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Review article

Despicable role of epithelial–mesenchymal transition in breast cancer metastasis: Exhibiting *de novo* restorative regimens

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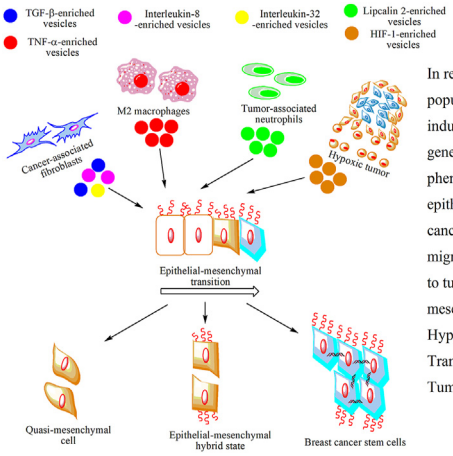
HIGHLIGHTS

- Epithelial-to-mesenchymal transition (EMT) induces phenotypic changes necessary for tumor cell survival and progression.
- Other cell populations, such as fibroblasts, immune and hypoxic cells, introduce EMT-promoting factors.
- Stress-related stimuli such as hypoxia, chemotherapy, and nutrient deprivation promote EMT in tumors.

GRAPHICAL ABSTRACT



In response to various stimuli, cell populations generate EMT factors to induce EMT in cancer cells. EMT generates various cancer cell phenotypes, such as stem-like, hybrid epithelial-mesenchymal, and dormant cancer cells. These cells grant invading, migratory, and tumorigenic properties to tumor cells. EMT: Epithelial-to-mesenchymal transition; HIF-1: Hypoxia-inducible factor 1; TGF-β: Transforming growth factor-β; TNF-α: Tumor necrosis factor α.

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ABSTRACT

Breast cancer (BC) is the most prevalent cancer in women globally. Anti-cancer advancements have enabled the killing of BC cells through various therapies; however, cancer relapse is still a major limitation and decreases patient survival and quality of life. Epithelial-to-mesenchymal transition (EMT) is responsible for tumor relapse in several cancers. This highly regulated event causes phenotypic, genetic, and epigenetic changes in the tumor microenvironment (TME). This review summarizes the recent advancements regarding EMT using de-differentiation and partial EMT theories. We extensively review the mechanistic pathways, TME components, and various anti-cancer adjuvant and neo-adjuvant therapies responsible for triggering EMT in BC tumors.

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Information regarding essential clinical studies and trials is also discussed. Furthermore, we also highlight the recent strategies targeting various EMT pathways. This review provides a holistic picture of BC biology, molecular pathways, and recent advances in therapeutic strategies.

Introduction

Breast cancer (BC) is the most common type of cancer diagnosed in women. In 2020, approximately 2.26 million women were diagnosed with BC, with 685,000 deaths globally. BC leads to the most disability-adjusted life years in women compared to other cancers. By 2040, new BC cases will increase by 31.2%.[1,2]

BC can arise in the duct epithelium (85%) or the lobules in glandular tissues (15%).[3] As with other solid tumors, BC metastasis is primarily responsible for patient mortality.[4] Epithelial-to-mesenchymal transition (EMT) is crucial in tumor pathophysiology, where epithelial cells lose their polarity, morphology, and cell–cell adhesion. BC cells gain migratory and invasive phenotypes to transform into mesenchymal cells.[5] EMT also induces stem-like phenotypes such as self-renewal, pluripotency, and tumorigenicity to circulating tumor cells (CTCs) responsible for the tumorigenic potential. The tumorigenic potential is essential for CTCs to adapt and develop secondary tumors in a new environment.[6] Owing to the massive impact of EMT on BC progression, various clinical trials have been initiated to target EMT in BC progression [Table 1].

EMT is responsible for differentiating and de-differentiating cancer cells in the tumor microenvironment (TME). EMT partially de-differentiates cancer cells from their progenitors, breaking the barriers to differentiate into new cell types.[14] During EMT, terminally differentiated cells are reprogrammed to acquire new phenotypes required for invasion, migration, and stem cell-like behavior.[15]

However, whether EMT leads to CSC development is unknown. Cancer epithelial cells rarely achieve complete mesenchymal phenotypes after EMT and only attain partial mesenchymal characteristics.[16] However, EMT transforms epithelial cells to express epithelial and mesenchymal characters[17]; Grosse-Wilde *et al*[18] reported epithelial and mesenchymal phenotypes in the same cell after EMT. These hybrid cells form during the mesenchymal conversion of epithelial cells during EMT or the epithelial conversion of mesenchymal cells during the mesenchymal-to-epithelial transition (MET). Hybrid cells demonstrate better stemness, self-renewability, and spheroid-forming abilities than completely differentiated epithelial or mesenchymal cells.[18] This event is known as partial EMT and is responsible for developing stemness in the cancer cells.[19]

In this article, we reviewed the role of EMT in tumor progression, metastasis, chemoresistance, and cancer cell differentiation. We also discussed the molecular mechanisms and epigenetic modulations responsible for triggering EMT in BC cells. The phenotypic changes caused by EMT in the cancer cells and the various strategies to inhibit EMT-causing pathways were elucidated. We also discussed the importance of phenotypic changes caused by EMT in tumor progression and metastasis. The role of the TME in triggering metastasis is mechanistically reviewed, and we discuss the role of cancer-associated fibroblasts (CAFs) and immune cells such as macrophages and neutrophils. Additionally, regarding the mechanistic role of EMT in inducing the events above, we summarize recent drugs and potential strategies to inhibit metastasis and EMT-induced chemoresistance in BC.

Phenotypical changes caused by epithelial-to-mesenchymal transition in breast cancer cells

EMT is a reversible event that converts epithelial cells to quasi-mesenchymal cells, causing them to lose their cobble-shaped morphology to become spindle-shaped mesenchymal cells.[20] Actin stress fibers, a crucial part of the cellular cytoskeleton, are reorganized to transform the shape into quasi-mesenchymal cells.[21] Adherens, tight junctions, gap junctions, and desmosomes tightly hold cells in epithelial sheets. These junctions are formed with the assistance of E-cadherin molecules, and epithelial cells demonstrate apical-basal polarity and interact with the underlying basal membranes via α6β4 integrins and hemidesmosomes.[22] EMT-mediated gene alteration includes down-regulating genes associated with the epithelial cell phenotypes and upregulating mesenchymal genes. The expression of genes associated with epithelial adhesion proteins such as occludin, E-cadherin, and Traffic jam 1 are underexpressed with subsequent increases in mesenchymal adhesion protein (N-cadherin), fibronectin, and vimentin.[23] Basal membrane degradation is necessary for transformed cancer cell migration and dissemination.[24]

Studies have also supported EMT-related transcription factor (TF)-mediated dedifferentiation of cells. During embryogenesis, Slug expression converts mammary luminal cells to stem cells.[25] Twist overexpression in mesenchymal cells prevents their maturation to the cartilage and bones and maintains their immature phenotypes.[26] The signals for EMT originate from the tumor stroma surrounding cancer epithelial cells. Signals from the TME induce the expression of

Table 1
Drugs and combinations in various phases of clinical trials to target EMT-mediated tumor progression in breast cancer.

NCT No.	Title	Therapeutic intervention	Sponsor/collaborators	Phase	Reference
NCT01861054	Pilot study to evaluate safety & biological effects of orally administered reparixin in early breast cancer	Reparixin	DompÃ© Farmaceutici S.p.A	Phase 2	7
NCT01952054	Denosumab for breast cancer with bone mets	Denosumab	M.D. Anderson Cancer Center, Amgen	Phase 2	8
NCT02001974	Pilot study to evaluate reparixin with weekly paclitaxel in patients with HER2-negative metastatic breast cancer (MBC)	Paclitaxel + reparixin	DompÃ© Farmaceutici S.p.A, PRA Health Sciences	Phase 1	9
NCT03285607	MCS110 combined with neoadjuvant doxorubicin, cyclophosphamide, and weekly paclitaxel in patients with hormone-receptor Positive and HER2- breast cancer	Biological: MCS110, doxorubicin, cyclophosphamide, paclitaxel	Washington University School of Medicine, Novartis Pharmaceuticals	Phase 1	10
NCT04664829	The role of bexarotene in inducing susceptibility to chemotherapy in metastatic TNBC	Bexarotene, capecitabine	National Cancer Centre, Singapore, National Medical Research Council (NMRC), Singapore	Phase 1	11
NCT03306472	A pre-operative window study of letrozole plus PR agonist (megestrol acetate) versus letrozole alone in post-menopausal patients with ER-positive breast cancer	Megestrol acetate, letrozole	Cambridge University Hospitals NHS Foundation Trust, Anticancer Fund, Belgium	Phase 2	12
NCT02602938	Aspirin on CTCs of advanced breast and colorectal cancer	Aspirin	Zhejiang Provincial People's Hospital	Phase 2	13

CTC: Circulating tumor cells; ER: Estrogen receptor; HER: Human epithelial growth factor receptor; MBC: Metastatic breast cancer; MCS110: Lacnotuzumab; NCT: National Clinical Trial; NHS: National Health Service; NMRC: National Medical Research Council; PR: Progesterone receptor agonist; TNBC: Triple-negative breast cancer.

