**Name of Journal: *World Journal of Stem Cells***

**Manuscript No. 32849**

**Manuscript type: Minireviews**

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

Garg M. Epithelial plasticity in cancer progression and therapy resistance

**Minal Garg**

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

**Author contributions:** Garg M contributed to this paper.

**Conflict-of-interest statement:** Garg M declares no conflict-of-interest related to this publication.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

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

**Telephone:** +91-93-35820857

**Received:** January 24, 2017

**Peer-review started:** February 2, 2017

**First decision:** March 28, 2017

**Revised:** May 22, 2017

**Accepted:** June 19, 2017

**Article in press:**

**Published online:**

**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 andmetastasis. 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 developradio-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:** Anticancer therapies; Cancer stem cells; Epithelial-mesenchymal transition; Molecular pathogenesis; Tumor relapse

**© The Author(s) 2017.** Published by Baishideng Publishing Group Inc. All rights reserved.

# 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.

Garg M. Epithelial plasticity and cancer stem cells: Major mechanisms of cancer pathogenesis and therapy resistance. *World J Stem Cells* 2017; In press

**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 epigeneticchangesmay 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 cancerrespectively[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 andtransition 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)[2] (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 andrelease 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/ZEB1 [dEF1/zinc-fingerE-box-binding homeobox 1 (ZEB1)] and SIP1 (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 pathwaysare 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[5-7]. 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[8] (Figure 3).

As per the tumor progression model described by Brabletz and colleagues, 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[9].

**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 (AML) 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 immunodeificient mice and tumorosphere formation in non-adherent 3D cultures[11,12]. 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 (HMGA2), Nanog, Nestin, Bcl-2, Bmi-1, c-Myc, and c-Metare required for the differentiated cells to reprogram to pluripotent stem cells, drive the production and maintenance of pluripotent cells with stemness properties[13-15]. Crosstalk between pleiotropically acting molecules, EMT associated genes and transcriptional mediators such as Snail/Twist or TGF-β 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[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 CD44high/CD117high stem cell profile and induces EMT in more-differentiated cells to generate cells with CSC phenotype[17].

Experimental studies underline 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[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)[20]. miR-200 by inhibiting LIN28; NF-κB, 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[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[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[25]. Epithelial plasticity thereby facilitates metastasis formation, confers long term survival advantages 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 oncostatin 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 radio therapeutic 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, MMMP9, 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[33].

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[34]. 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[35]. Reduced expression levels of EMT-ATFs including SNAI2; self-renewalgenes Nanog and BMI1; 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[36].

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 HIF-1α (hypoxia inducing factor-1α) expression identifies and isolates breast cancer stem cells (BCSCs) with ALDH activity (CD44+/CD24-/Aldefluorpos). 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[37]. 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[38].

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-renewalability and reducedmetastatic 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[39-53] (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 the 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, TNF-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 propagationinto 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.

**REFERENCES**

1 **Nieto MA**, Huang RY, Jackson RA, Thiery JP. EMT: 2016. *Cell* 2016; **166**: 21-45 [PMID: 27368099 DOI: 10.1016/j.cell.2016.06.028]

2 **Grigore AD**, Jolly MK, Jia D, Farach-Carson MC, Levine H. Tumor Budding: The Name is EMT. Partial EMT. *J Clin Med* 2016; **5**: [PMID: 27136592 DOI: 10.3390/jcm5050051]

3 **Sánchez-Tilló E**, Liu Y, de Barrios O, Siles L, Fanlo L, Cuatrecasas M, Darling DS, Dean DC, Castells A, Postigo A. EMT-activating transcription factors in cancer: beyond EMT and tumor invasiveness. *Cell Mol Life Sci* 2012; **69**: 3429-3456 [PMID: 22945800 DOI: 10.1007/s00018-012-1122-2]

4 **Garg M**. Epithelial-mesenchymal transition - activating transcription factors - multifunctional regulators in cancer. *World J Stem Cells* 2013; **5**: 188-195 [PMID: 24179606 DOI: 10.4252/wjsc.v5.i4.188]

5 **Hurteau GJ**, Carlson JA, Spivack SD, Brock GJ. Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. *Cancer Res* 2007; **67**: 7972-7976 [PMID: 17804704 DOI: 10.1158/0008-5472.CAN-07-1058]

6 **Gregory PA**, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 2008; **10**: 593-601 [PMID: 18376396 DOI: 10.1038/ncb1722]

