J Cancer Immunol. 2023;5(1):40-60.

Journal of Cancer Immunology Review Article

Epithelial Mesenchymal Transition: The Ultimate Driver of Cancer on Difficult Paths

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Received date: May 15, 2023, **Accepted date:** June 26, 2023

Citation: Tripathi D, Gupta PK, Tripathi S. Epithelial Mesenchymal Transition: The Ultimate Driver of Cancer on Difficult Paths. J Cancer Immunol. 2023;5(1):40-60.

Abstract

Metastasis is the perilous aspect of cancer and is responsible for 90% of deaths due to cancer. It represents an enigmatic and complex biological cascade that is poorly understood. The constant development in cancer research and the advent of new principles in metastasis have discovered some of the molecular keystones like epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs) of this cascade. Acknowledgment of the communications between cancer cells and their micro-environment enlightens the biology of metastasis and allows us to understand the mechanism of EMT induction and its role in governing invasion, migration, plasticity, colonization, and therapeutic resistance. EMT is the principal reason behind the cancer cells' complex behavior, tremendous plasticity, survival, and adoption in a constantly changing environment. Thus, EMT is a perfect driver mechanism to execute metastasis and develop resistance against conventional and targeted therapies. Studies have also discovered the role of EMT in CSCs generation and offered us prospects for evolving more effective treatments to target metastasis and improved patient prognosis.

Our primary aim in the present review is to summarize the induction and role of EMT in cancer. This review not only discusses the role of EMT in metastasis but also uncovers the role of EMT in survival, metabolism, and CSCs generation. Further, in this review, we also discuss the strategy to target the EMT for the development of new and effective therapeutics for cancer management.

Keywords: Cancer Stem Cells (CSCs), Cell signaling and cancer, Epithelial Mesenchymal Transition (EMT)

Introduction

Metastasis directly bears the responsibility for the survival of cancer patients and is responsible for 90% of deaths due to cancer [1]. Continuous cancer research and histopathological studies have shown an extensive role of Epithelial-mesenchymal transition in metastasis [2-6]. EMT is a core cell cytoskeleton redefining program. In the course of EMT, epithelial cells lose their structural and functional characteristics to attain the mesenchymal attributes for cell migration. This embryological developmental program plays an important role in cancer pathogenesis also. EMT influences various aspect of cancer metastasis from invasion metastasis cascade to cancer cells survival in a constantly changing environment for the resistance against therapies. The notion of this review is to illustrate the EMT, events of EMT, EMT induction, and its pleiotropic role in cancer. This transdifferentiation program (EMT) endorses the invasion-metastasis cascade, and promotes apoptotic resistance, therapeutic resistance, immune evasion, and the acquisition of stemness in tumor cells. EMT also rewires cellular metabolism to achieve the above aspect of cancer [7]. In the last section of this review, strategies to target the EMT for the management of cancer are also illustrated.

Elizabeth D Hay observed the conversion of epithelial cells into mesenchymal cells first time during limb growth, limb regeneration, and neural tube formation and coined the term "Epithelial-mesenchymal transition." She also reported that EMT was not an irreversible phenomenon and introduced

"Mesenchymal- epithelial transition" also. EMT is a classical embryonic developmental program for gastrulation, neural delamination, embryonic lung, heart valve, and brush border membrane formation [8]. Various reviews had described the history and timeline of EMT research with reference to embryonic development and cancer metastasis [1,2].

Type 1 EMT

During developmental or Type 1 EMT, cells undergo several rounds of EMT and MET processes in a sequence for the formation and differentiation of dedicated cells and internal organs' three-dimensional structures. Hence, developmental EMT is divided into primary, secondary, and tertiary EMT. Primary EMT includes parietal endoderm, mesoderm formation, and neural crest delamination. Secondary EMT includes the transition of transient epithelial structures like notochord, somites, somatopleure, and splanchnoplure into dermomyotome, sclerotome, the connective tissue of body muscles, and angioblasts. Canonical Wnt, BMPs, Nodal & Vg1 (TGF β superfamily), and fibroblast growth factor (FGF) signaling play an important role in Type 1 EMT. Type 1 EMT is not linked with fibrosis and invasion [8,9] (**Table 1**).

Type 2 EMT

Type 2 or Physiological EMT activated in response to wound healing, tissue fibrosis, tissue repair, and regeneration. In response to tissue injury and an inflammatory environment, Type-2 EMT gives rise to fibroblasts and myofibroblast from epithelial tissue. Type 2 EMT ceases with the end of inflammatory stimuli. TGF β, PDGF, EGF, and FGF-2 signaling play an important role in type 2 EMT [10]. If the inflammation persists for a long time owing to recurrent Type 2 EMT may result in organ damage and have potentially serious repercussions. Type 2 EMT is associated with fibrosis but not invasion [10-12] (**Table 1**).

Type 3 EMT

Type-3 EMT or pathological EMT is associated with cancer metastasis. During conversion from a benign to a malignant tumor, tumor cells originating in epithelial tissue start breaching the basement membrane of epithelial tissue and develop metastatic disease. In this conversion, epithelial tissue architecture and organization is the natural obstacle. Epithelial cells rested on a basement membrane, consisting of apical-basal polarity, and various junctions (Adheren junction, Tight junction, Desmosomes, and Gap Junctions) for their sheet-like architecture and maintenance (**Figure 1**) [13]. Hence, tumor cells of epithelial origin undergo a significant phenotypic change (Type-3 EMT) to execute the metastasis (**Table 1**). The most important event to dissolve the epithelial characteristics is the loss of E-cadherin, and it is considered a central event in the initiation of EMT [14,15]. Loss of E-cadherin weakens the epithelial cell attachment with neighbor epithelial cells and makes the cells free from the epithelial sheet-like organization. Down-regulation of E-cadherin is achieved through transcription repressor (Snail, Slug, Zeb 1, Zeb2, Twist and E-12/E47) (**Table 2**) of E cadherin gene (CDH1), posttranscriptional control (LnCRNA- NEF, MITA1, p21, ATB,) (Micro RNA- MiR 1993p, MiR186, MiR122, MiR 200c,) or epigenetic control of E-cadherin gene promoter (DNMT1, DNMT3A1, DNMT3A2, and HDAC1/2) [13,16-20]. PAR complex proteins and Scribble complex proteins are responsible for the apical-basal polarity of epithelial cells. Down regulation of E-cadherin stops SCRIB protein (Scribble complex protein) interaction with the lateral plasma membrane and reduces cell adhesion [21].

EMT induction also leads dissolution of tight junction through down regulation of claudin, occluding, and zona occludens. Tight junction, present on the apical side of epithelial cells, maintains the apical-basal polarity and controls transport of water, ion, and macromolecules movement. Claudin and occludin, transmembrane proteins, form the core of the complex. While Zona occludens proteins (ZO-1, -2, and -3), present on the cytoplasmic side of tight junction, link junctional occludin and claudin to the actin cytoskeleton. Tight junction proteins also interact with signaling pathways which influence cell proliferation, morphogenesis, differentiation, and migration. Generally, Claudin family protein, occluding, and ZO proteins expression is downregulated in EMT activation and associated with tumor invasion and migration. Overexpression of Snail in mouse mammary epithelial cells downregulate claudin and occludin expression at mRNA and protein level [7]. In pancreatic cancer, Zeb-1 mediated downregulation of claudin and ZO-1 is associated with tumor

Table 1. Types of EMT.

