Epithelial plasticity and cancer stem cells: Major mechanisms of cancer pathogenesis and therapy resistance

Minal Garg

Minal Garg, Department of Biochemistry, University of Lucknow, Lucknow 226007, India

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Correspondence to: Dr. Minal Garg, Assistant Professor, Department of Biochemistry, University of Lucknow, University Road, Lucknow 226007, India. garg_minal@lkouniv.ac.in

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Abstract

Epithelial-mesenchymal transition (EMT) has been linked with aggressive tumor biology and therapy resistance. It plays central role not only in the generation of cancer stem cells (CSCs) but also direct them across the multiple organ systems to promote tumor recurrence and metastasis. CSCs are reported to express stem cell genes as well as specific cell surface markers and allow aberrant differentiation of progenies. It facilitates cancer cells to leave primary tumor, acquire migratory characteristics, grow into new environment and develop radio-chemo-resistance. Based on the current information, present review discusses and summarizes the recent advancements on the molecular mechanisms that derive epithelial plasticity and its major role in generating a subset of tumor cells with stemness properties and pathophysiological spread of tumor. This paper further highlights the critical need to examine the regulation of EMT and CSC pathways in identifying the novel probable therapeutic targets. These improved therapeutic strategies based on the co-administration of inhibitors of EMT, CSCs as well as differentiated tumor cells may provide improved anti-neoplastic response with no tumor relapse.

Key words: Epithelial-mesenchymal transition; Anticancer therapies; Cancer stem cells; Molecular pathogenesis; Tumor relapse

Core tip: Frequently observed reason for the failure in the treatment of malignant carcinomas is the biological programming of epithelial cells called epithelial-mesenchymal transition (EMT). It confers cancer cells, an ability to lose epithelial traits; gain mesenchymal traits; acquire stem-like properties; disseminate and colonize to distant organ sites and show elevated resistance to cancer therapies. Partial elimination of cancer stem cells and their propagation into secondary tumors post-treatment are the limitations associated with currently available standard of care including radio/chemotherapies, surgical resection or combination of these. Differentiation-based therapeutic strategies utilize the variable and regulatory powers of EMT program, lead to successful eradication of stem-like population of cancer cells by reverting the EMT phenotype and may hold great promise in improving the clinical outcomes.
INTRODUCTION
Despite significant advancements in standard treatment modalities including surgical resection, radio and chemotherapeutic procedures to treat cancer, there has been a tremendous increase in cancer related deaths globally. Although current therapeutic strategies have been successfully implicated and resulted improved tolerance and organ preservation in patients with locally advanced cancer but fail to prevent tumor from relapse. Therapeutic resistance leads to cancer pathogenesis, tumor recurrence and metastasis.

Accumulation of multiple genomic mutations in cells leads to genetic instability or oncogene-induced plasticity. Genetic and epigenetic changes may transform normal stem cells, differentiated cells or progenitor cells into cancer stem cells (CSCs) and allow the development of tumors. Existence of quiescent CSCs has been reported during intense growth of tumor and examined to possess the potential to self-renew, ability to proliferate and aberrantly differentiate into heterogeneous lineages of cancer cells. Cancer cells leave primary tumor site, extravasate to distant organs through blood or lymphatic system, colonize into new environment and develop resistance to therapeutic drugs.

Dissolution of intercellular adhesions and loss of epithelial polarity as a result of epithelial-mesenchymal transition (EMT) program has been associated with uncontrolled proliferation of cells and malignant progression. EMT regulates the apico-basal polarity of epithelial cells and turns them into cells with mesenchymal traits. Eventually cancer cells proliferate extensively, invade, acquire migratory capabilities and metastasize. Altered epithelial functions enable the CSCs to survive and exhibit resistance to growth inhibitory drugs thereby contributes to long term tumor recurrence and cancer progression.