7 **Dykxhoorn DM**, Wu Y, Xie H, Yu F, Lal A, Petrocca F, Martinvalet D, Song E, Lim B, Lieberman J. miR-200 enhances mouse breast cancer cell colonization to form distant metastases. *PLoS One* 2009; **4**: e7181 [PMID: 19787069 DOI: 10.1371/journal.pone.0007181]

8 **Tian XJ**, Zhang H, Xing J. Coupled reversible and irreversible bistable switches underlying TGFβ-induced epithelial to mesenchymal transition. *Biophys J* 2013; **105**: 1079-1089 [PMID: 23972859 DOI: 10.1016/j.bpj.2013.07.011]

9 **Brabletz T**, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. *Nat Rev Cancer* 2005; **5**: 744-749 [PMID: 16148886 DOI: 10.1038/nrc1694]

10 **Bonnet D**, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997; **3**: 730-737 [PMID: 9212098]

11 **O'Brien CA**, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007; **445**: 106-110 [PMID: 17122772 DOI: 10.1038/nature05372]

12 **Vermeulen L**, Todaro M, de Sousa Mello F, Sprick MR, Kemper K, Perez Alea M, Richel DJ, Stassi G, Medema JP. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. *Proc Natl Acad Sci USA* 2008; **105**: 13427-13432 [PMID: 18765800 DOI: 10.1073/pnas.0805706105]

13 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: 16904174 DOI: 10.1016/j.cell.2006.07.024]

14 **Xu N**, Papagiannakopoulos T, Pan G, Thomson JA, Kosik KS. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell* 2009; **137**: 647-658 [PMID: 19409607 DOI: 10.1016/j.cell.2009.02.038]

15 **Di J**, Duiveman-de Boer T, Zusterzeel PL, Figdor CG, Massuger LF, Torensma R. The stem cell markers Oct4A, Nanog and c-Myc are expressed in ascites cells and tumor tissue of ovarian cancer patients. *Cell Oncol* (Dordr) 2013; **36**: 363-374 [PMID: 23928726 DOI: 10.1007/s13402-013-0142-8]

16 **Mani SA**, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; **133**: 704-715 [PMID: 18485877 DOI: 10.1016/j.cell.2008.03.027]

17 **Kurrey NK**, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, Bapat SA. Snail and slug mediate radioresistance and chemoresistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. *Stem Cells* 2009; **27**: 2059-2068 [PMID: 19544473 DOI: 10.1002/stem.154]

18 **Riethdorf S**, Wikman H, Pantel K. Review: Biological relevance of disseminated tumor cells in cancer patients. *Int J Cancer* 2008; **123**: 1991-2006 [PMID: 18712708 DOI: 10.1002/ijc.23825]

19 **Garg M**. Urothelial cancer stem cells and epithelial plasticity: current concepts and therapeutic implications in bladder cancer. *Cancer Metastasis Rev* 2015; **34**: 691-701 [PMID: 26328525 DOI: 10.1007/s10555-015-9589-6]

20 **Jolly MK**, Tripathi SC, Jia D, Mooney SM, Celiktas M, Hanash SM, Mani SA, Pienta KJ, Ben-Jacob E, Levine H. Stability of the hybrid epithelial/mesenchymal phenotype. *Oncotarget* 2016; **7**: 27067-27084 [PMID: 27008704 DOI: 10.18632/oncotarget.8166]

21 **Jolly MK**, Huang B, Lu M, Mani SA, Levine H, Ben-Jacob E. Towards elucidating the connection between epithelial-mesenchymal transitions and stemness. *J R Soc Interface* 2014; **11**: 20140962 [PMID: 25339690 DOI: 10.1098/rsif.2014.0962]

22 **Jolly MK**, Jia D, Boareto M, Mani SA, Pienta KJ, Ben-Jacob E, Levine H. Coupling the modules of EMT and stemness: A tunable 'stemness window' model. *Oncotarget* 2015; **6**: 25161-25174 [PMID: 26317796 DOI: 10.18632/oncotarget.4629]

23 **Hendrix MJ**, Seftor EA, Seftor RE, Trevor KT. Experimental co-expression of vimentin and keratin intermediate filaments in human breast cancer cells results in phenotypic interconversion and increased invasive behavior. *Am J Pathol* 1997; **150**: 483-495 [PMID: 9033265]