Type of EMT	Function	Signaling Pathway	Reference
Type 1	Primary EMT-Embryonic development, Gastrulation, mesoderm formation, neural crest, Secondary EMT- Dermomyotome and sclerotome formation Tertiary EMT- Cardiac valves	(Gastrulation-EGF, Wnt3, Wnt8c, Nodal, Vg1, FGF) [3] (BMP signaling and inhibition of it through Noggin during Neural crest EMT [4])	[3,4]
Type 2	Tissue repair, wound healing, tissue regeneration [5]	TGF-β, PDGF, EGF, and FGF-2	[4]
Type 3	Cancer metastasis	Receptor tyrosine kinase, Wnt, TGF-β, Notch	[6]

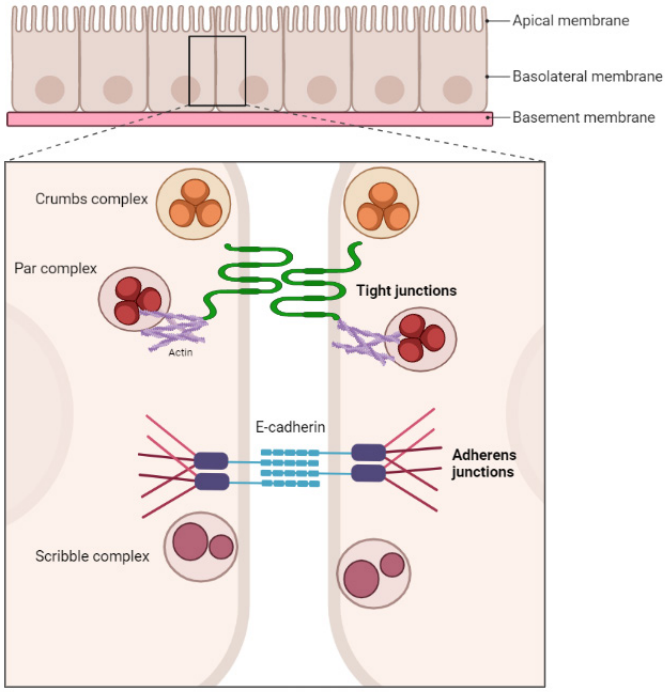

Figure 1. Architecture of epithelial tissue. In epithelial tissue, the ectodomain of E-cadherin establishes a complex with the extracellular domain of E-cadherin present on the membrane of adjacent epithelial cells. This homophilic interaction between E-cadherin proteins, stabilized with calcium ion (Ca^{2+}), between two adjacent epithelial cells creates an interface for the cell-cell adhesion and forms adherens junction for the structural integrity of epithelial tissue. The cytoplasmic tail of E-cadherin protein contains a juxta membrane domain (JMD) (responsible for the clustering of E-cadherin and adhesive strength) and a catenin binding domain (CBD) (to interact actin fiber through γ and β catenin, and α catenin for the stabilization of adherens junction) [189,190]. This interaction further provides tensile strength to the cells. By joining together, the actin cytoskeleton of adjacent epithelial cells, E-cadherin molecules resist mechanical stress and maintain the structural organization of epithelial cells.

Table 2. EMT transcription factors.

EMT-TF (E-cadherin repressor)	Type	Reference
Zeb1 and Zeb2	Zincfinger/ homedomain	[12,13]
Snail and Slug	C2H2 type Zinc finger	[14,15]
Twist	bHLH	[16,17]
E-12/E47	bHLH	[18,19]

metastasis and invasion [8]. However, deregulated and mis localized claudin expression is also reported in cancer and linked with tumor metastasis [9,10]. In colon cancer cells, claudin-1 activates Wnt/β-catenin and PI3K/Akt cell signaling pathway for the upregulation of E-cadherin repressor Zeb-1 [11]. Hence, the role of claudin is not well established.

EMT induction also reduces the expression of cytokeratin intermediate filament in epithelial cells. EMT exhibiting cells have increased expression of vimentin intermediate filament and N-cadherin. Vimentin and N- cadherin are markers for the mesenchymal state [20,21]. Vimentin expression is found from the early stages of embryo development in mesenchymal cells. In epithelial cells, cytokeratin is the main intermediate filament (IF filament), whereas in a malignant tumor, vimentin expression is upregulated, and simultaneously cells lose cytokeratin. Its link with cellular migration can be supported by the fact that fibroblasts lacking vimentin show reduced mechanical stability and directional migration towards different chemo-attractant agents [22,23]. Vimentin upregulation in carcinoma cells has been shown to be enough for the tumor cells to adopt fibroblast-like morphology. Vimentin is important for maintaining cellular integrity and providing protection against stress in normal physiological

conditions. Further, vimentin is also involved in tumor invasion, survival and it is often associated with the poor prognosis in cancers of epithelial origin [24-26]. Increased expression of vimentin in the epithelial state induces an increase in cell migration, responsible for the change in cellular shape and cell-cell contact loss after EMT activation [27]. Vimentin intermediate filaments are more plastic than other intermediate filaments and suitable for cancer cells' enhanced migration and invasion. Vimentin phosphorylation is crucial for vimentin structure and physiological functions. Phosphorylation and organization of vimentin play an important role in dynamics, migration, differentiation, mitosis, and stress response [28]. Further, vimentin phosphorylation creates sites for interactions with different proteins for cellular transformation and metastasis promotion. Inagaki reported that site-specific phosphorylation of vimentin induces disassembly of vimentin [29]. Site-specific phosphorylation of serine residues from the N-terminal domain of vimentin filaments through various kinases prevent polymerization of vimentin filament and leads to disassemble pre-existing vimentin filaments [30]. Likewise, Serine 39 residue of vimentin is phosphorylated by Protein kinase A, Protein kinase B (Akt), and Protein kinase C, are important for its disassembly and function [31]. Phosphorylation of vimentin protein at serine residues inhibits its polymerization and promotes the solubilization of subunits, resulting in its disassembly from the cell periphery, and retrograde movement of vimentin towards nucleus. Retrograde movement of vimentin subunits further prevents nuclear collapse during cell migration [32]. After serine phosphorylation, constant exchange between vimentin filament and dissembled vimentin subunits make vimentin a highly dynamic intermediate filament [33]. Zhuo *et al*. had further highlighted the role of vimentin in directional cell migration using quantitative live-cell imaging in genome edited cells. They also reported that, during cell migration, turnover of vimentin is slower than microtubules. Slow turnover of the vimentin acts as a template for the growing microtubules. The creation of persistent cell polarity is the hallmark of directional cell migration. Vimentin also plays an important role in providing persistence to cell polarity and acts as a stabilizer for the microtubule's organization [34]. This change in morphology of carcinoma cells is followed by other components of the cytoskeleton like actin filament, microtubules, and desmosomes internalization to coordinate the cytoskeletal change for the invasion and migration [35]. EMT expressing cells also increase secretion of fibroblast and matrix metalloproteinases in the tumor microenvironment [21]. With the downregulation of epithelial features and gain of mesenchymal markers, EMT exhibiting cells now have the potential to invade the adjacent tissue and blood system to initiate the metastasis invasion cascade.

EMT Induction in Cancer

Wnt signaling (Conanical and non conanical pathway), Notch signaling, hedgehog signaling, receptor tyrosine kinase signaling, TGF-β (Smad dependent and Smad independent pathway), and inflammatory tumor microenvironment play an important role in cancer associated EMT [5,36-41].

Wnt/β-catenin Cell Signaling

Abnormal Wnt/β-catenin cell signaling especially upregulation of β-catenin is associated with the development of various cancer. APC, AXIN, casein kinase-1, and GSK3β made destruction complex marked the phosphorylation and ubiquitination of β-catenin for the proteasomal degradation of β-catenin. Binding of Wnt ligand (Wnt1, Wnt2, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10b, or Wnt16) on frizzled receptor and LRP5 leads to recruitment of destruction complex towards the plasma membrane and β-catenin translocate to the nucleus. Translocated β-catenin activates TCF/LEF through the displacement of HDAC for the activation of SNAIL, SLUG, TWIST, ZEB1/2, and N-cadherin to induce EMT in cancer cells. In various carcinomas, especially in colon cancer, mutations were observed in the components of the destruction complex. APC mutation, GSK3β deletion, Axin1/2, and E-3 ubiquitin ligase inactivation were frequently detected in cancer tissue samples [57-59].

In non-canonical activation, Wnt signaling depends upon Wnt5a class ligands and is divided into Wnt/Planar cell polarity (PCP) and Wnt/calcium signaling pathway/PCP pathway, is important for embryonic development, stem cell maintenance, cell adhesion, cell migration, and cell polarity complex. Wnt/PCP pathway interacts with receptor tyrosine kinases like ROR1, ROR2, and RYK for the activation of PI3K/Akt signaling. WNT/PCP also interacts with Rho and Rac for the activation of the JNK pathway. In the Wnt/Ca^{2+} pathway activation involved the binding of Wnt5a with the FZD receptor and co-receptor ROR1. The activated pathway increases calcium ion concentration in the cell for the activation of calcium-dependent kinases (calmodulin-dependent kinase, calcineurin, and protein kinase C). These kinases influence various cell activities like adhesion, migration, and differentiation [60]. Both canonical and non-canonical pathways play an important role in EMT induction and metastasis. The upregulated EMT-TFs (Snail, Slug, Twist, and Zeb1/2) through canonical and non-canonical Wnt signaling pathways suppress the expression of E-cadherin. Suppression of E-cadherin releases β-catenin in the cytoplasm. Free β-catenin translocated to the nucleus to further strengthen the EMT-TFs expression and form a positive feedback loop [57].