EMT-associated TFs in the epithelial cells. These TFs are responsible for developing EMT-associated machinery inside the epithelial cells.[27] Paracrine signaling by CAFs[28] and macrophages[29] induces EMT in primary tumors.[27,30]

The epithelial phenotype demonstrates high proliferation capabilities, whereas the mesenchymal phenotype demonstrates migration and invasion capabilities. Hence, converting epithelial cells to mesenchymal cells and vice versa is crucial for secondary tumor generation and proliferation.[31] Spheroid formation is positively correlated with CSCs and their tumorigenic potential.[32] Primary tumors that undergo EMT demonstrate a 10-fold higher spheroid forming potential. Twist1 and Snail overexpression are correlated with a 30- and 2-fold increase in the tumor spheroid forming and tumor-initiating potential, respectively.[33,34] Furthermore, $CD24^{-}$ cancer cells demonstrate greater stemness and migratory potentials, and downregulating CD24 is achieved by transforming growth factor-β (TGF-β)-triggered EMT signaling.[35,36] Figure 1 represents the phenotypic and metastatic changes caused in epithelial cells after EMT.[22]

Role of epithelial-to-mesenchymal transition in tumor migration and initiation at distant anatomical sites

EMT is a pleiotropic event affecting several TME processes, including cell migration, morphological changes, chemoresistance, and genetic alterations.[37] Changes in metastatic cells are closely associated with modifications caused by EMT. However, whether EMT is responsible for tumor metastasis is still unclear. Nonetheless, ZEB1 upregulation is reportedly associated with cell migration and tumor initiation in adenocarcinoma.[38] Additionally, Snail was necessary for cancer cell dissemination in a mouse model.[39] Both these markers are upregulated during EMT in cancer cells.[39]

However, CTCs present as single cells and cell clusters. These cell clusters express E-cadherin; hence, the theory of EMT, i.e., that "The expression of E-cadherin is inhibited after EMT takes place" is contradicted.[40,41] In contrast, the hypothesis of partial EMT is strengthened by this observation. However, cells leading the invading cell clusters demonstrate gene expressions agreeing with activation by EMT.[42]

However, EMT alone fails to explain colonization in new tissues after cell shedding.[43] Subsequent steps after cell shedding involve seeding disseminated clusters in the parenchyma of the destination tissue and forming secondary tumors. After arrival at the new tissue environment, the cells must undergo adaptive mechanisms to survive in the new environment. However, the environment in new tissues is likely unfavorable for secondary tumor growth.[44,45] Thus, the chances of successful colonization are extremely low. Therefore, after migration, the migratory mesenchymal cells revert to epithelial cells to acquire the required epithelial phenotypes to grow new tumors successfully.[46] This event is known as MET. MET is characterized by inhibiting EMT-related TF release. Messengers such as cyclic adenosine monophosphate (cAMP) are released, assisting in MET.[47] Furthermore, epithelial cell markers such as E-cadherin are re-expressed. This process is responsible for forming secondary tumors, in which cells are organized hierarchically in their epithelial and stromal states to form new tumors.[22] The absence of the signals produced by the TME of primary tumors is also hypothesized to be responsible for restoring the epithelial phenotypes in migratory mesenchymal cells.[48]

Mechanistic pathways involved in triggering epithelial-to-mesenchymal transition

The signals from tumor stroma initiate EMT pathways in epithelial cells, resulting in them developing mesenchymal phenotypes. EMT is performed by several TFs, such as Twist, Snai1 (Snail), Snai2 (Slug), Zeb1, and Zeb2.[49] These TFs downregulate epithelial gene expression in epithelial cancer cells.[50]

Transforming growth factor-β pathway

The TGF-β pathway is one of the most important pathways in cancer EMT.[51] This pathway is triggered by binding specific ligands to TGF-β

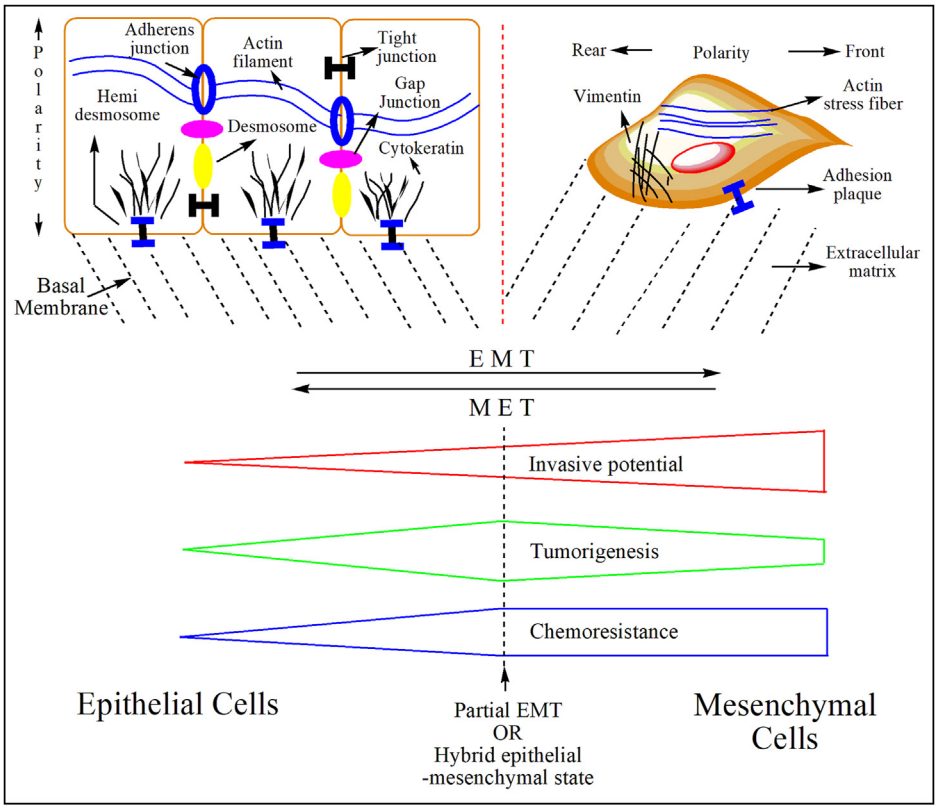

Figure 1. Representation of phenotypic changes induced by EMT. The epithelial cells maintain polarity and interact with each other and the basal membrane through junctions. However, cancer cells lose their polarity and interactions with neighboring cells after losing the epithelial phenotype. The variation in invasive, tumorigenic, and chemoresistant potential when epithelial cells transform during EMT is also epitomized. EMT: Epithelial-to-mesenchymal transition; MET: Mesenchymal-to-epithelial transition.

receptor type 1 (TGF-βR1) and TGF-βR2 [Figure 2]. The specific ligands activating this pathway include TGF-β (three isoforms), bone morphogenetic proteins, and activins.[52] TGF-βs binding to TGF-βR1 and TGF-βR2 leads to SMAD2 and SMAD3 phosphorylation, further complexing with SMAD4. Activating TGF-β receptors with bone morphogenetic proteins leads to SMAD1 and SMAD5 phosphorylation, which is also complex with SMAD4.[22] This SMAD trimeric translocates to the cell nucleus and is a transcription regulator for acquiring mesenchymal phenotypes in epithelial cells. SMAD complexes downregulate E-cadherin and upregulate mesenchymal proteins N-cadherin, vimentin, and fibronectin.[53] Various TFs associated with EMT, such as Twist, Slug, Snail, and Zeb1, are overexpressed in the transforming epithelial cells. These TFs increase TGF-β receptor expression through a positive feedback mechanism.[54]

Another member of the TGF-β family, growth differentiation factor 10 (GDF-10), is associated with BC progression. Zhou *et al*[55] conducted micro ribonucleic acid (miRNA) sequencing of specimens obtained from human triple-negative BC (TNBC) and observed a negative correlation between GDF-10 and BC progression. *In vitro* studies on TNBC cells revealed downregulating GDF-10 upregulated EMT phenotypes through the TGF-β pathway. GDF-10 expression inhibited cell proliferation and EMT in BC cells by upregulating SMAD7 and blocking nuclear SMAD4 translocation. The anti-metastatic activity of GDF-10 is also translated in *in vivo* BC xenograft models, thus showing its potential for clinical translation.[55]

MHP-1, a polysaccharide isolated from *Mortierella hepiali*, inhibited BC metastasis *in vitro* and *in vivo* by selectively inhibiting the TGF-β pathway. MHP-1 re-sensitized the topotecan-resistant MCF-7 cell line with mesenchymal phenotypes. MHP-1 also decreased MDA-MB-231 cell metastasis in the xenografts model and reduced vimentin and ALK5 expression.[56] Alpha-lipoic acid suppresses metastatic MDA-MB-231 and 4T1 cell migration and inhibits TGF-β-induced angiopoietin-like 4.[57]

Wingless-related integration site signaling pathway

Nineteen ligands activate the wingless-related integration site (Wnt) signaling pathway upon binding with the frizzled family of Wnt membrane receptors. The Wnt pathway includes canonical and non-canonical signaling pathways. Activating the canonical pathway leads to β-catenin nuclear translocation.[22] Nuclear β-catenin is a cofactor for expressing various genes related to cell proliferation, differentiation, and tumorigenesis [Figure 2].[58] E-Cadherin uses β-catenin to form adherens complexes. β-Catenin also connects the cytoskeleton with cell surface adherens junctions. Hence, when the epithelial character is lost in epithelial cells, β-catenin is free for nuclear translocation.[59]

Activating Wnt pathways is further responsible for upregulating EMT-inducing TFs.[60] Canonical Wnt pathway activation through recombinant Wnt3A led to Slug and Twist upregulation in BC.[61] To increase their expression, β-catenin directly interacts with the promoters of EMT-inducing TFs, such as Twist, Zeb1, and Slug.[48] Inhibiting secreted frizzled-related protein-1 (SFRP1), a Wnt signaling pathway inhibitor, induces EMT in BC cells, confirming the central role of Wnt signaling in EMT.[62] In contrast, the non-canonical Wnt pathway induces EMT through the protein kinase-C-dependent route. Activating the non-canonical pathway leads to vimentin and Snail upregulation and E-cadherin downregulation.[63,64]

Manganese-12 acetate potentially blocks the Wnt/β-catenin pathway by suppressing AKT and phosphoinositide 3-kinase (PI3K) phosphorylation. Cell migration and invasion capabilities in MCF-7 and MDA-MB-231 cell lines were significantly reduced. Furthermore, N-cadherin downregulation and E-cadherin upregulation were confirmed through western blotting. Real-time-polymerase chain reaction (PCR) confirmed the downregulation of EMT-related TFs such as Zeb1, Slug, Snail, and Twist1. Notably, the messenger RNA (mRNA) and protein levels of programmed death-ligand 1 (PD-L1) were also suppressed. Thus, manganese-12 acetate showcased a huge potential to manage BC metastasis by targeting major metastatic pathways.[65]

The long noncoding RNA (lncRNA) HOTTIP also triggers EMT and induces migration and invasion in BC cells. Silencing HOTTIP using short hairpin RNA inhibited the activity of the lncRNA in MDA-MB-231 and MDA-MB-468 cell lines. This EMT inhibitory activity was also translated in *in vivo* BC models by blocking the Wnt/β-catenin pathway.[66]