Understanding the molecular mechanisms that control cancer pathobiology and therapeutic resistance may allow us to identify the biomarkers of potential clinical significance and novel therapeutic targets to treat cancer for their effective eradication and improved clinical outcomes. This paper summarizes the major findings on mechanistic regulation of EMT that transforms stem cells into CSCs, its major functions in metastatic activities, drug resistance and therapeutic implications.

EPITHELIAL PLASTICITY
EMT program is classified as EMT type 1, EMT type 2 and EMT type 3 and considered as an important physiological phenomenon in organogenesis during embryonic development, wound healing and cancer respectively.[1] It is characterized by the chain of events that starts from cells’ inside to extracellular matrix (ECM) and includes loss of epithelial cell-cell junctions and cell polarity, stress fiber redistribution and transition from epithelial phenotype to mesenchymal (fibroblastic) phenotype. Epithelial tumor cells exhibit cellular plasticity, undergo transition from epithelial (E) to mesenchymal (M) phenotype, dismantle basement membrane, infiltrate the surrounding tissues and metastasize to distant sites. This is followed by the growth of secondary tumors and regain of epithelial characteristics, required during differentiation through the activation of reverse program-mesenchymal to epithelial transition (MET)[5] (Figure 1).

Cellular dissociation, morphological change to more prolonged forms and increased migration abilities of cells are described by molecular characteristics which include increased expression of N-cadherin, vimentin, type 1 collagen, β-catenin stabilization, repression of E-cadherin, claudins, zona occludens 1, occludins, cytokeratins, basement membrane components collagen IV and laminin 1 and release of matrix remodeling enzymes (matrix metalloproteinases). Molecular changes in the basic foundations in epithelial architecture are noted as a result of induction of EMT-activating transcription factors (EMT-ATFs) such as two-handed zinc-finger factors of d-crystallin/E2 box factor 1 (dEF1) family proteins, EF1/ZEβ1 [dEF1/zinc-fingerE-box-binding homeobox 1 (ZEB1)] and SIPI (Smad-interacting protein)/ZEB2, Snail family of zinc-finger transcription factors, Snail1 (Snail), Snail2 (Slug) and Snail 3 (Smuc), basic helix-loop-helix factors, Twist and E12/E47. Wnt, smad3 dependent transforming factor-beta (TGF-β), Hedgehog, Notch, fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin growth factor (IGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), estrogens, sonic hedgehog (Shh), and nuclear factor-κB (NF-κB) signaling pathways are regulated by micro-environmental stimuli and act as EMT inducers[3,4] (Figure 2). Besides the cooperation between signaling pathways through autocrine signaling loops, inflammatory cytokines, hypoxic or oncogenic signals also contribute to EMT during cancer progression.

Change in the expression of several microRNAs (miRNAs) has been observed during induction of EMT or MET. MicroRNAs are 21-23 nucleotide long non-encoding RNA molecules, modulate gene expression post-transcriptionally and act as master regulator in many pathological and physiological processes including tumor development. Suppression of E-cadherin through direct targeting of its transcriptional repressors and hence inhibition of EMT has been associated with the expression of miR-200 family, miR-205, miR-34 family[6,7]. Interaction of miRs with EMT-ATFs forms mutually exclusive inhibitory feedback loop and is responsible for bistable switch between E (miR-200high; miR-34high; Zeblow; and Snaillow) and M (miR-200low; miR-34low; Zebhigh; and Snailhigh) phenotypes.
and M (miR-200<low>; miR-34<low>; Zeb<high>; and Snail<high>) phenotypes (8) (Figure 3).

As per the tumor progression model described by Brabletz et al (9), migrating CSCs with stem-like characteristics at tumor-host interface acquire migratory capacities through EMT and are responsible for the formation of primary tumor, metastatic dissemination of cancer cells, recurrence and therapeutic resistance.

