruption, and microenvironmental remodelling. As the understanding of EMT plasticity deepens, new, precise, and effective treatments aimed at eradicating the most aggressive CSC-enriched tumour cell populations are anticipated to emerge, offering renewed hope for combating metastatic disease.

Abbreviations

2DG	2-deoxy-D-glucose
ACC	Acetyl-CoA carboxylase
ACLY	ATP citrate lyase
ACP	Acyl Carrier Protein
ACSL	Acyl-CoA synthetase long-chain
ACSL	Acyl-CoA synthetases
ADP	Adenosine diphosphate
AhR	Aryl Hydrocarbon Receptor
AI	Artificial Intelligence
ALDH	Aldehyde dehydrogenase
ALDH1A3	Aldehyde Dehydrogenase 1 Family, Member A3
ALDOA	fructose-bisphosphate aldolase A
AMP	Adenosine monophosphate
AMPA	Alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	AMP-activated protein kinase
ARG1	Arginase-1
ARNT	Aryl hydrocarbon Receptor Nuclear Translocator
ATGL	Adipose Triglyceride Lipase
ATRA	all-trans retinoid acid
AXL	Receptor Tyrosine Kinase
BCSCs	Breast cancer stem cells
BDNF	Brain- derived neurotrophic factor
BLI	Bioluminescence imaging
BMP-7	Bone morphogenetic protein-7
BTBD7	BTB/POZ domain-containing protein 7
c-MET	Hepatocyte growth factor receptor
c-Myc	cellular Myelocytomatosis protein
CAFs	Cancer-associated fibroblasts
CAMK2	Calmodulin-dependent protein kinase II
CAR-T	Chimeric Antigen Receptor T-cell
CAV1	Caveolin-1
CCL2	C-C motif chemokine ligand 2
CDH1	Epithelial cadherin, E-CADHERIN
CDH2	Neural cadherin, N-CADHERIN
CHD1	Chromodomain Helicase DNA Binding Protein 1
ChREBP	Carbohydrate-Responsive Element-Binding Protein
CK8	Cytokeratin 8
CLDN	Claudin
CLRKeap1	Cullin-RING E3 ubiquitin ligase complex
CPT1	Carnitine palmitoyltransferase 1
CRC	Colorectal Cancer
CreER	Cre recombinase fused with Estrogen Receptor
CRIF1	CR-6 interacting factor 1
CSCs	Cancer Stem Cells
CSF1R	Colony Stimulating Factor 1 Receptor
CT	Computed tomography
CTCs	Circulating Tumour Cells
D1x-2	Distal-less homeobox-2
DNMT	DNA- methyltransferase
DNMTis	DNA methyltransferase inhibitors
DPYD	Dihydropyridine dehydrogenase
DVL3	Disheveled 3
E-H-M	epithelial–hybrid–mesenchymal
E/M	Hybdrid Epithelial/Mesenchymal
ECM	Extracellular matrix
EMP	Epithelial Mesenchymal Plasticity
EMT	Epithelial-mesenchymal transition
EMT-TFs	EMT-inducing transcription factors
ENO	Enolase
EPCAM	Epithelial Cell Adhesion Molecule
ESRP1	Epithelial Splicing Regulatory Protein 1
EZH2	Enhancer of zeste homolog 2
FAK	Focal adhesion kinase
FAO	Fatty acid beta-oxidation
FAP	Fibroblast Activation protein
FASN	Fatty acid synthase
FAT1	FAT Atypical Cadherin 1
FBP1	fructose-1,6-bisphosphatase 1
FDA	Food Drug Association
FFAs	Free fatty acids
FH	Fumarate hydratase
FRET	Förster Resonance Energy Transfer
Fsp1	Fibroblast-specific protein 1
GADPH	Glyceraldehyde-3-Phosphate Dehydrogenase
GDH	Glutamate dehydrogenase
GEMMs	Genetically engineered mouse models
GLN	Glutamine
GLS1	Glutaminase 1
GLUT1	Glucose Transporter 1
GPR91	G protein-coupled receptor 91
GPx2	Glutathione peroxidase 2
GRHL2	Grainyhead-like 2
GRNs	gene regulatory networks
GTP	Guanosine-5′-triphosphate
H3K27me3	Histone H3 protein trymethylated Lysine 27
H3K9me3	Histone H3 protein trymethylated Lysine 9
HDACi	Histone deacetylase inhibitor
HIF-1α	Hypoxia-Inducible Factor 1-alpha
HK2	Hexokinase 2
HMG-CoA	3-hydroxy- 3-methylglutaryl–coenzyme A
HMGA1	High Mobility Group A1
HOTAIR	HOX transcript antisense RNA
HSL	Hormone-Sensitive Lipase
ICB	Immune checkpoint blockage
IDH	Isocitrate dehydrogenase
IGF	Insulin-like growth factors
integrin αvβ3	αvβ3 vitronectin receptor
integrin-α5β1	Fibronectin Receptor
IVM	Intravital microscopy
JAK2	Janus Kinase 2
KLF4	Krüppel-like factor 4
Krt14CreTg	Keratin 14 Cre recombinase Transgenic
LDHA	Lactate dehydrogenase A
LEF	Lymphoid enhancer factor
LIN28/let-7	Lin28 homolog-mediated control of Lethal-7 maturation
LKB1	Liver Kinase B1
LOXL2	Lysyl oxidase homolog 2
LSH	Lymphoid-Specific Helicase
MALAT1	Metastasis Associated Lung Adenocarcinoma Transcript 1
MAPK	Mitogen-activated protein kinase
MCT1	Monocarboxylate Transporter 1
MDSCs	Myeloid-derived suppressor cells
MET	Mesenchymal-epithelial transition
MGL	Monoacylglycerol lipase