NOTCH pathway

The NOTCH pathway controls cell proliferation and differentiation. Jagged family and delta-like ligands bind to four NOTCH receptor isoforms (NOTCH1–NOTCH4).[67,68] After ligands bind to NOTCH receptors, a cleavage process frees an intracellular NOTCH fragment. This fragment travels to the nucleus, causing EMT-specific genetic expressions [Figure 2].[69] The NOTCH pathway promotes Slug and Snail and downregulates E-cadherin expression.[70] This pathway also causes CD44 receptor overexpression in cancer cells.[71] However, NUMB represses the NOTCH pathway by metabolizing cleaved NOTCH fragments and inhibiting EMT in BC cells.[72] The epigenetic regulations performed by miRNAs and lncRNAs on the NOTCH pathway were previously reviewed.[73]

Phosphoinositide 3-kinase/protein kinase B signaling pathway

The PI3K/protein kinase B (AKT) signaling pathway involves different cellular physiological processes such as cell cycle, growth,

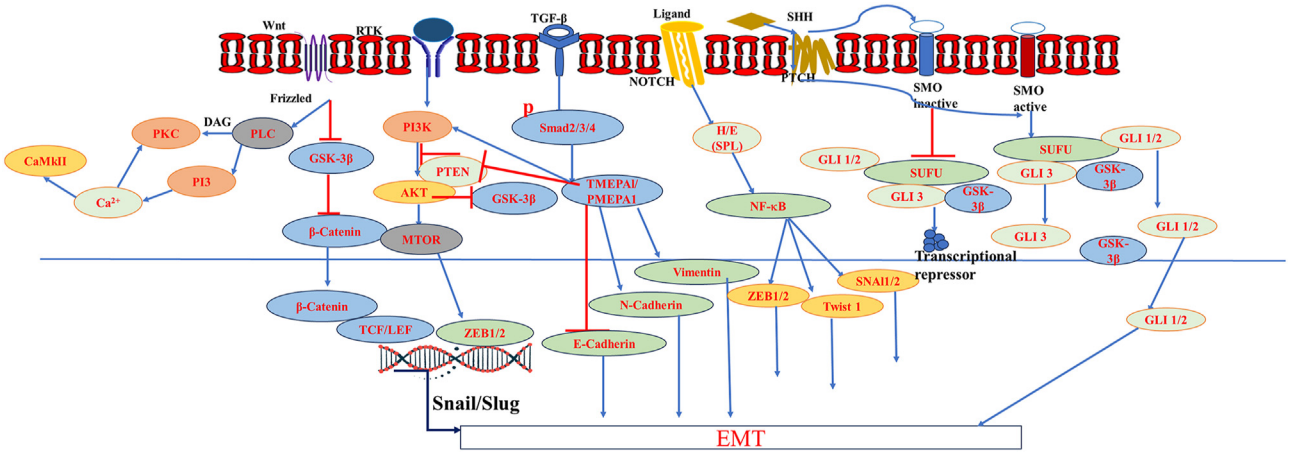

Figure 2. Mechanistic representation of the WNT, PI3K/AKT, NOTCH, Hedgehog, and TGF-β pathways responsible for EMT induction in BC. AKT: Protein kinase B; BC: Breast cancer; EMT: Epithelial-to-mesenchymal transition; H/E: Hematoxylin and eosin; NF-κB: Nuclear factor kappa B; PI3K: Phosphoinositide 3-kinase; TGF-β: Transforming growth factor-β; WNT: Wingless-related integration site.

proliferation, and apoptosis via effector molecules acting downstream of the pathway.[74] PI3K/AKT is a major regulatory factor during cell survival in stressful environments, such as in tumors; therefore, PI3K/AKT plays a crucial role in cancer development.[74,75] The association between PI3K/AKT and EMT has been established in different cancers. Twist overexpresses the *AKT2* gene, leading to invasion and drug resistance in BC cells. However, Twist-induced metastasis, invasion, and resistance to paclitaxel were inhibited by silencing AKT. Thus, Twist and AKT maintain a positive feedback loop in enhancing pro-EMT function [Figure 2].[76] PI3K and phosphoinositide-dependent kinase-1 (PDK) expression increase AKT activity and p65 subunit expression of nuclear factor kappa B (NF-κB), participants of EMT induction in BC.[77] Cyclin-dependent kinase-6 (CDK-6) upregulates PI3K expression and silences CDK-6-regulated PI3K, further downregulating the efflux P-gp protein and leading to chemo-sensitization of cancer cells.[78]

Hedgehog signaling

The Hedgehog (Hh) signaling pathway plays a major role during embryonic development. This signaling cascade activates after binding the Hh protein with a transmembrane receptor called "patched." Patched activation leads to glioma-associated oncogene (*GLI*) activation via another intermediate protein called "smoothened." Subsequently, *GLI* mediates the expression of downstream target genes. Canonical and non-canonical Hh signaling is crucial in BC development.[79] In the case of estrogen-positive (ER+) BC, estrogen increases SHH and GLI1, leading to an increase in invasive characteristics of T47D (HER2−) and BT-474 (HER2+) cells.[80] Furthermore, more than one pathway leads to GLI activation in TNBC. Apart from GLI activation via the Hh pathway, NF-κB and forkhead box C1 protein (FOXC1) reportedly activate GLI1 and GLI2 in TNBC. Moreover, GLI1 increases the metastatic potential of BC cells by increasing the levels of the C-X-C chemokine receptors CXCR4/CXCR7.[79] LCP1/L-PLASTIN, a chemokine ligand 12 (CXCL12)/CXCR4 signaling mediator, is expressed by GLI1.[81] These results indicate that inhibiting GLI1 could be a rational therapeutic approach to reducing the metastatic burden in BC. Figure 2 pictorially represents the major mechanistic pathways responsible for EMT in BC.

Role of the tumor microenvironment in epithelial-to-mesenchymal transition

Tumorigenesis is associated with disrupting the normal balance in surrounding tissues and developing a microenvironment more suitable for rapid cell growth. EMT in the neoplastic cells is controlled by stromal cells in TME. CAFs, immune cells, and vascular cells are primarily responsible for regulating EMT in epithelial cells.

Cancer-associated fibroblasts

CAFs involve various neoplastic cells crucial in tumor progression.[82] CAFs are differentiated from several cells, including CSCs, cancer fibroblasts, endothelial cells, and stellate cells.[83] The TME also contains non-neoplastic fibroblasts; however, they do not participate in tumor signaling and management like CAFs. CAFs express higher α-smooth muscle actin than normal breast fibroblasts (NBFs). CAFs release various cytokines and chemokines such as tumor necrosis factor (TNF)-β, TGF-β, interleukin-8 (IL-8), and CC motif chemokine ligand 17 (CCL17), which remodel the TME and cause phenotypic changes in BC cells [Tables 2 and 3].[82,84] TGF-β1 is also responsible for apoptotic resistance in BC CAFs via the p-44/42/mitogen-activated protein kinase (MAPK) signaling pathway.[85] CAFs co-cultured with MCF-7 cell lines induced higher N-cadherin and vimentin expression while reducing E-cadherin expression. CAFs increased the invasiveness of MCF-7 cells by inducing such changes, whereas NBFs could not.[86]

Mechanistic pathway for the continuous active state of cancer-associated fibroblasts

Tang *et al*[87] reported an essential pathway responsible for the continuous active state of CAFs to fuel BC progression and metastasis: the autocrine TGF-β1/miR-200s/miR-221/DNMT3B regulatory loop signaling pathway. CAFs could remain active without TME and cancer epithelial cells *in vitro*.[103,104] DNMT3B decreases tumor suppressor miRNA-200b, miRNA-200c, and miRNA-221 levels through DNA methylation of their promoters. This continuous suppression transformed the NBFs into CAFs. Inhibiting the TGF-β1/miR-200s/miR-221/DNMT3B regulatory loop led to miRNA-200b and miRNA-200c demethylation, restoring the NBFs phenotypes in CAFs. Thus, the epigenetic modulations performed by this feedback loop are critical to maintaining an active CAF status in BC cells.[87]

Conversion of normal breast fibroblasts to cancer-associated fibroblasts

Li *et al*[89] reported the role of BC cells in transforming NBFs into CAFs. BC epithelial cells release exosomes enriched with survivin. The survivin-rich exosomes activate NBFs into CAFs by upregulating superoxide dismutase 1 (SOD1). These activated fibroblasts induced BC progression and metastasis.[89] Furthermore, Soon *et al*[105] isolated CAFs and NBFs from patients with BC; CAFs had increased resistance towards doxorubicin compared to NBFs. CAFs also demonstrated an increased level of pro-invasion biomarkers such as IL-6, CCL2, fibroblast growth factor 7 (FGF7), matrix metalloproteinase 2 (MMP2), MMP9, and MMP11 compared to the NBFs.[105]

TGF-β1 orchestrates EMT in BC cells via autophagy; TGF-β1 stimulates CAF-orchestrated tumor progression, EMT, and pulmonary metastasis through autophagy and overexpression of fibroblast activation

Table 2
Pathways for CAF-induced EMT for its prevention.

Biomarker	Mechanistic pathway	Pharmacological action	Reference
TGF-β1	p-44/43/MAPK pathway	Resistance to apoptosis in BC CAFs	85
DNMT3B	TGF-β1/miR-200s/miR-221/DNMT3B regulatory loop	Transformation of NBFs to CAFs	87
TGF-β1	Autophagy and FAP-α overexpression	EMT and pulmonary metastasis	28
IL-32	p-38/MAPK signaling pathway	EMT	88
Exosomal survivin	Upregulation of SOD1	Transformation of NBFs to CAFs	89
TGF-β1	Upregulation of lncRNA HOTAIR	EMT	90
IL-8	Upregulation of S100A8	Enhanced invasive capabilities	91
Exosomal miR-181d-5p	Downregulation of CDX2 and HOXA5	EMT	92
miR-146a	Suppressing TXNIP that potentiated Wnt signaling	EMT	93
miR-335	Downregulation of CDH11	Downregulation of EMT	94
miRNA Let-7b	IL-8 inhibition	Inhibition of CAF-mediated EMT	95
CCL17	HIC1 inhibition	Tumor progression and metastasis	96

BC: Breast cancer; CAF: Cancer-associated fibroblast; CCL17: CC motif chemokine ligand 17; EMT: Epithelial-to-mesenchymal transition; FAP-α: Fibroblast activation protein-alpha; HOTAIR: HOX antisense intergenic ribonucleic acid; IL: Interleukin; lncRNA: Long noncoding ribonucleic acid; MAPK: Mitogen-activated protein kinase; miRNA: Micro ribonucleic acid; NBF: Normal breast fibroblast; SOD1: Superoxide dismutase 1; TGF-β1: Transforming growth factor beta 1; Wnt: Wingless-related integration site.