**CSCs**

Theory of CSCs and the fact that cancer arises from the rare subset of cells with stemness properties was conceptualized around 150 years ago. Small population of tumor cells expressing specific surface markers including CD34+ and CD38+ in human acute myeloid leukemia has been identified as CSCs or cancer initiating cells (CICs) by Bonnet and Dick (10). These cancer cells were later described in other tumors including head and neck, breast, prostate, lung, liver, pancreas, colon and bladder. CSCs and stem cells share similarities in terms of expression of specific surface markers, regulation by stem cell niche and signaling pathways as well as their self-renewal abilities. Tumorigenic activities exhibited by CSCs allow them to differ significantly from normal stem cells. Studies from hematological
as well as solid organ malignancies characterize CSCs with the ability to mediate angiogenesis, develop tumor upon their serial transplantation into immunodeficient mice and tumorsphere formation in non-adherent 3D cultures\(^\text{[21,22]}\). The other hallmark feature of CSCs reported is aberrant differentiation where these cells undergo asymmetrical division to produce non-tumorigenic population of cells and symmetrical division to develop tumorigenic daughter cells.

Regulation of CSCs, stemness properties and their enhanced migratory characteristics has been shown to be orchestrated by the interplay of complex pathways and various transcription factors. Hedgehog, Notch, Wnt/beta-catenin, octamer-binding transcription factor 4 (Oct4), SRY (sex-determining region Y)-box 2 (Sox2) and Kruppel-like factor 4 (Klf4), high-mobility group AT-hook 2 (HMG2), Nanog, Nestin, Bcl-2, Bmi-1, c-Myc, and c-Met are required for the differentiated cells to reprogram to pluripotent stem cells, drive the production and maintenance of pluripotent cells with stemness properties\(^\text{[13-15]}\). Crosstalk between pleiotropically acting molecules, EMT associated genes and transcriptional mediators such as Snail/Twist or TGF-\(\beta\) treatment has been linked with expression of tumor cells with CD44\(^+\)/CD24\(^-\) surface markers, enhanced mammosphere formation and induction of EMT in immortalized human mammary epithelial cells\(^\text{[16]}\). Transfection of ovarian cancer cells with EMT-ATFs Snail/Snail 2 results in derepression of stemness genes including Nanog and KLF4, allows cells to acquire CD44\(^hig\)/CD117\(^hig\) stem cell profile and induces EMT in more-differentiated cells to generate cells with CSC phenotype\(^\text{[17]}\).

Experimental studies underlie the important functions of EMT in the generation of CSCs and conferring self-renewal ability to the differentiated tumor cells. Transition from E to M phenotype contributes to the enhanced migration of CSCs, their dissemination in the circulation and colonization to a particular site. Thus migratory CSCs form secondary metastatic nodules and exhibit E phenotype via MET\(^\text{[18,19]}\).

EMT gradient model describes the bimodal nature of EMT program in epithelial cells. Cancer cells with E phenotype display stemness properties during early stage of tumor development but loose it when they acquire M phenotype. Nevertheless robust association of hybrid E/M phenotype of cancer cells that co-express epithelial and mesenchymal markers through the partial activation of EMT program has been examined with increased stemness, plasticity, self-renewability, migration capabilities and poor cancer outcomes (Figure 1). Role of phenotypic stability factors (PSFs) including OVOL and GRHL2 has been characterized in stabilization of E/M hybrid state when coupled with miR200/ZEB (EMT-decision making circuit)\(^\text{[20]}\), miR-200 by inhibiting LIN28; NF-\(\kappa\)B, but not c-Myc by regulating LIN28/let-7; and OVOL by coupling with miR200/ZEB/LIN28/let-7 circuit have been examined to increase the stemness of the hybrid E/M phenotype\(^\text{[21,22]}\) (Figure 3).