TGF-β Signaling

TGF-β signaling pathway is the major cell signaling pathway in EMT. TGF-β is the prototype of bone morphogenetic proteins, Growth & differentiation factors (GDF) & Mullerian inhibiting substance (MIS) protein family. TGF-β binds to the type I and type II receptors (TβRI and TβRII p, serine/threonine kinase) present on the plasma membrane. In canonical activation (SMAD-dependent activation), the TGF-β receptor

phosphorylates R-SMAD proteins (SMAD2 and SMAD3). Activated SMAD2/3 forms a complex with SMAD4. The complex of SMAD2/4 and SMAD4 translocate to the nucleus [53]. Translocated complex upregulate metastasis-promoting genes (IL-11, PTHrP, MMP-9), Tumor angiogenesis genes (CTGF, VEGF), and EMT-inducing genes (SNAI, SLUG, ZEB, and SLUG) [61].

In addition to the standard SMAD-mediated TGF-signaling pathway, the activated receptors also interact via other cell signaling pathways independent of SMAD. Non-SMAD signaling pathways include the interaction of activated TGF-β receptor to MAPK pathways (Erk, JNK, and P38 pathways), PI3K/Akt pathways, Rho GTPases & IkB kinase pathways. Both SMAD-dependent and independent TGF-β signaling play an important role in EMT. The complex of SMAD2, SMAD3, and SMAD4 upregulates the expression of EMT-TF genes (SNAI1/2, Zeb1/2, and twist) for the downregulation of E-cadherin, ZO-1, laminin, Occludin, and claudin. The activated complex of SMAD2, 3, and 4, also increase the expression of N-cadherin, Vimentin, and fibronectin for the gain of mesenchymal characteristics [62]. SMAD3 and SMAD 4 complex interact with SNAIL-1 to downregulate E-cadherin and tight junction proteins (CAR, occluding, and claudin-3) [63].

Notch Signaling

Notch cell signaling is an example of juxtracrine cell signaling and is important for organ formation, tissue function, and tissue repair. In mammals, the NOTCH receptor (NOTCH 1,2,3,4) consists of an extracellular region (containing EGE-like repeats and a negative regulatory region, a transmembrane region, and an intracellular NICD region. Intracellular NICD region have recombination signal binding protein-J (RBPJ) association module, ankyrin repeats (7 repeats) and nuclear transfer signal sequence on the ankyrin domain. Mammals have five acknowledged ligands (Delta-like ligand 1,2,3, and Jagged 1, and 2) for NOTCH pathway activation. Ligand binding to the Notch receptor induces conformational change for proteolytic cleavage from A disintegrin and Metalloproteinase (ADAM) family of proteases and γ-secretase complex. Proteolytic cleavage liberates the NICD region and is translocated to the nucleus. In the nucleus, NICD interacts with RBPJ (also known as CSL proteins) and induces recruitment of co-activators like Mastermind-like protein (MAML) to promote the expression of NOTCH pathway target genes.

Ligand-independent activation of the NOTCH signaling pathway comes under the non-canonical NOTCH pathway. Endocytosis of the NOTCH receptor and cleavage of the NOTCH receptor in endosomes through protease releases NICD [64-66]. Both pathways play an important role in EMT induction. Studies have shown that Notch pathway activation plays an important role in EMT-TFs (Snail, Slug, Zeb1/2) and vimentin expression [66]. Released NICD also interacts with Wnt, Hippo, Akt, TGF-β, and NF-κB cell signaling pathways to strengthen the expression of genes responsible for EMT. In various carcinomas, this cross-talk especially between Notch and NF-κB influence metastasis [64].

Receptor Tyrosine Kinase (RTK) Signaling Pathways

Receptor tyrosine kinase signaling is involved in cell growth, proliferation, differentiation, and metabolism. Various growth factors (EGF, FGF, PDGF, HGF) signaling through their respective receptor also play an important role in cancer and EMT activation. Autocrine activation, overexpression of RTK receptors, gain of mutation, and translocation are mechanisms of aberrant activation of RTK signaling in cancer. In several carcinomas, Ras or Raf proteins are mutated. Mutated Ras and Raf also influence the expression of EMT transcription factors (Snail and Zeb-1). Either RTKs activate PI3K signaling or MAPK pathway or support the TGF-β or Wnt/βcatenin signaling to induce EMT in cancer cells [6]. In Urothelial carcinoma cell lines, FGF treatment induces PLCγ expression for stress fibre formation and MAPK pathway activation to downregulate E-cadherin for the induction of EMT [42]. EGF treatment in breast cancer cell line induces ERK signaling to induce nuclear co-localization of Smad2, 3 and promote Snail expression to induce EMT [43]. TGF-β and EGF work synergistically to induce snail and slug expression in ovarian cancer cells [44]. TGF-β signaling pathway either through stimulation of PDGF and EGF production or activated TGF-β receptor phosphorylate receptor tyrosine kinase to activate MAPK kinase signaling [39]. In breast cancer cell line (MCF-7 and T-47D), exposure of TNF-α/ TGF-β induces activation of ERK and p38 activation to upregulate Slug expression [45]. Activated EGF signaling induces downregulation of Wnt5a, inhibitor of EMT, through Arf6/ERK in SGC-7901 cell line [46].

Inflammatory Tumor Microenvironment

The tumor microenvironment is the major factor in the induction of EMT. The inflammatory tumor microenvironment (TME) is the hallmark of a tumor. TME of solid malignant tumors represents a complex and dynamic interaction between diverse cells. It comprises of tumor cells, stromal cells, fibroblast cells, and immune cells, particularly macrophages. Continuous heterotopic communication between cancer cells and other components of TME through various messengers (growth factors, chemokines, and cytokines) plays a dominating role in the formation of the inflammatory microenvironment **(Figure 2)**. This inflammatory TME reinforces cancer development, heterogeneity, clonal selection, tumor metastasis, apoptotic resistance, and therapeutic resistance [47,48]. Two types of mechanisms have been identified to link inflammatory TME with cancer. Genetic events (Oncogene activation, gene translocation, and inactivation of tumor suppressor genes) induced inflammation and chronic inflammation induced through pathological conditions play an important role in carcinogenesis and progression [49]. For example, RET oncogene mutation in thyrocytes causes initiation and development of PTC. RET/PTC3 fusion oncogene activation in growing PTC cells also activates NF-κB and causes MCP-1 and

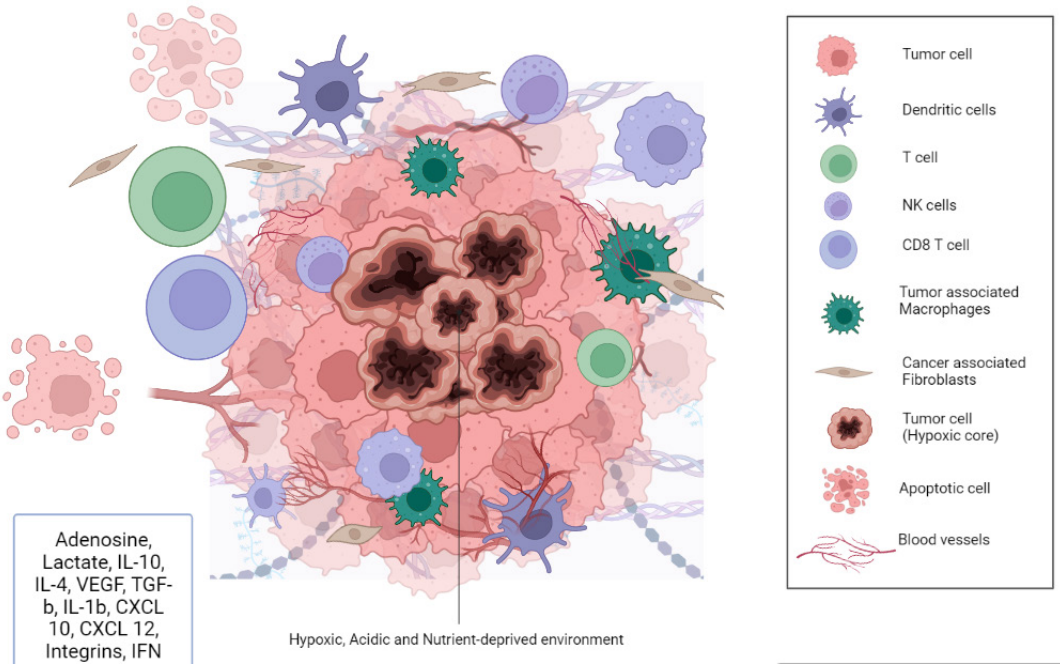

Figure 2. Tumor Microenvironment. Tumor microenvironment composed of Tumor cells and non-tumor cells (Macrophages, Fibroblast, T cells, B cells, and Dendritic cells). Non-tumor cells and tumor cells are in communication with help of chemical messengers like cytokines and chemokines. Tumor microenvironment plays a vital role in EMT induction.