Table 3
Recent targets and strategies to target CAF-mediated EMT in BC.

Therapeutic entity	Target	Mechanism of action	Reference
Pirfenidone	Repression of PD-L1 expressions	Inhibiting cytokines	97
Pirfenidone	Suppression of YAP and CD44	Reduction of EMT and stemness in BC cells	98
Artemisinin	Suppression of TGF-β	Inhibition of EMT and inactivation of CAFs	99
Inhibition of TGF-β1 by siRNA	Suppression of p-44/42 leading to suppression of MAPK signaling pathway	Sensitization of CAFs to 5-FU and tamoxifen	85
NBFs released OPG	Blocking the Wnt/β-catenin pathway	Inhibition of EMT and metastasis	100
miR-4516	Expression of FOSL1	Suppression of CAF metastatic abilities	101
Valsartan	Decrease in IL-6 mRNA levels	Inhibition of pulmonary metastasis	102

5-FU: 5-Fluorouracil; BC: Breast cancer; CAF: Cancer-associated fibroblast; EMT: Epithelial-to-mesenchymal transition; IL: Interleukin; MAPK: Mitogen-activated protein kinase; NBF: Normal breast fibroblast; OPG: Osteoprotegerin; PD-L1: Programmed death-ligand 1; siRNA: Small interfering RNA; TGF-β1: Transforming growth factor beta 1; Wnt: Wingless-related integration site.

protein-alpha (FAP-α). However, autophagy inhibitor 3-methyladenine blocked TGF-β1 mediated metastasis in BC.[28] IL-32, another cytokine overexpressed in the CAFs, binds to BC cells via integrin β3 receptors overexpressed on the BC epithelial cells. IL-32 overexpression causes EMT-related changes in the epithelial cells by acting through the p-38/MAPK signaling pathway.[88]

Mechanistic pathways responsible for cancer-associated fibroblast-induced epithelial-to-mesenchymal transition in breast cancer

Tumor suppressor breast cancer gene 1 (*BRCA1*) is essential in orchestrating CAF-induced metastasis. Mutated *BRCA1* cells were co-cultured with HCC1937 cells, and CAFs demonstrated increased EMT-specific biomarkers. These transformed CAFs triggered EMT in BC cells.[106] Furthermore, TGF-β1 is responsible for inducing EMT in BC cells. The CAFs-conditioned culture medium enhanced lncRNA HOX antisense intergenic RNA (HOTAIR) in BC cells, and the promoter site of HOTAIR is directly bound to downstream TGF-β proteins, i.e., SMAD2, 3, and 4. Hence, TGF-β pathway stimulation leads to HOTAIR upregulation. Downregulating HOTAIR inhibits EMT in BC cells.[90] The mechanistic pathways and therapeutic targets to prevent CAF-induced EMT are summarized in Tables 2 and 3.

CAFs also induce EMT in breast epithelial cells by upregulating IL-8. IL-8 upregulates S100A8 expression in non-invasive BC cells to render them invasive characteristics. S100A8 is a calcium-binding protein belonging to the S-100 family. S100A8 overexpression is also associated with cell migration in colon, breast, and lung tumors. S100A8 is upregulated by IL-8-mediated TFs p65, NF-κB, and CEBPβ upregulation.[91] BC CAFs treated with 20 ng/mL of docetaxel upregulated IL-8 in BC CAFs. Hence, IL-8 may be important in chemoresistance and tumor survival.[107]

CAFs also play a role in mediating epigenetic modulations via miRNAs. CAFs secrete miR-181d-5p-enriched exosomes that potentiated cell proliferation and EMT in BC cells. miR-181d-5p promoted BC cell proliferation and EMT by downregulating CDX2 and HOXA5. HOXA5 overexpression in BC cells increases proliferation, migration, invasion, and EMT.[92] Furthermore, BC cell-derived miR-146a can enrich exosomes to convert NBFs to CAFs and induce EMT in the BC epithelial cells. miR-146a orchestrated this action by suppressing TXNIP, which stimulated the Wnt pathway.[93] *CDH11* genes in CAFs are associated with metastasis in BC cells. CDH11 overexpression leads to vimentin, fibronectin, and β-catenin overexpression in BC cells.[108] miR-335 expression downregulates CDH-11 and the biomarkers above in BC cells. Hence, miR-335 epigenetically suppresses EMT in BC cells. Furthermore, anti-CDH11 antibodies suppressed the EMT in BC cells and inhibited BC metastasis.[94]

Let-7b miRNA of the Let-7 family is an IL-8-mediated BC metastasis inhibitor.[109] Hence, Let-7b is mechanistically inhibited in CAFs to continue IL-8-mediated metastasis in BC cells.[95] However, NBFs demonstrate higher Let-7b expression than CAFs. Inhibiting Let-7b in NBFs activates CAFs through the IL-6-dependent positive feedback loop. Increased Let-7b expression in CAFs led to CAF inactivation by blocking the IL-6-dependent positive feedback loop. Furthermore, an increase in Let-7b also suppressed IL-8 expression, suppressing BC metastasis and tumor growth.[95]

CAFs release CCL17 in high concentrations, leading to EMT and BC progression.[110] Inhibiting the HIC1 protein in pre-metastatic BC cells is essential for CCL17-dependent metastasis. $HIC1^-$ epithelial cells released CXCL14, which bound to its cognate GPR85 receptors on the mammary fibroblasts to transform them to CAFs following the AKT, extracellular signal-regulated kinase 1/2 (ERK1/2), and neddylation pathways. These activated CAFs then release CCL17 for tumor progression and metastasis in BC.[96]

Strategies to prevent cancer-associated fibroblast-induced epithelial-to-mesenchymal transition in breast cancer

Pirfenidone (PFD), a small pyridine class molecule, has recently gained attention for its ability to target CAFs. PFD is an orally active U.S. Food and Drug Administration-approved drug commercially used to treat lung fibrosis.[111] PFD inhibited IL-6, IL-8, TNF-β, CCL17, and CXCL14 release from CAFs and downregulated PD-L1 expression by downregulating inflammatory mediators. PD-L1 overexpression inactivates T cells in the BC TME.[97] The potential of PFD to suppress EMT-induced stemness in BC cells has also been reported. Overexpressing yes-associated protein-1 (YAP1) was associated with increased stromal characteristics and induced EMT. A positive correlation was also observed between YAP1 and EMT-associated gene expression, such as ZEB and vimentin in BC cells. PFD also reduced the invasiveness of invasive BC cells and CAFs. The treatment could reduce EMT-associated biomarkers. Notably, stemness-associated proteins, such as YAP1 and CD44, were also reduced.[98] Hence, PFD shows potential to be used with first-line chemotherapeutic and immunotherapeutic drugs in BC therapy.

Artemisinin (ART), a sesquiterpene lactone, and its derivatives were tested for their anti-CAF activity. ART has anti-malarial and anticancer activities.[112] ART suppressed the TGF-β signaling pathway in TME. Because TGF-β-mediated signaling is crucial for the trans-differentiation of CAFs in the BC TME,[99] inhibiting TGF-β inactivated CAFs in BC cells. ART and its derivatives also inhibited TGF-β-mediated metastasis and BC growth in orthotopic models *in vivo*.[113] Furthermore, small interfering RNA (siRNA)-mediated genetic inhibition of TGF-β1 sensitized CAFs to 5-fluorouracil (5-FU) and tamoxifen treatment. Suppressing p-44/42 proteins inhibited the MAPK signaling pathway. Notably, the genetic knockdown of *TGF-β1* also orchestrated a 3-fold decrease in the anti-apoptotic B cell lymphoma 2 protein (Bcl-2), leading to apoptosis in drug-resistant BC CAFs.[85] Alrouji *et al*[100] recently demonstrated the suppressing ability of normal NBFs. NBFs release osteoprotegerin (OPG), a decoy receptor for the NF-κB ligand-receptor activator. BC cells were treated with human recombinant OPG *in vitro* and *in vivo*. Recombinant OPG suppressed the EMT, metastasis, and proliferation in the BC tumor *in vivo*. Recombinant OPG also inhibited the metastasis in BC tumors by blocking the Wnt/β-catenin pathway.[100]

miR-4156 has tumor-suppressing activities.[114] CAFs in patients with TNBC demonstrated 5-fold lower concentrations of miR-4156 compared to NBFs. Exosomal miR-4156 expression is inversely related to FOSL1

expression in patients with TNBC. FOSL1 expression is associated with poor prognosis, high proliferation, and metastasis in TNBC cells. Hence, miR-4156 and FOSL1 inhibitors could soon be used as an anticancer agent.[101]

Angiotensin II, an essential peptide of the renin-angiotensin system, plays an important role in vasoconstriction, blood volume, aldosterone secretion, and maintaining water and sodium in the body.[115] Angiotensin II potentiates cancer progression, metastasis, migration, and progression.[116] Takiguchi *et al*[102] studied the potential of the angiotensin blocker valsartan to block BC progression and pulmonary metastasis. Angiotensin II significantly increased BC proliferation, EMT, and pulmonary metastasis in the presence of CAFs *in vivo*. The mRNA expression of IL-6 was significantly increased in xenograft tumors. Furthermore, valsartan significantly reduced the pulmonary metastasis of BC cells.[102]

Role of immune cells and inflammation in epithelial-to-mesenchymal transition

Primary tumors release pro-inflammatory mediators such as TGF-β, TNF-α, IL-1, IL-6, and IL-8 recruit infiltrating immune cells into the TME.[117] Rather than performing anti-tumor actions, immune cells, mainly lymphocytes, are chemically programmed by the TME to aid tumor progression and metastasis [Tables 4 and 5].[118] Inflammatory exogenous chemicals such as lipopolysaccharides also induce EMT by downregulating E-cadherin by upregulating mesenchymal markers such as smooth muscle actin and S100A by binding to Toll-like receptor 4.[119] Immune cells and inflammation-related mechanistic pathways and therapeutic targets are reviewed in Tables 4 and 5.