24 **Grosse-Wilde A**, Fouquier d'Hérouël A, McIntosh E, Ertaylan G, Skupin A, Kuestner RE, del Sol A, Walters KA, Huang S. Stemness of the hybrid Epithelial/Mesenchymal State in Breast Cancer and Its Association with Poor Survival. *PLoS One* 2015; **10**: e0126522 [PMID: 26020648 DOI: 10.1371/journal.pone.0126522]

25 **Strauss R**, Li ZY, Liu Y, Beyer I, Persson J, Sova P, Möller T, Pesonen S, Hemminki A, Hamerlik P, Drescher C, Urban N, Bartek J, Lieber A. Analysis of epithelial and mesenchymal markers in ovarian cancer reveals phenotypic heterogeneity and plasticity. *PLoS One* 2011; **6**: e16186 [PMID: 21264259 DOI: 10.1371/journal.pone.0016186]

26 **Creighton CJ**, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, Rimm DL, Wong H, Rodriguez A, Herschkowitz JI, Fan C, Zhang X, He X, Pavlick A, Gutierrez MC, Renshaw L, Larionov AA, Faratian D, Hilsenbeck SG, Perou CM, Lewis MT, Rosen JM, Chang JC. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. *Proc Natl Acad Sci USA* 2009; **106**: 13820-13825 [PMID: 19666588 DOI: 10.1073/pnas.0905718106]

27 **Wang Z**, Li Y, Ahmad A, Azmi AS, Kong D, Banerjee S, Sarkar FH. Targeting miRNAs involved in cancer stem cell and EMT regulation: An emerging concept in overcoming drug resistance. *Drug Resist Updat* 2010; **13**: 109-118 [PMID: 20692200 DOI: 10.1016/j.drup.2010.07.001]

28 **Garg M**. Targeting microRNAs in epithelial-to-mesenchymal transition-induced cancer stem cells: therapeutic approaches in cancer. *Expert Opin Ther Targets* 2015; **19**: 285-297 [PMID: 25563894 DOI: 10.1517/14728222.2014.975794]

29 **Smigiel JM**, Parameswaran N, Jackson MW. Potent EMT and CSC Phenotypes Are Induced By Oncostatin-M in Pancreatic Cancer. *Mol Cancer Res* 2017; **15**: 478-488 [PMID: 28053127 DOI: 10.1158/1541-7786]

30 **Dai X**, Ahn KS, Wang LZ, Kim C, Deivasigamni A, Arfuso F, Um JY, Kumar AP, Chang YC, Kumar D, Kundu GC, Magae J, Goh BC, Hui KM, Sethi G. Ascochlorin Enhances the Sensitivity of Doxorubicin Leading to the Reversal of Epithelial-to-Mesenchymal Transition in Hepatocellular Carcinoma. *Mol Cancer Ther* 2016; **15**: 2966-2976 [PMID: 27765853 DOI: 10.1158/1535-7163.MCT-16-0391]

31 **Chiou GY**, Cherng JY, Hsu HS, Wang ML, Tsai CM, Lu KH, Chien Y, Hung SC, Chen YW, Wong CI, Tseng LM, Huang PI, Yu CC, Hsu WH, Chiou SH. Cationic polyurethanes-short branch PEI-mediated delivery of Mir145 inhibited epithelial-mesenchymal transdifferentiation and cancer stem-like properties and in lung adenocarcinoma. *J Control Release* 2012; **159**: 240-250 [PMID: 22285547 DOI: 10.1016/j.jconrel.2012.01.014]

32 **Adam L**, Zhong M, Choi W, Qi W, Nicoloso M, Arora A, Calin G, Wang H, Siefker-Radtke A, McConkey D, Bar-Eli M, Dinney C. miR-200 expression regulates epithelial-to-mesenchymal transition in bladder cancer cells and reverses resistance to epidermal growth factor receptor therapy. *Clin Cancer Res* 2009; **15**: 5060-5072 [PMID: 19671845 DOI: 10.1158/1078-0432.CCR-08-2245]

33 **Luo Z**, Li Y, Zuo M, Liu C, Yan D, Wang H, Li D. Effect of NR5A2 inhibition on pancreatic cancer stem cell (CSC) properties and epithelial-mesenchymal transition (EMT) markers. *Mol Carcinog* 2017; **56**: 1438-1448 [PMID: 27996162 DOI: 10.1002/mc.22604]