GM-CSF secretion to recruit macrophages. Macrophage and transformed thyrocyte interactions increase pro-inflammatory cytokines levels in the TME [50-52]. Karin *et al*. also reported that loss of p53 triggers secretion of Wnt ligand (Wnt1, Wnt 6 and Wnt7a). Wnt secretion causes activation of macrophages to secrete IL-1β. Further, they concluded that p53 mutation in breast cancer reinforces systemic metastatic inflammation [53].

Similarly, *H. pylori* infection causes gastric cancer, Hepatitis virus causes liver carcinogenesis, and inflammatory bowel disease promotes colon carcinoma [54]. Ulcerative colitis increases colon cancer risk by 5 to 7-fold. Similarly, tobacco exposure, chronic airway conditions, and tuberculosis also increase cancer development and metastasis through chronic inflammation [55,56]. The Hallmark of inflammation-induced cancer is the presence of inflammatory cells and inflammation mediators. Both pathways lead to the activation of transcription factors like NF-κB, STAT-3, and HIF-1 in tumor cells. Thus, both pathways prime to the release of growth factors, cytokines (TNF-α, IL-6, IL-1β, NO, prostaglandins) and chemokines (CC-chemokine ligand-2, CXC-chemokine ligand 8) in TME through tumor cells. Inflammatory mediators produced, in turn, help in the recruitment of immune cells at the tumor site and increase interaction between the immune cells and tumor cells. Increased interaction causes activation of NF-κB and STAT-3 in the tumor cells and immune cells, which further reinforces inflammatory TME [57]. Inflammatory TME impacts cell survival, proliferation, angiogenesis, invasion, EMT activation, and cell migration [58].

Fetching a dynamic negotiation with stromal cells and immune cells under the influence of inflammatory TME associated signaling, tumor cells exhibit EMT/MET plasticity to adopt the constantly changing microenvironment at different steps of metastasis invasion cascade [59]. Among the cellular components of TME, tumour associated macrophages (TAM) and tumor engagement have a crucial role in orchestrating inflammatory TME for the induction of EMT. Cancer cells initiate this engagement with the secretion of macrophage colony-stimulating factor-1 (CSF-1) to recruit TAM at the tumor site, that in response releases EGF and activates EMT plasticity for the invasion metastasis cascade. The contribution of TAM-secreted inflammatory messengers like TGF-β, TNF-α, Il-1β, and IL-6 in the induction of EMT, at the invasive edge of tumors, has been extensively reported [60-62]. TGF-β emerges as the master regulator of pro-invasive TME and powerful inducers of EMT. Cancer-associated fibroblast (CAF) is the chief source of TGF-β in TME. Various signals present in the TME cause activation of CAF. Growth factors (TGF-β, HGF,

PDGF, FGF), activation of transcription factors (NF-κB and HSF-1), cytokines (IL-6, IL-1), and reactive oxygen species derived from the interaction of tumor cells and other components of TME play a significant role in the activation of CAF. Activated CAF in turn increases levels of TGF-β, HGF, and IL-6 levels in TME, which play an important role in EMT induction [63]. TGF-β interactions with various cytokines signaling pathways maintain pro-inflammatory milieu throughout the beginning and development of the EMT program in tumors. TNF-α and IL-6 rewrite inflammatory TME through effects on several diverse tumor cell types. IL-6 is a keystone cytokine that links inflammation and cancer. IL-6 has the capability to initiate EMT through Janus kinase (JAK)/signal transducer and activator of transcription-3 (STAT-3) signaling for the expression of EMT-TF. Increased levels of IL-6 in serum samples or tumor tissues have been linked with the dire prognosis of patients [64]. Similarly, TNF-α has a crucial role in inflammation-induced tumor growth and metastasis. TNF-α facilitates EMT in tumor cells through activation of NF-κB and expands AKT signaling pathway through stabilization of EMT-TF [65-67]. Tumor cells continuously communicate with cancer associated fibroblast (CAF) and macrophages in tumor microenvironment. Interaction between CAF, TAMs, and tumors increases TGF-β, IL-6 and IL-1β cytokines which in turn induce EMT in tumor cells [68].

Role of EMT in Cancer

Role in metastasis: Driver of a difficult journey

The role of EMT was studied in various cancer cell lines, mouse models, and human tissue samples. Histopathological studies of various tumor tissue samples were used to check the EMT-markers expression and correlated its prognosis of cancer patients [58-65]. Hage *et al*. observed an increase in metastasis with the increase of grade based on the expression of EMT markers expression in breast cancer tissue samples. Vimentin was found to be upregulated while cytokeratin 5/6 was found to be downregulated as the grade of the cancer increases. Further, they suggested using vimentin and cytokeratin 5/6 to evaluate EMT to predict the risk of metastasis [66]. Similarly, in a study containing 3,218 patients from metastatic breast cancer, EMT-TFs, specially overexpression of Slug, were associated with poor prognosis [67]. In gastric cancer also, loss of epithelial markers (E-cadherin and γ-catenin) and gain of mesenchymal markers (Vimentin, N-cadherin, and S1004A) were significantly associated with unfavorable prognosis and poor outcome [68]. In papillary thyroid cancer (PTC), overexpression of vimentin in gene expression profiles of invasive regions of PTC patient samples and cell lines revealed the role of vimentin in nodal metastasis and maintenance of morphology [69]. Similarly, anaplastic thyroid cancer (ATC) metastasis, lethal endocrine malignancy, linked with EMT in ATC tissue samples. Most ATC tumor tissue samples showed frequent expression of Zeb-1, Snail, and Slug and loss of E-cadherin compared with differentiated thyroid cancer (DTC) tissue samples [70-72]. Similarly, Sandy et. al. examined the EMT markers expression in different prostate tissue samples and observed the expression of Zeb-1 significantly upregulated as disease grade increased from clinically localized to castrate-resistant to metastatic. They further suggested the use of EMT markers expression in prostate cancer to predict the recurrence and survival of prostate cancer [60]. In hepatocellular carcinoma also, loss of E-cadherin and increased expression of Zeb-1 were associated with metastasis and poor prognosis based on the study of tissue samples [73].

Various mouse model-based studies were also performed to evaluate the role of EMT in cancer metastasis [74]. In the metastatic breast cancer model (MMTV-PyMT), deletion of Snail stops in early-stage metastasis premalignant site and late lung metastasis [75]. Similarly, the deletion of Zeb-1 in pancreatic ductal adenocarcinoma (PDAC) mouse model down the tumor stage, dissemination, and distant metastasis development [76]. Zhao *et al*. had tracked the conversion of epithelial cells (RFP positive) to mesenchymal cells (GFP positive) in the triple transgenic mouse model to characterize the invasion and migration of EMT-exhibiting cells [77]. Evidence of EMT involvement in early metastasis was described by Andrew et. al. study in a mouse model. They used a cre-lox-based PDAC mouse model, having KRAS and p53 mutation, for the detection of EMT with the help of lineage tracing [78].

Hence, it is postulated from migration patterns and *in vivo* studies that tumors originating from epithelial origin generally show mesenchymal invasion or collective invasion with the help of EMT [69]. Mesenchymal invasion is also known as single-cell migration, in which tumor cells exhibit the complete signature of EMT. In most carcinomas and tumors, collective invasion is observed. One or several cells present on the invasive front are the leader cells, and these cells show characteristics of the EMT program [70]. In these migration patterns, tumor cells show different types of EMT states like complete EMT, partial EMT, or hybrid EMT state. The tumor invasive leading front cells show signatures of EMT execution with weakened cell adhesion; however, the main tumor remains of epithelial characteristics. Hence, an EMT gradient ranging from complete EMT, partial EMT, and No EMT is present in the tumor tissue. This EMT gradient also depends upon the tissue in which malignancy arises [71,72]. Nicole *et al*. revealed involvement of re localization or internalization like E-cadherin was responsible for the pEMT induction in tumor cells based on PDAC mouse model having KRAS and p53 mutation. Further, they reported that pEMT association with cluster migration [73].