Effect of smoking in triggering epithelial-to-mesenchymal transition in breast cancer by increasing cytokine levels

Pham *et al*[135] studied the effect of electronic (E)-cigarette smoke on BC cell metastasis. The vapor condensate increased CCL5, vascular cell adhesion protein 1 (VCAM-1), and other major pro-inflammatory cytokine concentrations in BC cells. CCL5 mechanistically binds to CXCR1 receptors to mediate EMT, migration, and invasion in BC cells.[136] E-cigarette smoke also increased the survival of BC cells by regulating the tumor-invading macrophages through VCAM-1 and integrin $\alpha_4\beta_1$. Immunohistochemical studies showed that E-cigarette smoke increased macrophage infiltration into the BC cells and increased BC cell proliferation, EMT, and lung metastasis *in vivo*.[135]

Role of tumor-associated macrophages in regulating epithelial-to-mesenchymal transition in breast cancer

Tumor-associated macrophages (TAMs) are the most extensively explored recruited immune cells. TAMs release TNF-α, which mediates EMT, activating the p38 MAPK pathway.[137,138] TNF-α mediated EMT is regulated by NF-κB in BC cells.[139] TAMs also secrete vascular endothelial growth factor (VEGF), leading to tumor angiogenesis.[140] Zhang *et al*[141] studied samples from 278 patients with TNBC and reported $CD163^+$ TAMs in the TME. Cell infiltration was associated with lymph node metastasis and tumor recurrence. They also observed lower E-cadherin expressions in BC cells; however, multivariate analysis showed that TAM infiltration in the TNBC and E-cadherin downregulation were independent prognostic markers.[141] BC cells were incubated with activated M1 macrophages in a conditioned media, inducing metastasis and EMT in the epithelial cells, indicating that the M1 macrophages communicate signals to trigger EMT.[142] Additionally, Nie *et al*[121] investigated clinical BC tissues and observed high TAM-released CXCL8 expression. CXCL8 overexpression was also associated with migration, invasion, and EMT in patients with BC via binding to CXCR2. CXCL8 also improves the self-renewal capability of BC stem-like cells.[121] Immune cells infiltrating the tumor induce EMT by releasing MMP enzymes, urokinases, and cathepsins.[143,144]

Table 4
Immune cells and inflammation-related pathways in BC EMT.

Biomarker	Mechanistic pathway	Pharmacological action	Reference
CD163 expression in TAMs	–	Lymph node metastasis	120
CXCL8	CXCL2 binds to CXCR2	EMT and increased stemness	121
IL-8	Binds to IL-8Rα and IL-8Rβ	Conversion of neutrophils to TANs	122
Nicotine	Recruitment of TANs in TME	EMT by releasing lipocalin	123
Histidine decarboxylase expressed MDSCs	Potentiating Wnt/β-catenin pathway	Increased metastatic potential	124
Operative stress	Increased infiltration of MDSCs in tumors	Increased EMT	125
CXCL17	Chemotaxis of MDSCs and upregulation of platelet growth factor-BB	Increased lung metastasis	126
CCL3	Activation of the PI3K-AkT-mTOR signaling pathway	Infiltration of MDSCs in the TME	127
MDSCs released NO and IL-6	Activation of NOTCH and STAT3 signaling	Increased TGF-β signaling and stemness in BC cells	128
Overexpression of transcription factor ΔNp63	Upregulation of CXCL2 and CCL22	Enhanced recruitment of MDSCs the breast TME	129

AkT: Protein kinase B; BC: Breast cancer; CCL: CC motif chemokine ligand; CXCL: Chemokine ligand; CXCR: C-X-C chemokine receptor; EMT: Epithelial-to-mesenchymal transition; MDSC: Myeloid-derived stem cell; NO: Nitric oxide; PI3K: Phosphoinositide 3-kinase; TAM: Tumor-associated macrophage; TAN: Tumor-associated neutrophil; TGF-β: Transforming growth factor beta; TME: Tumor microenvironment; Wnt: Wingless-related integration site; –: No data.

Table 5
Recent drugs and targets to suppress immune cell-mediated EMT.

Therapeutic entity	Mechanistic pathway	Pharmacological activity	Reference
Ginsenoside RG3	Inhibition of STAT3 pathway, NOTCH pathway, and downregulation of chemokines	Downregulation of EMT and MDSCs infiltration in TME	130
BML-111	Inhibition of inflammatory mediators. Suppression of ILK signaling pathway	Inhibition of EMT	131
Danirixin	Suppression of stem-cell phenotypes	Inhibition of cell proliferation and lung metastasis	121
Ruyiping	Inhibition of STAT6-mediated macrophage activation	Inhibition of EMT and lung metastasis	132
Progranulin	Downregulation of exosomal miR-5100, which suppressed CXCL2 levels	Inhibition of metastasis	133
OVOL2	Inhibition of TGF-β signaling by epigenetically inhibiting SMAD4/7 levels	Reduced EMT	134
OVOL2	Suppressing IL-10	Inactivation of TAM2	29
Salidroside	Suppression of STAT3 activation	Conversion of pro-tumor TAN2 to anti-tumor TAN1	123

CXCL: Chemokine ligand; EMT: Epithelial-to-mesenchymal transition; ILK: Integrin-linked kinase; MDSC: Myeloid-derived stem cell; miR: Micro ribose nucleic acid; TAM: Tumor-associated macrophage; TAN: Tumor-associated neutrophil; TGF-β: Transforming growth factor beta; TME: Tumor microenvironment.

Targeting tumor-associated macrophages to prevent epithelial-to-mesenchymal transition in breast cancer

Targeting the immune system in the BC TME is an effective strategy to inhibit cancer progression. BML-111, an analog of lipoxin A4, is an effective inhibitor of inflammatory mediators and is used to manage inflammation-mediated cancer metastasis,[145] and Lin *et al*[131] used BML-111 to target TAMs in the TNBC TME. BML-111 inhibited EMT in MDA-MB-231 and 4T1 cells. A concentration of 800 ng/mL of BML-111 induced significant cell death and reduced metastasis in BC cells by inhibiting the integrin-linked kinase (ILK) pathway and downregulating ILK, p-AKT, and p-GSK3β protein expression.[131] CXCR2, a CXCL2 receptor, is overexpressed in BC cells and TAMs. Nie *et al*[121] used danirixin, a CXCR2 antagonist, to inhibit metastasis in BC cells. Danirixin suppressed the cell proliferation rate and lung metastasis of BC cells, suppressing BC stem cell activity *in vivo*.[121] Ruyiping is a traditional Chinese medicine to treat BC and inhibits TAM2-mediated EMT of TNBC cells by preventing STAT6-mediated macrophage activation. Furthermore, *in vivo* studies using ruyiping demonstrated a reduced TAM2:TAM1 ratio and lung metastasis of TNBC.[132]

Progranulin (PGN), a glycoprotein secreted by several cells, is a metastasis biomarker in BC. High PGN expression on TAMs is associated with EMT and metastasis.[146] Yue *et al*[133] knocked out PGN from TAMs, and the $PGN^{-/-}$ TAMs inhibited BC migration and invasion. $PGN^{-/-}$ released exosomes enriched with miR-5100, which downregulated CXCL12. Thus, the CXCL12/CXCR4 pathway was suppressed, inhibiting BC lung metastasis in $PGN^{-/-}$ mice *in vivo*.[133]

Zinc finger TF OVOL2 has gained interest in BC research due to its ability to inhibit the TGF-β pathway.[134] OVOL2 has activity by binding to the DNA sequences in the promoter region.[147] OVOL2 blocked the TGF-β pathway at multiple levels by inhibiting SMAD4 and SMAD7 mRNA expression, blocking complex formation between SMAD4 and SMAD2/3, and inhibiting SMAD4 binding to target DNA.[134] Additionally, OVOL2 can inhibit M2 macrophage activation by antagonizing IL-10. IL-10 was downregulated by OVOL2 directly binding to the promoter.[29]

Role of neutrophil infiltration in regulating epithelial-to-mesenchymal transition in breast cancer

IL-8 plays a major role in the chemo-attraction of neutrophils towards TME. IL-8 binds to two receptors, IL-8Rα and IL-8Rβ, expressed on neutrophils. Neutrophils convert into tumor-associated neutrophils (TANs) when responding to immune mediators.[148,149] Like TAMs, TANs can behave as anti-tumor (TAN1) or pro-tumor (TAN2).[150] Neutrophils orchestrate EMT in BC by releasing neutrophil extracellular traps (NETs).[151] Factors present in the TME, such as IL-8 and granulocyte colony-stimulating factor, trigger the formation of NETs from TANs. NETs comprise double-stranded DNA and nuclear and granular neutrophil proteins. NETs can capture CTCs and encourage secondary tumor formation.[151] Martins-Cardoso *et al*[152] studied the effects of isolated NETs on MCF-7 cell lines and found EMT-related gene alteration *in vitro*. They also observed a positive correlation between NETs and metastasis in the cancer genome atlas.[152]

Prevention of tumor-associated neutrophil-induced epithelial-to-mesenchymal transition by salidroside

Nicotine can recruit TAN2 in the pre-metastatic TME. Recruited TANs released lipocalin 2 via STAT3 activation, inducing EMT in the BC cells. Elevated lipocalin 2 blood and urine levels are observed in early-stage patients with BC, thus making it a promising prognostic marker for BC disease.[123] Salidroside, a natural antioxidant isolated from *Rhodiola rosea*, has anti-cancer potential at high concentrations.[153] However, low-dose salidroside inhibited neutrophil conversion to TAN2 and enhanced anti-tumor TAN1 phenotypes by blocking STAT3 activation. Thus, salidroside treatment inhibited BC cell metastasis to the lungs *in vivo*.[123]

Induction of epithelial-to-mesenchymal transition by myeloid-derived suppressor cells in breast cancer

Myeloid-derived suppressor cells (MDSCs) are crucial for suppressing immune action against tumors.[154] MDSCs trigger cancer cell metastasis by releasing MMP9, epidermal growth factor (EGF), and TGF-β.[155] MDSCs are a heterogeneous population of cells comprising immature macrophages, dendritic cells, and granulocytes.[156] The genesis of MDSCs occurs through altered myelopoiesis during the existence of a tumor.[157] MDSCs play an important role in tumor progression and metastasis by remodeling the TME by promoting EMT, stem-like phenotypes in tumor cells, releasing metalloproteases and angiogenic factors, and suppressing the immune response.[158] Furthermore, MDSCs promote tumor growth by suppressing the immune action exhibited by natural killer, $CD4^+$ T, and $CD8^+$ T cells.[159,160] MDSCs are characterized by Gr-1, Ly6G, and Ly6C molecules on their surface in mice. In humans, MDSCs are characterized by CD33 receptor expression on their membranes and histamine receptors.