Breast CSCs with E/M hybrid behavior are examined to show increased ALDH1\(^+\) (aldehyde dehydrogenase 1) activity, mammosphere formation, self-renewal capability and stemness as compared to highly differentiated M cells that exhibit less cellular plasticity and E cells which show less self-renewability\(^\text{[23,24]}\). Subset of ovarian cancer cells with hybrid E/M state has been identified with low membranous and high cytoplasmic E-cadherin, high CD133, high CD44, low Tie2 expression, increased plasticity and in vivo xenograft tumor growth upon their transformation\(^\text{[25]}\). Epithelial plasticity thereby facilitates metastasis formation, confers long term survival advan-
tages to the disseminated cancer cells at distant sites, makes tumor cells resistant to conventional therapies and allows the cancer to relapse.

**THERAPEUTIC IMPLICATIONS**

Chemotherapy and radiotherapy as non-invasive as well as surgical resection or the combination of these are the most commonly used cancer therapies in clinics. These therapies although can be employed to kill bulk of the tumor and provide maximal benefit to the overall survival of the patients, nevertheless, therapies have always been associated with systemic or local toxicity, aggressive cancer relapse and drug resistance. Population of pancreatic cancer cells exhibiting resistance to gemcitabine, ovarian carcinoma cells to paclitaxel, breast cancer cells to tamoxifen or lapatinib, lung cancer cells to gefitinib have been identified with the co-existence of subset of cancer cells, CSCs with mesenchymal traits and multiple resistant mechanisms associated with them[26-28]. Relative dormant behavior, high expression of anti-apoptosis proteins and multiple drug resistance membrane transporters, epithelial plasticity, hypoxia are some of the potential reasons of CSCs’ survival and therapeutic failure. Use of combinational approaches to target EMT which is responsible for the survival of CSCs and their tumor functions offers new possible strategy for cancer therapy. Multiple powers of EMT have crystallized an emerging concept of differentiation based cancer therapies as attractive targets for therapeutic intervention (Figure 4).

Prevention of STAT3-mediated transcription of ZEB1, SNAI1 via suppression of JAK1/2 by ruxolitinib and ZEB1 silencing through shRNA-mediated knockdown in oncstatin M (OSM, an IL-6 cytokine family member) driven mesenchymal/CSC phenotype has been examined to revert it back to an epithelial/non-CSC state in pancreatic ductal adenocarcinoma[29]. Dai et al[30] studied the therapeutic effects of ascochlorin (ASC) in increasing sensitivity to doxorubicin treatment through inhibiting STAT3 binding to the Snail promoter, reverting EMT phenotype, inhibiting metastasis in the treatment of hepatocellular carcinoma.

Delivery of anti-miR-145 using polyurethane-short branch-PEI (PU-PEI) to the mice bearing xenograft tumors has been examined to regulate Oct4/Sox2/Fascin1, Tcf4 (immunoglobulin transcription factor 4, also known as E2-2) and Wnt5a, inhibit EMT and metastatic potential and sensitize lung adenocarcinoma CSCs to chemo and radiotherapeutic drugs[31]. Forced expression of miR-200 family has been validated to restore the sensitivity of EGFR inhibitor, induces MET in mesenchymal bladder cancer cell lines, reduces tumor aggressiveness and metastatic spread[32]. Study by Luo et al[33] reports the reduced expression levels of CSCs markers LIN28B, Nanog, Oct4, and Notch1; lower expression of EMT markers MMP2, MMP3, MMP9,
Biological mechanism(s) of resistance

High levels of circulating IGF1 lead to EMT induction and CSC maintenance

Use of IGF1R inhibitors sensitize cancer cells to killing effects of carboplatin, paclitaxel, docetaxel, and vinorelbine

Anti-sense FAK oligonucleotides, adeno-viral dominant-negative FAK-CD, FAK siRNA, pharmacological inhibitors affect tumor cells and microenvironment