34 **Kanamoto A**, Ninomiya I, Harada S, Tsukada T, Okamoto K, Nakanuma S, Sakai S, Makino I, Kinoshita J, Hayashi H, Oyama K, Miyashita T, Tajima H, Takamura H, Fushida S, Ohta T. Valproic acid inhibits irradiation-induced epithelial-mesenchymal transition and stem cell-like characteristics in esophageal squamous cell carcinoma. *Int J Oncol* 2016; **49**: 1859-1869 [PMID: 27826618 DOI: 10.3892/ijo.2016.3712]

35 **Chen HA**, Kuo TC, Tseng CF, Ma JT, Yang ST, Yen CJ, Yang CY, Sung SY, Su JL. Angiopoietin-like protein 1 antagonizes MET receptor activity to repress sorafenib resistance and cancer stemness in hepatocellular carcinoma. *Hepatology* 2016; **64**: 1637-1651 [PMID: 27530187 DOI: 10.1002/hep.28773]

36 **van der Horst G**, Bos L, van der Mark M, Cheung H, Heckmann B, Clément-Lacroix P, Lorenzon G, Pelger RC, Bevers RF, van der Pluijm G. Targeting of alpha-v integrins reduces malignancy of bladder carcinoma. *PLoS One* 2014; **9**: e108464 [PMID: 25247809 DOI: 10.1371/journal.pone.0108464]

37 **Shiraishi A**, Tachi K, Essid N, Tsuboi I, Nagano M, Kato T, Yamashita T, Bando H, Hara H, Ohneda O. Hypoxia promotes the phenotypic change of aldehyde dehydrogenase activity of breast cancer stem cells. *Cancer Sci* 2017; **108**: 362-372 [PMID: 28012234 DOI: 10.1111/cas.13147]

38 **Wu SL**, Li YJ, Liao K, Shi L, Zhang N, Liu S, Hu YY, Li SL, Wang Y. 2-Methoxyestradiol inhibits the proliferation and migration and reduces the radioresistance of nasopharyngeal carcinoma CNE-2 stem cells via NF-κB/HIF-1 signaling pathway inactivation and EMT reversal. *Oncol Rep* 2017; **37**: 793-802 [PMID: 28000883 DOI: 10.3892/or.2016.5319]

39 **Garg M**. Epithelial plasticity in urothelial carcinoma: Current advancements and future challenges. *World J Stem Cells* 2016; **8**: 260-267 [PMID: 27621760 DOI: 10.4252/wjsc.v8.i8.00]

40 **Ruan D**, He J, Li CF, Lee HJ, Liu J, Lin HK, Chan CH. Skp2 deficiency restricts the progression and stem cell features of castration-resistant prostate cancer by destabilizing Twist. *Oncogene* 2017 [PMID: 28346424 DOI: 10.1038/onc.2017.64]

41 **Nurwidya F**, Andarini S, Takahashi F, Syahruddin E, Takahashi K. Implications of Insulin-like Growth Factor 1 Receptor Activation in Lung Cancer. *Malays J Med Sci* 2016; **23**: 9-21 [PMID: 27418865]

42 **Golubovskaya VM**. Targeting FAK in human cancer: from finding to first clinical trials. *Front Biosci* (Landmark Ed) 2014; **19**: 687-706 [PMID: 24389213]

43 **Infante JR**, Camidge DR, Mileshkin LR, Chen EX, Hicks RJ, Rischin D, Fingert H, Pierce KJ, Xu H, Roberts WG, Shreeve SM, Burris HA, Siu LL. Safety, pharmacokinetic, and pharmacodynamic phase I dose-escalation trial of PF-00562271, an inhibitor of focal adhesion kinase, in advanced solid tumors. *J Clin Oncol* 2012; **30**: 1527-1533 [PMID: 22454420]

44 **Naujokat C**, Steinhart R. Salinomycin as a drug for targeting human cancer stem cells. *J Biomed Biotechnol* 2012; **2012**: 950658 [PMID: 23251084 DOI: 10.1155/2012/950658]

45 **Benigni A**, Zoja C, Corna D, Zatelli C, Conti S, Campana M, Gagliardini E, Rottoli D, Zanchi C, Abbate M, Ledbetter S, Remuzzi G. Add-on anti-TGF-beta antibody to ACE inhibitor arrests progressive diabetic nephropathy in the rat. *J Am Soc Nephrol* 2003; **14**: 1816-1824 [PMID: 12819241]