Role of myeloid-derived suppressor cells in colonizing migratory breast cancer cells

MDSCs play a crucial role in BC metastasis. A subpopulation of BC cells with overexpressed cytokeratin 14 colonized and raised secondary tumors. Histidine decarboxylase-labeled MSDCs have a close spatial relationship with cytokeratin 14-expressing BC cells by promoting Wnts expression, potentiating Wnt/β-catenin signaling in BC cells.[124]

Myeloid-derived suppressor cells induce epithelial-to-mesenchymal transition after surgical ablation of breast cancer tumors

Surgical ablation is a major treatment for BC. However, MDSCs promote BC metastasis after the primary tumor is removed through surgery. The operative stress possibly increased MDSCs infiltration in the TME. Infiltrated MDSCs were isolated and incubated with 4T1 cells *in vitro*. The co-incubation led to metastatic changes in epithelial cells via TGF-β, VEGF, and IL-10 upregulation.[125] Thus, post-operative MDSC down-regulation can potentially overcome BC metastasis.

Mechanistic and epigenetic pathways responsible for myeloid-derived suppressor cell-induced epithelial-to-mesenchymal transition

Chemokine CXCL17 orchestrates $CD11b^+Gr\text{-}1^+$ MDSC chemotaxis to secondary tumor sites in the lungs and aids in colonizing BC cells by releasing platelet growth factor-BB.[126] CCL3 infiltrates MDSCs in TME, and CCL3-activated MDSCs trigger EMT in epithelial cells via the PI3K-AKT-mTOR signaling pathway.[127] MDSCs induce nitric oxide-mediated NOTCH pathway activation and IL-6-mediated STAT3 phosphorylation. These two pharmacological modulations lead to increased TGF-β signaling pathway activation via prolonged STAT3 activation, inducing stem cell-like phenotypes in BC cells and suppressing T cell-mediated immune action.[128]

High levels of TF ΔNp63 are observed in samples from patients with TNBC. ΔNp63 mediates MDSC chemotaxis by upregulating CXCL2 and CCL22. ΔNp63 levels correlated positively with MDSC infiltration in breast tumors, and ΔNp63 also increased BC cell proliferation, metastasis, and colonization in the lungs. Owing to its roles in immunosuppression and metastasis, blocking ΔNp63 can inhibit BC progression.[129]

RG3 prevents myeloid-derived suppressor cell-induced epithelial-to-mesenchymal transition

Ginsenoside RG3 has been studied for its role in inhibiting MDSC-mediated EMT in BC.[161] RG3 inhibited MDSC-mediated EMT by

blocking the STAT3 and NOTCH pathways and suppressing chemokines in the BC TME. In a mouse model, RG3 delayed MDSC-mediated tumor growth and metastasis and downregulated MDSCs in the blood samples at a non-cytotoxic dose. Furthermore, *in vitro* studies demonstrated the potential of RG3 to inhibit the stem-cell-like phenotypes in BC cells. Thus, RG3 demonstrated strong potential as an anti-metastatic agent for BC management by blocking multiple pathways essential for EMT.[130]

Role of programmed death-ligand 1 in triggering epithelial-to-mesenchymal transition and recent therapeutic strategies

PD-L1 is a crucial transmembrane protein suppressing acquired immune cells. PD-L1 binds to the inhibitory checkpoint receptor programmed cell death 1 (PD1) and transmits an inhibitory signal to the immune response, antagonizing it.[162] PD1 antagonization leads to antigen-specific cytotoxic T cell proliferation suppression, simultaneously decreasing apoptosis in anti-inflammatory regulatory T cells.[162] EMT is associated with PD-L1 overexpression in the BC TME by activating the PI3K/AKT pathway.[163] A study on 67 patients reported a high correlation between PD-L1 overexpression and EMT markers in the BC TME ($p = 0.005$).[164] Furthermore, EMT triggers PD-L1 expression by upregulating the CMTM6 protein.[165] Penisuloxazine A is a C-terminal terminal inhibitor of heat shock protein 90 that downregulated PD-L1 in invasive BC tumors. Penisuloxazine A also destabilized the epidermal- (EGFR) and FGF receptors (FGFR), blocking their downstream signaling. Penisuloxazine A significantly reduced EMT and its biomarkers in MCF-7, MDA-MB-231, and trastuzumab-resistant JIMT-1 cells *in vitro*.[166]

Noman *et al*[167] schematically silenced Zeb1, Snail, Slug, and Twist in BC cell lines *in vitro* to investigate the TFs involved in EMT-induced PD-L1 upregulation; silencing Zeb1 decreased PD-L1 expression significantly in BC cells. Overexpressing the miR-200 family also resulted in EMT-mediated PD-L1 overexpression.[167] PD-L1 was inhibited using the monoclonal antibody atezolizumab, and atezolizumab downregulated EMT-associated genes and upregulated apoptosis-inducing genes in MDA-MB-231 cells.[168] Sativan, an isoflavone isolated from *Spatholobus subrectus*, is a Chinese herbal medicine traditionally used for treating BC. Sativan demonstrated excellent apoptosis-inducing capabilities in MD-MB-231 cell lines by upregulating BCL-2-associated X (BAX) and downregulating BCL-2 expressions *in vitro*. Furthermore, a decrease in EMT-inducing protein was also reported. Sativan upregulated miR-200c expression in BC cells, epigenetically suppressing PD-L1 expression. Sativan also inhibited tumor growth and metastasis in BC *in vivo*.[169]

Poly (Adenosine diphosphate-ribose) polymerase inhibitors induce epithelial-to-mesenchymal transition in breast cancer gene-mutated breast cancer and the role of metformin

Poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) proteins are associated with spindle pole formation, inflammation, cell cycle regulation, and DNA repair. PARPs also play an important role in base excision repair and recruit repair enzymes at the site of single-strand breaks. PARP inhibitors act by blocking DNA double-strand break repair.[170] However, clinical and pre-clinical evidence shows that *BRCA*-mutated BC does not respond to PARP inhibitors, and the resistance to PARP inhibitors in BC is associated with EMT and chemoresistance.[171]

PD-L1 was upregulated in BC cells via p-AKT S473 activation after treatment with PARP inhibitors. PD-L1 upregulation also potentiated EMT through the B-catenin/STT3-mediated transcriptional pathway. However, PARP inhibitors could still initiate EMT after *PD-L1* knockout. Hence, anti-PD-L1 monotherapy was ineffective against PARP inhibitor-induced EMT. Metformin blocks the mTOR pathway responsible for tumor proliferation and chemoresistance and was used to treat PARP inhibitor-induced EMT. Metformin blocked p-AKT S473 phosphorylation to inhibit PARP inhibitor-induced EMT and subsequent chemoresistance. Therefore, co-administering metformin and PARP inhibitors holds potential in BRCA-mutated BC therapy.[172]

Role of hypoxia in epithelial-to-mesenchymal transition

Hypoxia plays an important role in BC progression and metastasis. Hypoxic tumors are characterized by more aggressive phenotypes, metastasis, and resistance to chemotherapy and radiotherapy.[173] The reasons for a hypoxic TME include a high metabolic rate and poor vascular network, causing insufficient oxygen supplies and poor dissemination of waste products from the tumor.[174] Hypoxia-inducible factor (HIF)-1 is a key regulator of hypoxia-induced gene expression in the TME. HIF-1 modulates gene expression related to hypoxia tolerance, apoptosis escape, chemo-resistance, and angiogenesis [Figure 3].[175] HIF-1 comprises two sub-units: HIF-1α and HIF-1β. Under normal oxygen concentrations, HIF-1α is readily ubiquitinated. In contrast, HIF-1α is more stable under hypoxic conditions, leading to increased expression.[176] Inhibiting HIF-1 in a triple TNBC orthotopic tumor model in mice resulted in growth arrest in the primary tumor and inhibited metastasis to lymph nodes and lungs.[177] Furthermore, a hypoxic environment upregulates vimentin in TNBC with E-cadherin downregulation. A zinc finger protein known as Gli-1 is upregulated in the hypoxic TNBC

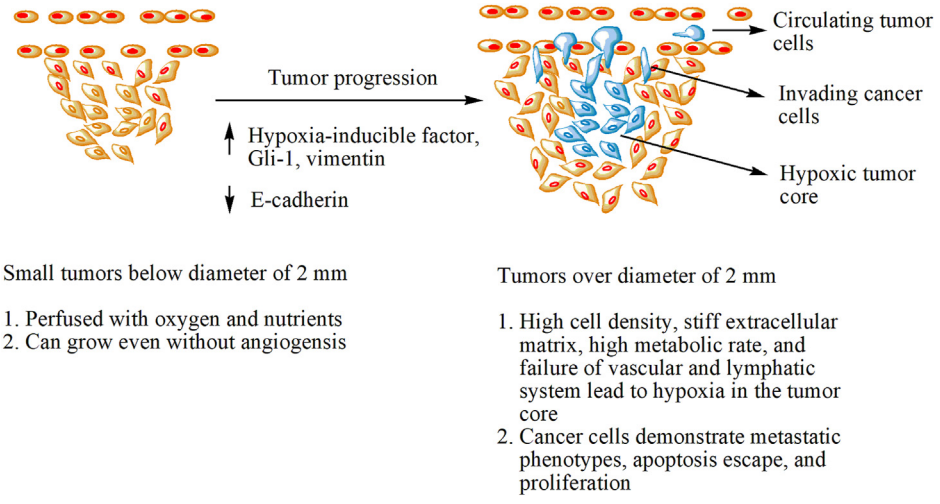

Figure 3. Role of hypoxia in promoting EMT in tumors. The cells undergo various metabolic and signaling changes due to various factors attributing to hypoxia in the tumor microenvironment, leading to EMT in the cancer cells and subsequent invasion and metastasis. EMT: Epithelial-to-mesenchymal transition.

microenvironment. *Gli-1* knockdown downregulated hypoxia-induced EMT.[178] Extracellular adenosine triphosphate (ATP) also plays an important role in BC tumor metastasis by upregulating HIF-1/2α signaling. *HIF-2α* knockdown in BC cells decreased ATP-driven EMT. A chromatin immunoprecipitation (ChIP) assay revealed that downstream HIF-2α proteins orchestrate the ATP-associated EMT, among which MMP-9 and LOXL-2 play significant roles.[179]