Clinical pilot studies

Clinical if any

Table 1 Cancer stem cells and epithelial-mesenchymal transition targeted therapy

| Cancer type | Biological mechanism(s) of resistance | Targeted therapy and therapeutic functions | Clinical trial if any | Ref. |
|-------------|--------------------------------------|--------------------------------------------|-----------------------|------|
| CRPC        | Skp2 regulates CRPC through Twist-mediated oncocigenic functions including EMT and CSCs acquisition | Genetic or pharmacological inactivation of Skp2 re-sensitize CRPC cells toward chemotherapies such as paclitaxel or doxorubicin | None | [40] |
| Lung cancer | High levels of circulating IGF1 lead to EMT induction and CSC maintenance | Use of IGF1R inhibitors sensitize cancer cells to killing effects of carboplatin, paclitaxel, docetaxel, and vinorelbine | Phase I trial | [41] |
| Ovarian cancer, advanced solid tumors | FAK linked with WNT, TGF-beta, Integrin and Hedgehog pathways, mediate cell invasion and metastasis | Anti-sense FAK oligonucleotides, adeno-viral dominant-negative FAK-CD, FAK siRNA, pharmacological inhibitors affect tumor cells and microenvironment | Phase I trial | [42,43] |
| Invasive ductal breast cancer | Elevated expression of ABC drug transporters, induction of Wnt/β-catenin, Hedgehog, Notch and PI3K/Akt/mTOR signaling pathways, and acquisition of EMT | Salinomycin promotes differentiation of CSCs, epithelial reprogramming of cells that had undergone EMT | Clinical pilot studies | [44] |
| Human lung epithelial cells | Activation of TGF-β/Smad signaling pathway, induction of EMT and therapy resistance | Use of drug, lerdelimumab, which acts as monoclonal antibody to TGF-β1 | Preclinical | [45] |
| Renal cell carcinoma, malignant melanoma | Activation of TGF-β/Smad signaling pathway, induction of EMT and therapy resistance | Use of drug, GC1008, which acts as monoclonal antibody to TGF-β1 | Phase I trial | [46] |
| Glioblastoma/anaplastic astrocytoma | Activation of TGF-β/Smad signaling pathway, induction of EMT and therapy resistance | Antisense oligonucleotide specific for the mRNA of human TGF-β2 | Phase I II trial | [47] |
| Renal cell carcinoma, advanced cancers | Inflammatory cytokines including TNF-α and IL6 promote EMT and tumor invasion | Infliximab, a TNF-α monoclonal blocking antibody suppresses the levels of IL6 and CCL2 | Phase II trial | [48,49] |
| Metastatic gastric adenocarcinoma, recurrent and metastatic head and neck squamous cell carcinoma | Activation of NF-κB and TNF-α signaling | Bortezomib, a proteasome inhibitor suppresses NFκB activation | Phase II trial | [50,51] |
| Advanced solid tumors; advanced lung cancer | Increased expression of HIF-1α | Drug, PX-478 inhibits HIF1α expression | Phase I trial | [52,53] |
|                                              |                                              | Topotecan along with conventional chemotherapies such as cisplatin or bevacizumab inhibit HIF-1α expression | Phase I II trial | |

CRPC: Castration-resistant prostate cancer; Skp2: S-phase protein kinase 2; EMT: Epithelial-mesenchymal transition; CSCs: Cancer stem cells; IGF1: Insulin-like growth factor 1; FAK: Focal adhesion kinase; TGF-beta: Transforming growth factor-beta; ABC: ATP-binding cassette; siRNA: Small interfering RNA; TNF-α: Tumor necrosis factor-α; IL6: Interleukin 6; NF-κB: Nuclear factor-κB; CCL2: C-C motif chemokine ligand 2; HIF-1α: Hypoxia inducible factor 1-alpha; PX-478: S-2-amino-3-[4'-N,N-bis(chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride.

SNAIL, TWIST, Vimentin; increased expression of E-cadherin and β-catenin, and reduced sphere formation through siRNA mediated knockdown of NR5A2 (pancreatic cancer susceptibility gene) in pancreatic cancer.