Intravasated tumor cells are present either in single-cell form (Single circulating tumor cells) or in clusters (Multicellular circulating tumor cells) in blood circulation. Various studies have shown expression of EMT markers in circulating tumor cells and also reported EMT status [74-78]. Circulating tumor cells (CTCs) present in the bloodstream encounter a challenge of anokis, the hydrodynamic shearing force of blood, and clearance from natural killer cells. EMT activates various cell

survival pathways and inhibits apoptotic pathways in CTCs. Expression of EMT markers in association with cell survival pathway proteins were detected in various carcinomas [79]. Analysis of CTCs from patients' sample of lung, breast, prostate, liver and colon cancer had linked EMT markers with prognosis and poor outcome. These studies on CTC also highlighted EMT generated phenotypes and co expression of epithelial markers and mesenchymal markers were detected in CTC samples [80].

In circulation, CTCs also interact with blood cells and these interactions play a pivotal role in survival, EMT maintenance, and metastasis. The high number of platelets in the blood of cancer patients were correlated with poor prognosis of patients in various cancers. In blood circulation, platelets physically coat the tumor cells. This coating of platelets works as a protective blanket around the CTC and protects it from the immune system, especially from NK cells. Coated platelets are an active source of TGF-β and PDGF that inhibits NK cells activity [81]. Platelet secreted factors are also important to maintain the EMT status of tumor cells. Platelets activate TGF-β and NF-κB signaling in tumor cells to maintain the EMT program, which further increases metastases *in vivo* [82]. CTCs also interact with neutrophils, most rich leukocyte, and neutrophils also help in activation of EMT in tumor cells. Clusters of CTCs and neutrophils were reported in mouse model and breast cancer patients. Neutrophils adhere on CTCs and this interaction guide CTCs cluster to vascular endothelium for extravasation and resist the shearing force [83].

Once tumor cells are circulated in the blood vascular system, they are stuck on blood vessels or get trapped on the blood vessels [84]. Tumor cells escape from the lumina of blood vessels to reach the surrounding tissue parenchyma. Finally, once tumor cells are lodged on the blood vasculature, they initiate growth and form a microcolony. This microcolony penetrates the vessels and places tumor cells directly with the contact of tissue parenchyma. Alternatively, carcinoma cells may directly interact with endothelial cells and pericytes cells and cross the blood vessel lamina to access the tissue parenchyma. Extravasated tumor cells now reach a completely new environment. Studies from various carcinomas have shown a new pattern of EMT in the colonization step of metastasis. In most of the carcinoma cells, EMT exhibiting cells undergo a reversion process, *i.e.,* mesenchymal-epithelial transition (MET). MET proves that EMT is not an irreversible process in carcinomas. When tumor cells clear multiple steps of metastasis cascade, they undergo reversion of the EMT process to achieve epithelial characters. After performing multiple steps of metastasis when tumor cells reach the distant anatomical organ, they experience a completely new microenvironment in which signals of EMT induction and maintenance are not present. Hence, in the absence of these signals, EMT exhibiting cells undergo the MET process and attain an epithelial state. Tumor cells present in micro metastases interact with the new environment, review, and adopt this environment to produce stroma and tumor micro-environment like the previous micro-environment for the induction of EMT and successful colonization.

Studies from various carcinomas have further enlightened the necessity of MET in the colonization step [6,85,86]. In breast cancer, E-cadherin re-expression is important for the establishment of micro-metastasis and further colonization [87]. Forced expression of EMT status in tumor cells inhibited the metastatic outgrowth and colonization of the tumor. In pancreatic tumors also, E-cadherin ^{high} and E-cadherin ^{low} tumor cells are detected in circulation but in metastatic tumors intensely E-cadherin ^{high} cells are present [88]. In ovarian cancer also, re-expression of E-cadherin is associated with metastatic outgrowth [89].

Hence, in summary, EMT dynamics are present in the tumors, and it is a reversible process. Tumor cells use this plasticity to complete the metastasis process. Loss of E-cadherin initiates the invasion metastasis cascade through activation of EMT. However, in the last step of metastasis, EMT exhibiting cells undergo the MET process to accomplish the colonization step **(Figure 3).**

EMT: An apparatus that energies therapeutic resistance

Despite the advancements in cancer research, resistance to chemotherapy, radiotherapy, and novel targeted therapies unceasingly is a significant challenge in cancer management. EMT is whispered to be one of the most prominent mechanisms of cytoskeleton change to facilitate tumor invasion. However, in recent years it has been postulated as one of the most crucial apparatuses responsible for therapeutic resistance. EMT confers survival and increases apoptotic resistance in tumor cells to successfully achieve the steps of metastasis. Its activation leads to upregulation of survival factors and downregulation of apoptosis signals to ensure cell survival. Tumor cells utilize this property of EMT in therapeutic resistance, and EMT exhibiting cells are more resistant to the cancer therapies. Exposure to chemotherapy, hormone therapy, targeted therapy, and radiotherapy also induce activation of EMT and EMT-TF, which subsequently enriches the mesenchymal phenotype. EMT either directly increases the apoptotic resistance or imparts stemness in the cancer cell to cause therapeutic resistance in cancer [90].

EMT markers and EMT-TF coordinate the invasion and interact with cell survival and apoptosis pathways for the survival and hindrance of apoptosis. In epithelial cells, E-cadherin increases the clustering of death receptors (DR4 & DR5) to form a death-inducing signaling complex (DISC) for activation of caspase-8. On the other hand, EMT activation diminishes DR4/DR5 mediated DISC formation caspase-8 activation and inhibits sensitivity towards DR4/DR5 facilitated apoptosis [91]. EMT activation induces E-cadherin switching to N-cadherin, and N-cadherin expression increases the expression of Slug, another EMT-TF, decoy receptor- DcR1 and DcR2 expression. Increased DcR1 and Dcr2 suppress TNF-α related apoptosis-

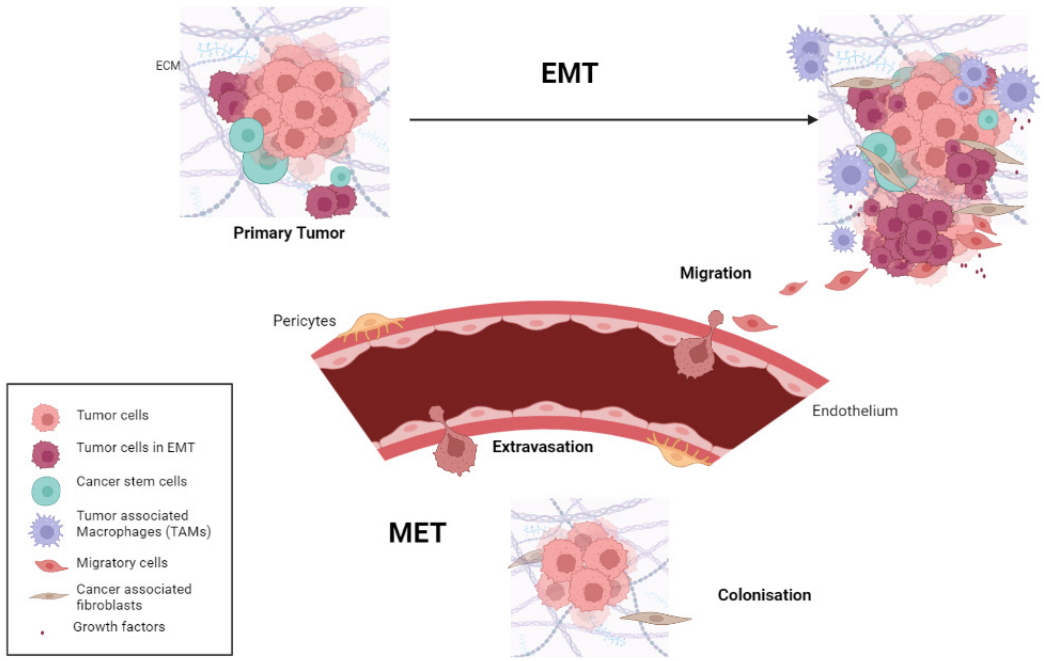

Figure 3. EMT and cancer metastasis. EMT play an essential role in each step of metastasis. EMT give invasive potential to tumor cells, help in survival, and extravasation. In the last step of metastasis, EMT exhibiting tumor cells undergo MET process for successful colonization.