Heme oxygenase-1 (HO-1) is crucial for cancer cell survival in hypoxia-induced oxidative stress. HO-1 is an antioxidant resulting from two TFs: activating TF-4 (ATF-4) and nuclear factor erythroid 2-related factor 2 (NRF-2). The cumulative cytoprotective actions induced by these two TFs include antioxidant enzyme upregulation and autophagy induction. ATF-4 and NRF-2 repression is positively related to anoikis and the inability to develop lung metastatic nodules.[180] ATF-4 upregulation leads to ZEB1 activation and E-cadherin downregulation.[181] A study in patients with TNBC concluded that ATF-4 promoted EMT through the TGF-β/SMAD pathway.[182]

Epigenetic modulations responsible for hypoxia-induced epithelial-to-mesenchymal transition

HIF-1α and hypoxia directly upregulate lncRNARP11-390F4.3, and lncRNARP11-390F4.3 expression is crucial for hypoxia-induced EMT and subsequent metastasis. Overexpression of lncRNARP11-390F4.3 overexpression leads to the expression of EMT-related phenotypes in BC cells.[183] The epigenetic modulation orchestrates the hypoxia-mediated suppression of apoptosis-promoting protein p52-2 via miR-205. miR-205 overexpression is also accompanied by the appearance of EMT-related markers in BC cells.[184]

Role of obesity in hypoxia-mediated epithelial-to-mesenchymal transition in breast cancer

Along with tumors, hypoxia in the adjacent adipose tissues causes EMT in BC cells. Obesity is a major cause of incidence and metastasis of BC in postmenopausal women.[185] Co-culturing MCF-7 cells and adipocytes led to upregulating TGF-β, HIF-1α, and lectin-like oxidized low-density lipoprotein receptor 1 (LOX1) receptor mRNA levels in MCF-7 cells. The levels of pro-EMT TFs Twist1 and FOXC2 were also increased. Notably, estrogen receptors on the surface of MCF-7 cells were downregulated, causing hormone therapy resistance. Adipose tissue hypoxia accompanies obesity; therefore, obesity is a significant risk factor for EMT and BC progression.[186] Adipokine resistin induces EMT and stemness in BC cells by binding to adenylyl cyclase-activated protein 1 (CAP1) receptors. Resistin-induced EMT in the MDA-MB-231 and MCF-7 cell lines by re-organizing F-actin filaments to form protrusions in the BC cells. Furthermore, the levels of epithelial markers (E-cadherin and claudin-1) and mesenchymal markers (Twist1, Zeb1, vimentin, Snail, and Slug) were downregulated and upregulated, respectively.[187]

Targets to prevent hypoxia-mediated epithelial-to-mesenchymal transition

Owing to the important role of hypoxia in tumor progression, various strategies have been designed to target hypoxia [Tables 6 and 7]. Paeoniflorin, a monoterpene glycoside, suppresses HIF-1α upregulation in hypoxic conditions. Paeoniflorin suppresses EMT in hypoxic MDA-MB-231 cell lines by repressing phosphorylated PI3K and AKT expressions in TNBC cells.[188] Fucoidan, a sulfated polysaccharide, downregulates the HIF-1α protein and hypoxia-induced EMT in MDA-MB-231 cells.[189]

A saponin, Escin la, inhibits LOXL2, a downstream protein of HIF-2α. Escin la suppressed *LOXL2* mRNA expression. Escin la prevented E-cadherin downregulation and suppressed EMT in the TGF-β-overexpressed MCF-7 cells. However, Escin la could not prevent hypoxia in the BC cells; hence, the mode of action can be credited to downregulating LOXL2 expression.[190] Mechanistic pathways in hypoxia-mediated EMT are represented in Tables 6 and 7.

Relationship between epithelial-to-mesenchymal transition and breast cancer stem cells

Apart from the role of EMT in BC metastasis, EMT also transforms epithelial cells to give them stem cell-like properties [Figure 4].[191] These properties include CD44 glycoprotein overexpression, CD24 glycoprotein downregulation, developing spheres in suspension culture, and tumorigenesis in mice or human systems.[192] However, CSCs, by EMT definition, should be able to differentiate into epithelial cells through MET. Epithelial cells can dedifferentiate into several alternative mesenchymal states according to the situation, one of which is BC stem cells (BCSCs). Thus, BCSCs can form new metastatic tumors when disseminated to distant tissues.[14]

Migratory cells undergo MET before forming the macrometastasis.[193,194] During EMT transformation, CSCs develop mature adhesion plaques (macromolecular structures comprising grouped integrins). The plaques contribute to the differentiation ability of CSCs by activating the focal adhesion kinase (FAK).[195]

Molecular and epigenetic modulations responsible for epithelial-to-mesenchymal transition-triggered breast cancer stem cell generation

EMT activation leads to the activation of the autocrine WnT/β-catenin and TGF-β/SMAD signaling pathways. Intracellular expression of Snail

Table 6
Mechanistic pathways to prevent hypoxia-mediated EMT in BC.

Biomarker	Mechanistic pathway	Pharmacological activity	Reference
Resistin	Binding to CAP1 receptors	Induction of EMT and stemness	187
Gli-1	Upregulation of hypoxia	Induction of EMT in TNBC	178
Extracellular ATP	Upregulation of HIF-1/2α signaling	EMT	179
lncRNARP11-390F4.3	lncRNARP11-390F4.3 is upregulated by hypoxic conditions	Hypoxia-induced EMT	183
miR-205	Suppression of apoptosis-promoting protein p52-2 in hypoxic conditions	EMT	184

ATP: Adenosine triphosphate; BC: Breast cancer; CAP1: Cyclase-activated protein 1; EMT: Epithelial-to-mesenchymal transition; HIF: Hypoxia-inducible factor; lncRNA: Long noncoding ribonucleic acid; miR: Micro ribose nucleic acid; TNBC: Triple-negative breast cancer.

Table 7
Recent drugs and targets to inhibit hypoxia-mediated EMT.

Therapeutic entity	Mechanistic pathway	Pharmacological response	Reference
Paeoniflorin	HIF-1α suppression by repressing phosphorylated PI3K and Akt	EMT suppression	188
Fucoidan	HIF-1α downregulation	Repression of hypoxia-induced EMT	189
Escin la	LOXL2 suppression	Suppression of TGF-β-mediated EMT	190

Akt: Protein kinase B; EMT: Epithelial-to-mesenchymal transition; HIF: Hypoxia-inducible factor; PI3K: Phosphoinositide 3-kinase.

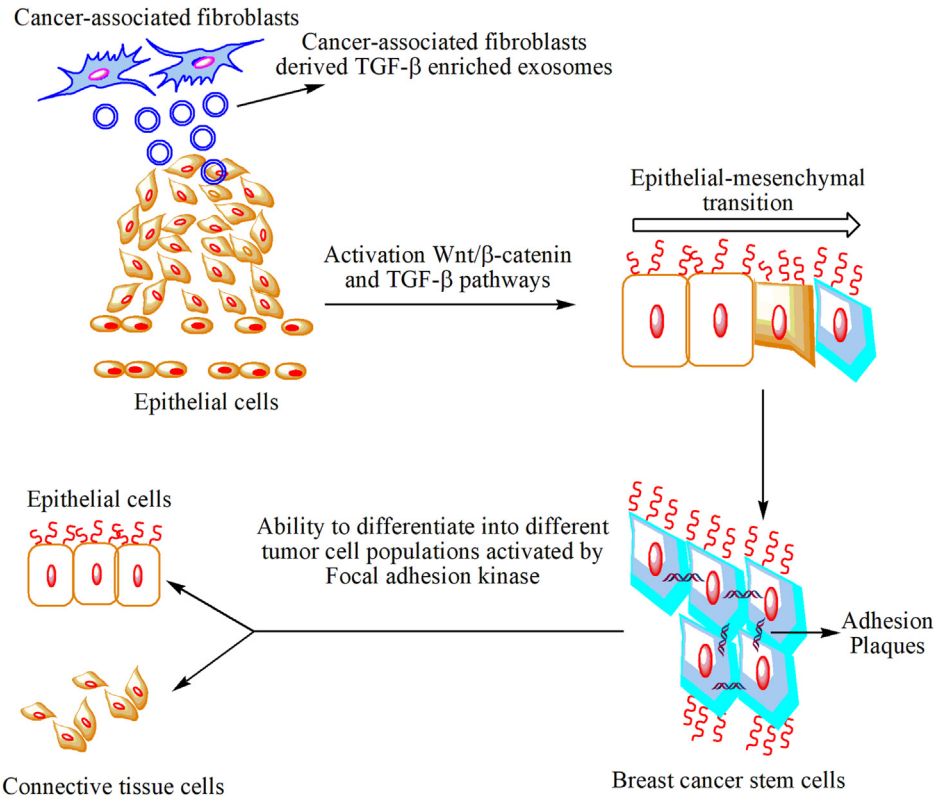

Figure 4. Schematic representation of EMT-induced breast cancer stem cells in the TME and the pathways involved in generating differentiated tumor cells at distant anatomical sites. EMT: Epithelial-to-mesenchymal transition; TME: Tumor microenvironment; TGF-β: Transforming growth factor-β; Wnt: Wingless-related integration site.

reduces the expression of the tumor suppressor *p53* gene through *p53* deacetylation. *p53* knockdown potentiated the tumorigenic potential of BC cells.[196] Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein that plays an important role in tumor progression and metastasis and induces stemness in hypoxic BC tumors.[197] EpCAM overexpression leads to upregulating stemness-related markers, including NaNOG, OCT4, and SOX2.[198] N-glycosylation is an important pre-step for EpCAM to regulate stemness and EMT in breast tumors by modulating NF-κB in tumor cells.[199]

The expression of cytokeratin 18 (CK18), a cytoskeleton protein, is important in BC metastasis and BCSC development. CK18 expression is downregulated in aggressive, metastatic, and TGF-β-treated MCF-7 cells. In contrast, CK18 depletion upregulates EpCAM expression by activating the Wnt-β-catenin pathway.[200] Moreover, radio-resistant BC cells isolated from the MDA-MB-231 cell line reportedly demonstrate BCSC markers such as notch-1, CD44, and aldehyde dehydrogenase 1.[201]

BCSCs have high telomerase activity associated with stemness,[202] EMT, and BC cell migration. Inhibiting telomerase reverse transcriptase (TRT) transforms BCSCs into cells with more epithelial phenotypes.[203] Telomerase inhibitor BIBR1532 exhibited apoptosis in BCSCs with an IC50 value of 38.71 μM at 72 h. BIBR1532 also decreased the sphere-forming and migration abilities of BCSCs *in vitro*.[204]