ML	Machine Learning
MMP	Matrix metalloproteinase
mtDNA	Mitochondrial DNA
mTOR	Mechanistic Target of Rapamycin
NAD+	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-B
NGF	Nerve growth factor
NICD	Notch Intracellular Domain
NK	Natural Killer
Notch-Dll4-JAG1	Notch- Delta-like 4 ligand -Jagged 1 ligand
NPC	nasopharyngeal carcinoma
NRF2	Nuclear factor erythroid 2-related factor 2
NRTN	Neurturin
NSCL	Non-small cell lung cancer
NTRK	Neurotrophic Tyrosine Receptor Kinase
OCT4	Octamer Binding Transcription Factor 4
OVOL2	Ovo Like Zinc Finger 2
OXPHOS	Oxidative phosphorylation
p75NTR	75 kDa neurotrophin receptor
PCGF1	polycomb group factor 1
PD-1	Programmed cell Death protein 1
PD-L1	Programmed death-ligand 1
PDAC	Pancreatic ductal adenocarcinoma
PDGF	Platelet-derived growth factor
PDGF-BB	Platelet-Derived Growth Factor-BB
PDOs	Patient-derived organoids
PFD	Pirfenidone
PFKFB-3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
PFKP	Phosphofructokinase, platelet
PGC-1α	Proliferator-activated receptor gamma coactivator 1-alpha
PGI	Phosphoglucose isomerase
PKCα	Protein kinase C alpha
PKM2	Pyruvate Kinase M2
PPARs	Peroxisome proliferator-activated receptors
PPP	Pentose phosphate pathway
PTX	Paclitaxel
RAS	Rat sarcoma virus family
Rho	Rho GTPase family
RNF4	Ring finger protein 4
ROS	Reactive oxygen species
SCD	Stearoyl-CoA desaturase
SCF	SKP1-Cullin-F box (E3-ubiquitin ligase complex)
scRNA-seq	Single-cell RNA sequencing
SDHAF2	Succinate Dehydrogenase Complex Assembly Factor 2
SDHB	Succinate dehydrogenase complex iron-sulfur subunit B
Siah2	Seven in absentia homolog 2 (E3-ubiquitin protein ligase)
SMAD	SMA (Caenorhabditis elegans worm) gene and MAD (Mothers Against Decapentaplegic) gene fusion
SOX2	Sex-determining Region Y-related High mobility group-box 2
Src	Proto-oncogene tyrosine-protein kinase
SREBP1	Sterol Regulatory Element-Binding Protein 1
STAT3	Signal Transducer and Activator of Transcription 3
SUCNR1	Succinate receptor 1
TAM	Tumor-associated macrophage
TAZ	Transcriptional coactivator with PDZ-binding motif
TCA	Tricarboxylic Acid Cycle
TCF	T-cell factor
TKI	Tyrosine Kinase Inhibitors
TLR	Toll-like receptor
TME	Tumor microenvironment
TNBC	Triple negative breast cancer
TNF-α	Tumor Necrosis Factor-alpha
TRAP1	Tumor necrosis factor receptor-associated protein 1
Trk	Tropomyosin receptor kinase B
Trp53Fl	"floxed"Transformation-related protein 53
VEGF	Vascular endothelial Growth Factor
VIM	Vimentin
Wnt	Wingless-related integration site
YAP1	Yes-associated protein 1
ZEB	Zinc finger E-box-binding homeobox
ZO-1	Zonula Occludens-1
α-KG	alpha-ketoglutarate
α-SMA	Alpha-smooth muscle actin
β-catenin	Beta catenin
β-TrCP	Beta-transducin repeat-containing protein

Authors' contributions

L.C., F.V., K.S., and S.D.F. wrote the main parts of the manuscript and prepared the figures. O.R.B., S.D.B., N.D.M., and S.D.F. contributed to the drafting of the manuscript. G.S. revised the manuscript during the editing process. S.D.F. conceptualized the manuscript, contributed to bibliography compilation, and supervised all stages of the manuscript preparation, including the manuscript structure and figure design. All authors have reviewed and approved the final version of the manuscript.

Funding

The research leading to these results has received funding from AIRC Individual Grant – MFAG (28761), PRIN Progetti di Rilevante Interesse Nazionale 2022 (Prot. 2022J8×7PJ) and 2022 PNRR (Prot. P2022YAKJY), and PNRR MCNT1 2023 (12378252) to S.D.F. S.D.B., G.S., and S.D.F. received funding from the European Union – NextGenerationEU initiative under the Italian Ministry of University and Research as a part of the PNRR–M4C2–I1.3 Project PE00000019 'HEAL ITALIA' CUP B73C22001250006. G.S. and S.D.F. received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101119464.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 11 February 2026 / Accepted: 13 May 2026
Published online: 25 May 2026

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