inducing ligand (TRAIL) mediated apoptosis [92]. Homophilic ligation of N-cadherin also initiates PI3K/AKT signaling, which upregulates anti-apoptotic protein expression and downregulates the expression of pro-apoptotic proteins levels [93,94]. EMT-TF also plays a substantial role in cancer resistance. It was shown that Snail, an EMT-TF, halts cell cycle and confers resistance to the cell death induced by TNF-α in the MDCK cell line [95]. Additionally, Wan *et al*. observed that in hepatocellular carcinoma, silencing of Snail causes increased TRAIL-mediated apoptosis and downregulation of anti-apoptotic proteins [96]. Further, it was demonstrated that interactions of Slug, EMT-TF, with pro-apoptotic protein PUMA protects tumor cells from apoptosis, and inhibition of Slug-PUMA axis through Slug silencing causes inhibition of lung colonization [97,98]. Similarly, Jiang *et al*. report that inhibition of Slug in oral squamous cell carcinoma increases radiosensitization of tumor cells through upregulation of PUMA [99]. Apart from its role in EMT induction, Twist, another EMT-TF, also halts terminal differentiation and acts as an oncogene to suppress the p53 mediated apoptosis [100]. Thus, the interaction of EMT and cell survival/ apoptotic pathway plays an important role in EMT-mediated therapeutic resistance.

Bcl-2 family proteins and EMT-TF interaction also play an important role in apoptotic resistance [101,102]. Exposure to chemotherapy initiates the expression of mesenchymal markers in tumor cells of epithelial tissue origin. In the MCF7 cell line, exposure to Adriamycin leads to clonal heterogeneity and the development of resistance. Adriamycin-resistant cells express EMT features and have a selective growth advantage [103]. Twist overexpression in gastric cancer cells promotes invasion and contributes to resistance against paclitaxel treatment via Akt and Bcl-2 pathway [104]. In the nasopharyngeal carcinoma (NPC) cell line, Twist develops acquired resistance against Taxol through its interaction with the Akt pathway [105]. Zeb-1 is also involved in cisplatin and paclitaxel resistance in cancer cell lines [106,107]. Dam *et al.* have reported the extensive role of EMT in various cell lines against chemotherapeutic drugs. In this review, authors have reported the role of Snail, Slug, Twist, and Zeb against chemotherapy (Paclitaxel, cisplatin, 5-fluorouracil, tamoxifen, gemcitabine, anthracycline, oxaliplatin, letrozole, and doxorubicin), radiation, and targeted therapy (Trastuzumab, gefitinib, crizotinib, and nitedanib) [108].

Experiments based on the EMT lineage tracing system have also shown the direct involvement of EMT in drug resistance. Fisher *et al.* have constructed fibroblast specific protein1 promoter regulated Cre recombinase, which activates GFP expression after cleavage of Lox. This model has shown that after treatment with cyclophosphamide, GFP^+ EMT exhibiting cells were resistant to the chemotherapy treatment and chemotherapy-treated mice have a greater number of GFP^+ EMT cells in lung metastasis [109]. Thus, EMT markers and EMT-TF are well connected with cell survival, and apoptotic

pathways and tumor cells utilize this connection for drug resistance.

EMT and cellular metabolism: Restructuring of energy for metastasis

EMT endows tumor cells with augmented metastatic capability for tumor dissemination. It is also connected with a complex cellular reprogramming of metabolism to meet the energy requirement of the malignant tumor. With the activation of EMT, differentiated cancer cells of epithelial origin become undifferentiated mesenchymal cells. The metabolic phenotype of both states is also changed. Scientific literature has indicated that mesenchymal cancer cells have different metabolic requirements than epithelial phenotype to gratify the metabolic requirements of increased invasion and migration [110,111]. EMT inducing signaling pathways and EMT-TFs influence cellular metabolism [112,113]. With EMT activation, cancer cells increase glucose transporters and the glycolytic flux as cancer cells promote glycolysis [114]. Enhanced glycolysis in mesenchymal cancer cells better satisfies the needs of invading tumor cells. Enhanced glycolysis is the fastest mode of ATP generation and is best suited for the increased energy requirement for invasion and migration. Increased uptake of glucose is also important for the EMT promotion. Enhanced glucose uptake (hyperglycemic condition) promotes O-glycosylation of Snail. O-glycosylation of Snail enhances its stability and strengthens EMT in tumor cells [115]. Further, enhanced glycolysis is best fitted for the increased macromolecular biosynthesis and enhanced maintenance of appropriate cellular redox status. It is not only important for the basic needs, the energy requirement of invasion and migration, but also helps in maintaining the dedifferentiated state and stemness in tumor cells [116].

EMT induced reprogramming of cellular metabolism also plays an important role in epigenetic regulation of EMT. Cellular metabolism impacts availability or levels of metabolites or co-factors (ATP, α- keto glutarate, NAD^+, FAD, Acetyl CoA, S-adenosyl methionine and oxygen) important for epigenetic changes. These metabolites work as substrate or co-factor in various epigenetic changes [117]. Hence, EMT induced metabolic reprogramming is dynamic process and important for the induction, maintenance, and promotion of EMT mediated effects in cancer.

EMT and Cancer Stem Cells

The dangerous side of EMT mediated drug resistance is the delivery of stemness in tumor cells. Clonal heterogeneity exists in tumors, and these heterogeneous clonal populations encompass cells with diverse molecular and phenotypic features. In this heterogeneous mass, a very rare population also exists, showing stem cell-like properties. These cells show self-renewal, the ability of differentiation to generate tumor heterogeneity, and possess the capability of tumor initiation. This scarce population is termed cancer stem cells (CSCs). CSCs have been found to initiate and sustain primary tumor growth, drive dissemination, and establish distal metastases [118]. In cancer, aldehyde dehydrogenase activity ($ALDH1^+$), Side population ($ABCG2^+$), CD133, $CD24^{low}/CD44^{high}$, SSEA1, EMT promoting pathways, and transcription factor defining stemness (Oct4, Sox-2, and Nanog) based assay are used to isolate and characterize the cancer stem cells [119,120].

CSCs are thought to be highly resistant to radiotherapy, chemotherapy, and targeted therapy. Efficient ROS scavenging system for the redox balance, hypoxia, DNA repair, anti-apoptotic system, metabolic plasticity (ALDH and NAD), metabolic reprograming, expression of transcription factors defining stemness, drug efflux through ABC transporter proteins, interaction with inflammatory TME and TME cells (CAF and TAM), activation of signaling pathways (NF-κB, Notch, PI3K/Akt, Il-6/ STAT3, Wnt and Hh) and finally EMT, play an important role in CSC mediated therapeutic resistance [121-126].

Property of therapeutic resistance makes CSCs capable of tumor relapse after cancer treatment [127]. Studies have further shown that chemotherapy and radiotherapy exposure induces EMT in cancer. Chemotherapy induces enrichment of CSCs either through selection or generation. Advancement in cancer research shows that conventional cancer therapeutics frequently fail to remove cancer cells that have achieved CSCs state by activating the EMT program, allowing CSC-mediated clinical recurrence [128].

Various scientific findings have concluded attainment of stem cell-like features following EMT activation in several carcinomas like colorectal, pancreas, prostate, and ovarian cancer [3,70,129-131]. TGF-β treatment or ectopic expression of Twist or Snail induces EMT in human epithelial mammary epithelial cells and also increases expression of $CD44^{high}/ CD24^{low}$ expression in EMT exhibited cells [132]. In human mammary epithelial cells, activation of the Ras-MAPK pathway leads to activation of stem-like characteristics, and this acquisition of stemness is achieved through EMT signaling [133]. Similarly, CSCs isolated from human hepatocellular carcinoma cell lines and primary tumors through sphere formation assay exhibit high vimentin expression [134]. Increased expression of EMT-TF in ovarian and colorectal cancer causes upregulation of stemness, defining further links between EMT and CSCs at the molecular level [135]. EMT-TFs (FOX-C2, Snail and Twist) are also linked with the ABC transporter gene promoter activation in breast cancer cells [136]. Liu *et al.* have sequentially introduced TFs (TF Oct-4, KIF4 first, followed by c-Myc, and in the last Sox-2) in mouse embryonic fibroblasts (MEFs) for pluripotent stem cells and reported that optimum stem cell reprogramming requires sequential activation EMT-MET mechanism [137]. Maria *et al*. had shown that co-expression of stemness marker (ALDH-1) and EMT marker (nuclear Twist) from circulatory tumor cells isolated from breast cancer patients [138]. In colorectal cancer (CRC) tissue samples, co-expression of Snail and IL-8 was corelated with expression of CSCs marker CD44. $CD44^+$ cells

derived from primary CRC tissue has shown Snail expression in colonosphere assay and Snail expression is essential for the CSCs and metastasis [139]. Yu *et al*. had shown expression of EMT pathway expression specially Snail in $CD44^+$ $CD24^-$ $ALDH1^+$ cancer stem cells derived from head neck squamous carcinoma tissue samples and shown knockdown of Snail impaired the stemness property [140].