Role of partial epithelial-to-mesenchymal transition in breast cancer stem cell generation and tumorigenic potential

BCSCs are present in two alternative epithelial- and mesenchymal-like stem cell states. These states are capable of inducing new tumors.[31,205] TNBC cells promote stemness under the influence of chemotherapeutic stress.[206] Activated $CD8^+$ T cells have also induced BCSC-like features in mammary epithelial cells by inducing EMT and giving them tumorigenic potential.[207] IL-6-mediated EMT led to BCSC generation from mammary epithelial cells.[208] Furthermore, IL-6-mediated EMT and subsequent stemness led to resistance against PI3K inhibitors.[209] The extent of EMT is crucial. Overactivating EMT leads to detrimental effects on the tumorigenic ability of cells. In low-claudin BC, cells with epithelial phenotypes expressed higher capabilities to generate new tumors than those without epithelial characteristics.[210] Thus, partial EMT leading to CSC generation is focused upon.[211]

Role of yes-associated protein/TAZ in breast cancer stem cell generation

YAP/TAZ transcriptional regulator overexpression is associated with increased metastasis and stemness in BC. YAP/TAZ promotes malignancy and stemness by directly interacting with transcriptional-enhanced associate domain (TEAD) TFs.[212] Various YAP/TAZ inhibitors, such as verteporfin,[213] dasatinib,[214] and celastrol,[215] inhibited cancer growth. However, luteolin, a natural flavonoid, exhibits YAP/TAZ inhibition in TNBC cells. Luteolin inhibits CYR61 and CTGF expression, two major YAP/TAZ-targeted genes. Luteolin also inhibited YAP/TAZ nuclear translocation and transcription in TNBC. However, luteolin increased YAP/TAZ phosphorylation via the non-canonical Hippo pathway, thus increasing its proteasome degradation and inhibiting its nuclear translocation.[216]

Targets and strategies to target breast cancer stem cells

Downregulating integrin α3 promotes stemness in BC cells. Integrin interacts with VASP and regulates the PI3K/AKT pathway, inhibiting BC promotion, EMT, and stemness. Metastatic BC cells expressed a low level of integrin α3. Integrin α3 treatment of metastatic BC cells reduced wound healing potential and spheroid formation abilities. Thus, integrin α3 should be explored as a possible anti-metastatic agent for BC

management.[217] Another marker, phosphatase and tensin homolog (PTEN), is downregulated in BC. PTEN suppresses tumors by inhibiting PI3K/AKT pathway-mediated proliferation, metastasis, and tumorigenesis.[218] PTEN was downregulated in BCSCs, and PTEN expression prevented Matrigel invasion by BCSCs. Furthermore, immunoprecipitation studies found that Abi1, a core adaptor protein in the WAVE regulatory complex, was downregulated by PTEN. Upregulation of Abi1 downregulated PTEN to induce EMT and BCSC phenotypes in BC cells.[219] Efflux proteins such as BC resistance protein (BCRP) have been implicated in secreting chemotherapeutic drugs out of cells, leading to drug-resistant phenotypes in CSCs.[220]

Effects of hyaluronic acid on breast cancer stem cells

Hyaluronic acid (HA), a common pharmaceutical ingredient, is a crucial targeting moiety in BC-targeted nanoscale formulations.[221] However, HA also plays a critical role in BC cell migration.[222] Jariyal *et al*[223] reported the effects of high molecular weight (HMW) and low molecular weight (LMW) HA on different sub-populations of BC cells and BCSCs. LMWHA induced EMT in the MCF-7 cell line *in vitro*, whereas HMWHA did not. LMWHA and HMWHA downregulated the stemness-regulating EpCAM glycoprotein in MCF-7 cell lines. In MDA-MB-231 cells, treating LMW and HMW HA downregulated stem cell-specific CD44 receptors.[223]

Epithelial-to-mesenchymal transition orchestrates chemoresistance in breast cancer

EMT was thought to be limited to the loss of epithelial phenotypes and the gain of mesenchymal phenotypes to migrate and colonize in distant tissues. However, studies indicate EMT leads to immune escape, chemotherapy resistance, and BCSC generation [Table 8].[6,224] The net effects of these phenotypic changes caused by EMT include chemoresistance and tumor relapse.[225] Chemoresistant BC samples were enriched with EMT-related genes.[226] Furthermore, cancer cell dormancy, EMT, and expression of multidrug resistance proteins such as P-gp go hand-in-hand.[227] We have summarized biomarkers and epigenetic modulators that induce chemoresistance via EMT-related pathways in Table 8.

Conclusion and perspective

This review describes the importance of EMT in tumor progression and promotion. We mechanistically discussed the pathways responsible for orchestrating EMT in cancer cells, the role of the TME in promoting EMT, and the role of EMT in tumor progression, metastasis, and chemoresistance. We also summarized various therapeutic targets to counter EMT in cancer cells.

EMT is essential in BC progression, metastasis, relapse, and chemoresistance. EMT is also crucial for generating various mesenchymal cells and BCSCs, aggravating BC, and raising secondary tumors. The highly regulated nature of EMT by various TME components demonstrates its utmost importance. Scientists have targeted various EMT-involved pathways, and several studies inhibited stemness in BC cells by targeting EMT. This approach can prevent the tumorigenic potential of BC cells, ultimately preventing disease relapse.

However, stress-induced metastasis due to chemotherapy or surgical ablation should be countered. Targeted delivery of TGF-β inhibitors and agents such as all-trans retinoic acid, which differentiate immature MDSCs,[243] should be explored to block metastasis post-approval, thus inhibiting tumor relapse. Lifestyle-associated factors, including obesity and smoking, also trigger EMT through cytokine-associated pathways. Thus, targeted delivery of TGF-β inhibitors should be explored for patients with these risk factors. Because several pathways can trigger metastasis, targeted delivery systems are required to neutralize disseminated migratory cell clusters in the systemic circulation. However, conventional nanocarriers cannot accumulate in the TME owing to high intra-tumor pressure, stiff extracellular matrix, poor extravasation of nanocarriers, and high cell density.[244,245] In contrast, cell-based drug delivery systems have recently demonstrated superior tumor-targeting properties.[246–248] Such systems should target primary and secondary

Table 8
EMT-based mechanisms for chemoresistance in the BC cells.

Biomarker	Mechanism	Pharmacological effects	Reference
Downregulation of miR-200b	Fibronectin 1 and increased luciferase activity overexpression	EMT and DOX resistance	228
Snail	Snail downregulation	5-FU resistance	229
AMP-activated protein kinase	Deactivation of AMP-activated protein kinase	EMT induced 5-FU, ADR, and PTX resistance in MCF-7 and MDA-MB-231 cell lines	230
Terminal differentiation-induced noncoding RNA (TINCR)	Overexpression of TINCR inhibits miR-125b	EMT and trastuzumab resistance	231
Downregulation of miR-489	SMAD3 upregulation	EMT and ADR resistance	232
S-phase kinase-associated protein-2 upregulation	p27 inhibition	EMT and PTX resistance	233
Downregulation of miR-137	Upregulation of dual-specific phosphatase 4	EMT and DOX resistance	234
Downregulation of miR-125b	Sema4C upregulation	EMT and PTX resistance	235
Upregulation of lncRNA H19	Snail upregulation	EMT and tamoxifen resistance. Curcumin prevented H19-mediated EMT and resistance	236
SIRT1	Wnt/β-catenin pathway activation	EMT and DOX resistance. Resveratrol blocked DOX resistance by blocking the SIRT1-β-catenin pathway	237
Ki67 upregulation	Upregulation of mesenchymal phenotypes	EMT and cisplatin resistance. Niclosamide suppressed Ki67 to inhibit EMT and cisplatin resistance	238
ADR treatment mediated EMT and subsequent chemoresistance	P53 triggered p21 activation. Disrupting the connection of p53 with mdm2	EMT by upregulation of Twist1 and ADR resistance	239
5-FU treatment mediated EMT and chemoresistance	Upregulation of stemness and EMT markers	5-FU resistance. Salinomycin re-sensitized the resistant BC cells to 5-FU	240
Zeb1	Increase in the autophagic genes resulting in LC3-II overexpression	DOX resistance	241
Lactate dehydrogenase A	Increase in pro-survival autophagy and downregulation of apoptosis	EMT and ADR resistance. Downregulation in lactate dehydrogenase A level inhibited autophagy and triggered apoptosis	242

5-FU: 5-fluorouracil; ADR: Adriamycin; AMP: Adenosine monophosphate; BC: Breast cancer; DOX: Doxorubicin; EMT: Epithelial-to-mesenchymal transition; LC3-II: Microtubule-associated protein 1A/1B-light chain 3 – phosphatidylethanolamine conjugate; lncRNA: Long noncoding ribonucleic acid; MCF-7: Michigan Cancer Foundation-7; MDA-MB-231: M.D. Anderson metastatic breast cancer cells; miR: Micro ribose nucleic acid; PTX: Paclitaxel; TINCR: Terminal differentiation-induced noncoding RNA; Wnt: Wingless-related integration site.

tumors and CTCs. Furthermore, re-purposed drugs such as statins with cytotoxic and anti-metastatic pharmacological activities should be used to treat BC.[249] Lastly, co-delivering anti-metastatic drugs and chemotherapeutic agents should be studied for enhanced antitumor effects.[250]

This review provides a comprehensive summary of BC biology, molecular pathways, and recent advancements in therapeutic strategies, molecular pathways, and recent advances in therapeutic strategies. We also highlight the need to investigate the pathophysiology of BC to elucidate novel targets for which therapeutic agents can be developed to treat BC.

Authors contribution

Paras Famta, Saurabh Shah, and Biswajit Dey – conceptualization, data curation, manuscript writing, data visualization, editing; Kondasingh Charan Kumar, Deepkumar Bagasariya, Ganesh Vambhurkar, Dadi A. Srinivasarao, Anamika Sharma, and Rahul Kumar – data curation; Santosh Kumar Guru, and Rajeev Singh Raghuvanshi – supervision and validation; Saurabh Srivastava – supervision, editing, and conceptualization. All the authors have read and approved the final version of the manuscript.

Ethics statement

Not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that generative artificial intelligence (AI) and AI assisted technologies were not used in the writing process or any other process during the preparation of this manuscript.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability statement

No data was used for the research described in the manuscript.

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