Clinical value of valproic acid (VPA), histone deacetylase (HDAC) inhibitor has been investigated to suppress irradiation-induced EMT, attenuate cell invasion, migration abilities and improve the effectiveness of radiotherapy in the treatment of esophageal squamous cell carcinoma (ESCC) TE9 cells. Angiopoietin-like protein 1 (ANGPTL1) has been examined to reduce EMT-driven sorafenib resistance and cancer stemness properties of hepatocellular carcinoma cells through the inactivation of MET-extracellular receptor kinase/protein kinase B-dependent early growth response protein 1-Slug (MET receptor-AKT/ERK-Egr-1-Slug) signaling cascade. Reduced expression levels of EMT-ATFs including SNAI2; self-renewal genes Nanog and BM11; low activity of ALDH; low ratio of CDH1 (E-cadherin) and CDH2 (N-cadherin), reduced invasion and metastatic growth are the observed therapeutic effects as a result of targeting αv integrins in bladder cancer cell lines.

Tumor microenvironment and its associated factors create immunosuppressive environment, regulate the plasticity program and determine the metastatic capacity and therapeutic resistance. Prolonged stimulation of hypoxia conditions and increased hypoxia inducing factor-1α (HIF-1α) expression identifies and isolates breast cancer stem cells (BCSCs) with ALDH activity (CD44+/CD24−/Aldefluor66). Further knockdown of HIF-1α has been shown to cause significant loss of stem cell properties through the reduction in the expression of mRNA genes associated with EMT (Snail, Slug and Vimentin low and E-cadherin high) and may influence breast cancer clinical outcomes. Inhibition of growth, migration and reduced radioresistance of NPCSC (nasopharyngeal carcinoma CNE-2 stem-like cell) has been observed to be a consequence of exposure of these cells with 2-Methoxyestradiol (2-ME2), a metabolic product of estrogen and X-ray. 2-ME2 has been reported to decrease NF-κB p65 and HIF-1α protein expression levels, downregulate NF-κB p65 nuclear localization and reversion of EMT.

Experimental studies identify novel connection between the pharmacological targeting of signaling molecules that contribute to cancer stem-like and EMT...
phenotype, restrained cancer stem cell growth, inhibition of self-renewability and reduced metastatic growth in vitro as well as in vivo. Creating an inhospitable microenvironment around the protective niche of CSCs through therapeutic check on epithelial plasticity may provide the basis for developing improved therapeutic strategy in complete elimination of CSCs and bulk tumor population at primary and distant sites"}[30-33] (Table 1).

Unravelling the complex interplay of molecules and understanding the functions of miRNA-mRNA interactions complex in cellular plasticity that influence the biology of CSCs, high throughput screening of drugs in combination and their clinical utility, development of effective and safe systems for the delivery of synthetic miRNA precursors in clinically relevant animal models are the major challenging issues in the development of therapeutics and their translation into clinical setting.

CONCLUSION

Frequently observed reason for failure in the treatment of malignant carcinomas is the biological programming of epithelial cells called EMT. EMT confers cancer cells, an ability to lose epithelial traits; gain mesenchymal traits; acquire stem-like properties; disseminate and colonize to distant organ sites and show elevated resistance to cancer therapies. Aberrant activation of signaling pathways including Wnt/beta-catenin, hedgehog, Notch, receptor tyrosine kinase, TGF-α, TGF-β has critical roles in EMT. Number of experimental studies reports the regulatory effect of miRNAs on the cross talk of these pathways, EMT, generation of CSCs, cancer invasion and metastasis.

Partial elimination of CSCs and their propagation into secondary tumors posttreatment are the limitations associated with currently available standard of care including radio/chemotherapies, surgical resection or combination of these. Recent research studies come up with alternate form of therapies that can directly target, eliminate CSCs and decrease tumor relapse. Differentiation-based therapeutic strategies utilize the variable and regulatory powers of EMT program, lead to successful eradication of stem-like population of cancer cells by reverting the EMT phenotype and may hold great promise in improving the clinical outcomes.

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