Various studies on cell lines and mouse models have shown link between EMT and CSCs as described above. EMT was once regarded as binary switch or process where tumor cells might either be in a mesenchymal state or an epithelial state. But various studies indicate that in growing metastatic tumor have spectrum of EMT rather than complete epithelial or mesenchymal characteristics. Ievegenia *et al.* shown in genetic mouse model of skin squamous cell carcinoma mouse model, generate YFP^+ $Epcam^+$ (epithelial and YFP^+ $Epcam^-$ (mesenchymal cells) after spontaneous EMT. Based on cell markers (CD61, CD51 and CD106) they identified EMT generated heterogeneity. Within $Epcam^-$ tumor population, different tumor subpopulation also exists with different degree of vimentin and K14 expression and some subpopulations represent hybrid tumor phenotype. They further show that EMT transition consist different transition states (including hybrid or partial transition state) and each transition state represent differential clonogenic potential, stemness and invasive property [141]. Thus, EMT is the source of heterogeneity in tumors. Hence, Epithelial-mesenchymal plasticity (EMP), a recent term for this EMT spectrum, is used to describe the existence of complete epithelial state tumor cells, partial or hybrid tumor cells, and mesenchymal state of tumor cells. EMP is also postulated to be the reason behind the cancer cells complex behavior, adaptability, and resistance in the constantly changing environment. The development of this high adaptive capacity is closely related to the properties of cancer stem cells. Studies have investigated the relationship between complete EMT program and CSCs development. Studies suggest that complete epithelial or complete mesenchymal state has low probability of CSCs development [142]. Tumor cells exhibiting hybrid or partial EMT state have maximum potential to develop cancer stem cells [6,142-145]. Gener *et al.* described that E/M hybrid phenotype is the best suited state for CSC generation [146].

Hence, it can be concluded that heterotypic interactions between tumor cells and inflammatory TME lead to EMT induction which further creates tumor heterogeneity. Tumor heterogeneity generated by the EMT spectrum causes activation of stemness defining transcription factors that generate cancer stem cells in EMT exhibiting cells. This axis of EMT and CSCs is the ultimate evil that roots for metastasis, tumor relapse, therapeutic resistance, poor prognosis, and death **(Figure 4).**

EMT: As Therapeutic Target

The pleiotropic role of EMT makes this process an appropriate target for cancer management. Several strategies like targeting EMT signaling pathways, EMT-TF, epigenetic control of EMT, and cellular metabolism are used to target the EMT (**Table 3**). Various signaling pathways (TGF-β, Wnt/ STAT-3, NF-κB, RTKs) play an important role in EMT induction. Hence, several inhibitors of these pathways are in clinical trials to target the EMT. Goossens *et al.* had described the current status of various small molecule inhibitors, monoclonal antibodies, and synthetic peptides under clinical trials designed for the TGF-β pathways, Wnt/β catenin pathways, and Notch pathway. In this review, the current status of EGFR, c-Met, and NF-κB targeting therapeutic agents was also given [160]. However, this approach has several challenges and limitations. EMT

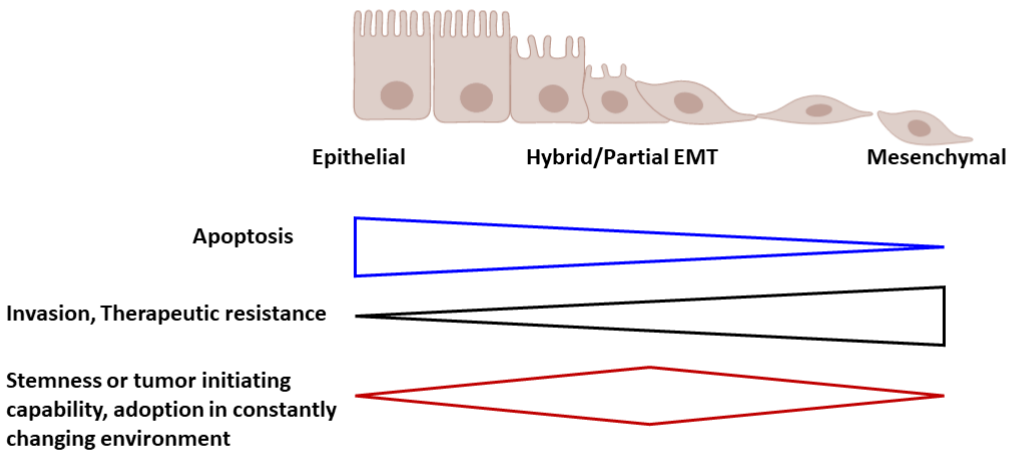

Figure 4. EMT mediated plasticity. In tumor, EMT is the source of heterogeneity and tumor cells show a spectrum of EMT phenotype. In this spectrum, tumor cells exhibiting hybrid or partial EMT have maximum potential to acquire the property of stemness for the formation of CSCs.

Table 3.

Therapeutic Agent	Target	Clinical Trial	Type of Cancer	Reference/ Clinical trial (Gov Identifier)
Fresolimumab	TGFβ1, TGFβ2, and TGFβ3	II	Refractory breast cancer	[152,153]
Galunisertib	TβRI	I/II	Glioblastoma, Colon cancer, Ovarian cancer	[152,153]
PF-03446962	TβRI	I, II	Advance solid tumors, Refractory hepatocellular carcinoma, Urothelial cancer	[152,153]
Belagenpumatucel	TβR1	I/II	Colon cancer, Ovarian cancer, NSCLC	[152,153]
Gemogenovatucel	TβR1	I/II	Ewing's Sarcoma, NSCLC	[152,153]
Bintrafusp Alfa	TβRII and PD-L1	I	Advanced squamous cell carcinoma, Esophageal Adenocarcinoma	[154,155]
WNT974	Porcupine inhibitor	I	Advanced solid tumor	[156]
Vantictumab	Anti Frizzled antibody	Ib	HER-2 negative breast cancer	[157]
Ipafricept	Decoy receptor for Wnt ligands	I	Solid Tumor	[158]
Foxy-5	Wnt 5a mimic	II	Colon cancer	[159]
LY2090314	GSK3β	II	Acute leukemia	[160]
Tarextumab (In combination with Etopside and platinum therapy)	NOTCH2	Ib/2	Stage IV Small cell lung cancer	NCT01859741
Tarextumab (In combination with Etopside and platinum therapy)	NOTCH2	Ib/2	Stage IV Pancreatic cancer	NCT01647828
Brontictuzumab	NOTCH1	I	Solid tumor	[161]
Navicixizumab	DLL4 and VEGF	Ib, III	Platinum resistant ovarian cancer	[162], NCT5043402
Crenigacestat	γ-secretase	I	Advanced Solid Tumor	[163]
Nirogacestat	γ-secretase	III	Desmod tumors	[164]
Ibrutinib (With paclitaxel and Gemcitabine)	Bruton tyrosine kinase	III	Pancreatic cancer	[165]
ACT001	NFkB and STAT3	I	Advanced Solid tumors	[166]
OPB-31121	STAT3	I	Hepatocellular carcinoma Advanced Solid tumors	[167,168]
BBI608(Napabucasin) with pembrolizumab	STAT3	I/II	Metastatic colorectal cancer	[169]
Danvatirsen	STAT3	I	Advanced solid malignancies	[170]
Ipatasertib	AKT	III	Metastatic castration resistant prostate cancer	[171]
LY2780301	AKT	IB/II	HER-2 Negative breast cancer	[172]
MK2206	AKT	II	Advanced Brast Cancer	[173]
Capmatinib	Type I B inhibitor of MET	I/II	Non-small cell lung cancer	[174]
Afatinib	ErbB family blocker	III	Lung Adenocarcinoma	[175]

Erlotinib	EGFR inhibitor	III	Non small cell lung cancer	[176]
Lapatinib	EGFR inhibitor	III	Advanced Brast Cancer	[177]
Azacitidine with Carboplatin	DNA methyl-transferase	I/Ib	Pediatric solid tumor	NCT03206021
Guadecitabine with pembrolizumab	DNA methyl-transferase	I	Solid tumor	[178]
Valporic acid (comination with bevacizumab and oxaliplatin/ fluoropyrimidine)	Histone deacetylase	II	Metastatic colorectal cancer	[179]
Vorinostat (with temozolomide and radiation therapy)	Histone deacetylase	I/II	Glioblastoma	[180]
Belinostat (with cis-Retinoic acid)	Histone deacetylase	I	Advanced Solid tumor	[181]
Entinostat	Histone deacetylase	I	Advanced breast cancer	[182]
Panobinostat	Histone deacetylase	FDA Approved	Multiple Myeloma	[183]
Mocetinostat	Histone deacetylase	I/II	Advanced pancreatic tumor and other soild tumors	[184]
Tazemetostat	Histone deacetylase	II	Solid tumor	NCT05023655
Pritumumab	Vimentin	I	Glioma	[185]
GSK2879552	Lysine Demethylase 1A	I	Small Cell Lung Cancer	[186]
CC-90011	Lysine Demethylase 1	I	Advanced Solid tumor	[187]
Mocetinostat (Platinum treated patients)	Histone deacetylase	II	Advanced Urothelial cancer	[188]

inducing pathways also play an important role in various physiological conditions. Likewie, the Wnt/β-catenin signaling pathway is critical for the maintenance of stem cells present in the hematopoietic system, hair, and intestinal tract, and the toxic effect of Wnt/ β-catenin inhibitors limit their use. Similarly, antibodies (Freslimumab to target TGF-β1 ligand and LY3022859 to target TGF-βRII receptor), ligands traps (sBetaglycans), and small molecules were developed which are under clinical trials to target TGF-β signaling. The majority of TGF-signaling target strategies work by directly inhibiting either the kinase activity of TGF receptors or the action of TGF-cytokines. However, these strategies also render the normal physiological function of this pathway. Several times, these signaling pathways also activate non-canonical pathways and induce EMT. EMT inducing pathways interact with various receptor tyrosine signaling pathways also. This integration of EMT signaling pathways with other signaling pathways is the major hurdle in the development of inhibitors to target the EMT. Further, EMT is a context-specific process and depends upon the mutation status. Hence, these therapeutic agents must be used specifically for cancer to reduce the serious side effects on non-malignant cells. Therefore, to overcome the above limitations various combinations of therapeutic agents and oligonucleotide-based agents are under clinical trials. Oligonucleotide based therapeutics include development of single strand DNA or RNA backbone against a specific gene or protein sequence. Oligonucleotide based therapeutics include development of microRNAs, small RNA, oligonucleotide, aptamers, and decoy [147]. Antisense oligonucleotide AP12009 (against TGF-β2) assessed for safety and efficacy (phaseI/II) in Glioma patients for dose escalation study and presently under phase III clinical trial. Similarly, AP110014 and AP15012 (Antisense oligonucleotide) are under preclinical trials for the treatment of various carcinomas [147].

EMT-TF interaction with various epigenetic machinery is also used to target EMT in cancer. DNA methylation and histone modification play an important role in EMT induction and maintenance. For example, histone acetyltransferase (HAT) adenosine 3',5' mono phosphate response element-binding protein (CBP) and p300 causes acylation of Snail protein at lysine 146 and 187 residue. This acylation of Snail strengthens

the stability of Snail protein. Zhao *et al*. had shown that a potent inhibitor CYD 19 form high binding affinity with Snail family proteins (Snail and Slug). This binding of CYD19 with Snail disrupts the interaction of Snail with CBP and P300. This inhibitory effect of CYD 19 causes inhibition of Snail induced EMT, metastasis (pulmonary) and increase of CSCs in MMTV-PyMT transgenic mice [148]. Likewise, Mocetinostat, a histone deacetylase, inhibits the function of Zeb-1 to prevent EMT, CSCs increase, and reversal of drug resistance [149]. Hence, various inhibitors are under clinical trials to target DNA methylation (Azacitidine, Decitabine, Guadecitabine, 5-Fluro-2 deoxy cytidine, and Hydralazine based), histone acetylation (Valproic acid, Phenylbutyrate, Phenylacetate, Vorinostat, Trichostatin, Belinostat, Entinostat, Panobinostat, Mocetinostat, CI-994, Romidepsin, GSK525762, CPI-0610, RO6870810, Tazemetostat, and Pinometostat) [161]. However, in this approach several challenges and limitations are also present. Epigenetic drugs generally show global results rather than specific ones and lead to genetic instability. The efficacy of epigenetic inhibitors is not good in solid tumors, especially in slow growing. Individual epigenetic signature and tissue variation also pose serious challenges against epigenetic inhibitors [162,163].

Another Anti-EMT strategy is to target mesenchymal characteristics like (Vimentin, N-cadherin, fibronectin, and MMPs) and induction of MET. Monoclonal antibodies, small molecules, and natural polyphenols were used to target the mesenchymal characteristics. Bollong *et al.* revealed that a small molecule, FiVe1, binds to vimentin protein leading to mitotic devastation and subsequent multinucleation, which causes loss of stemness in breast cancer cells having mesenchymal phenotype [164]. Pritumumab, a monoclonal antibody against vimentin, is in clinical trials (Phase II) for brain cancer [165]. ADH-1 (A cyclic peptide containing classical cadherin motif, HAV) functions as an antagonist against N-cadherin and is under phase II clinical trial [94,150]. To target Snail transcription factor, Co^{III} – Ebox is developed. Snail protein binds E box (CAGGTG) region. In Co^{III} – Ebox, an Ebox oligonucleotide is attached to Co (III) complex [151]. Similarly, various inhibitors (Ilomastat, Tanomastat, Rebimastat, Prinomastat, and Halofuginone hydrobromide) are under clinical trials to inhibit the MMPs activity in various cancers [160,166-169]. To target the EMT, the process of MET was also used. The concept of this strategy is to use small molecules or therapy for the redifferentiation of CSCs to original epithelial ancestors through the process of MET [170]. Diwakar *et al.* had shown proof of this concept and reported the role of protein kinase A in the redifferentiation of tumor-initiating cells (CSCs) present in the mesenchymal state to the epithelial state [171]. Retinoic acid signaling, peroxisome proliferator-activated receptor-γ signaling, metabolic reprogramming, epigenetic mechanisms, and EMT feedback loops are under investigation to induce differentiation in EMT-exhibiting cells. However, this induction of MET and re-appearance of epithelial characteristics may promote the colonization step of metastasis. Further, forced expression of epithelial characteristics may be lost if the tumor cell regains plasticity through another round of EMT [172].

In the last decade, natural phytochemicals were also extensively evaluated against EMT in various *in vivo* and *in vitro* studies [173]. Natural phytochemicals had shown promising results against EMT and EMT-TF [174,175]. Phytochemicals inhibit or modulate various cell signaling pathways involved in EMT, repress EMT-TFs, restore E-cadherin, and downregulate mesenchymal characteristics (Vimentin, Fibronectin, and MMPs) [176-181]. Natural phytochemicals have specific action and selective killing against cancer cells and have shown no harmful effects on non-malignant cells [182]. Despite the great potential of phytochemicals, one of the key apprehensions to using phytochemicals as anticancer agents is the poor solubility, limited bioavailability, and rapid metabolism in the human body. However, the combination of phytochemicals or employing nano formulation-based delivery of phytochemicals, which maximize the bio-efficacy and their bioavailability for focused delivery into cancer cells, may overcome the limitations of natural polyphenols [183,184].

Conclusion

EMT influences various aspects of cancer and creates an axis with CSCs for ultimate survival against therapies. EMT is a highly dynamic, context-specific, and complex program. Still, the mechanism of EMT activation and EMT-mediated effect are not well explored. Hence, the foremost question is to decipher the mechanism of EMT at the molecular level to understand this complex process comprehensively and design novel therapeutic approaches to target EMT for cancer management.

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