46 **Flavell RA**, Sanjabi S, Wrzesinski SH, Licona-Limón P. The polarization of immune cells in the tumour environment by TGFbeta. *Nat Rev Immunol* 2010; **10**: 554-567 [PMID: 20616810 DOI: 10.1038/nri2808]

47 **Bogdahn U**, Hau P, Stockhammer G, Venkataramana NK, Mahapatra AK, Suri A, Balasubramaniam A, Nair S, Oliushine V, Parfenov V, Poverennova I, Zaaroor M, Jachimczak P, Ludwig S, Schmaus S, Heinrichs H, Schlingensiepen KH. Targeted therapy for high-grade glioma with the TGF-β2 inhibitor trabedersen: results of a randomized and controlled phase IIb study. *Neuro Oncol* 2011; **13**: 132-142 [PMID: 20980335 DOI: 10.1093/neuonc/noq142]

48 **Harrison ML**, Obermueller E, Maisey NR, Hoare S, Edmonds K, Li NF, Chao D, Hall K, Lee C, Timotheadou E, Charles K, Ahern R, King DM, Eisen T, Corringham R, DeWitte M, Balkwill F, Gore M. Tumor necrosis factor alpha as a new target for renal cell carcinoma: two sequential phase II trials of infliximab at standard and high dose. *J Clin Oncol* 2007; **25**: 4542-4549 [PMID: 17925549]

49 **Brown ER**, Charles KA, Hoare SA, Rye RL, Jodrell DI, Aird RE, Vora R, Prabhakar U, Nakada M, Corringham RE, DeWitte M, Sturgeon C, Propper D, Balkwill FR, Smyth JF. A clinical study assessing the tolerability and biological effects of infliximab, a TNF-alpha inhibitor, in patients with advanced cancer. *Ann Oncol* 2008; **19**: 1340-1346 [PMID: 18325912]

50 **Shah MA**, Power DG, Kindler HL, Holen KD, Kemeny MM, Ilson DH, Tang L, Capanu M, Wright JJ, Kelsen DP. A multicenter, phase II study of bortezomib (PS-341) in patients with unresectable or metastatic gastric and gastroesophageal junction adenocarcinoma. *Invest New Drugs* 2011; **29**: 1475-1481 [PMID: 20574790]

51 **Chung CH**, Aulino J, Muldowney NJ, Hatakeyama H, Baumann J, Burkey B, Netterville J, Sinard R, Yarbrough WG, Cmelak AJ, Slebos RJ, Shyr Y, Parker J, Gilbert J, Murphy BA. Nuclear factor-kappa B pathway and response in a phase II trial of bortezomib and docetaxel in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. *Ann Oncol* 2010; **21**: 864-870 [PMID: 19850643]

52 **Onnis B**, Rapisarda A, Melillo G. Development of HIF-1 inhibitors for cancer therapy. *J Cell Mol Med* 2009; **13**: 2780-2786 [PMID: 19674190]

53 **Powell SF**, Beitinjaneh A, Tessema M, Bliss RL, Kratzke RA, Leach J, Dudek AZ. Phase II study of topotecan and bevacizumab in advanced, refractory non--small-cell lung cancer. *Clin Lung Cancer* 2013; **14**: 495-501 [PMID: 23816875]

**P-Reviewer:** Noisa P **S-Editor:** Ji FF **L-Editor: E-Editor:**

**Specialty type:** Cell and tissue engineering

**Country of origin:** India

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): 0

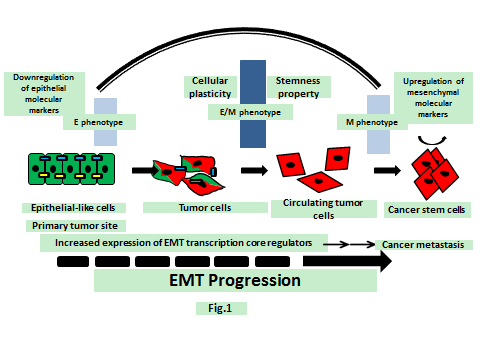
Grade D (Fair): 0

Grade E (Poor): 0

**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 oncogenic 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, adenoviral 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 oligodeoxynucleotide specificfor the mRNA of human TGF-β2 | Phase I/II trial | [47] |
| Renal cell carcinoma, advanced cancers | Inflammatory cytokines including TNFα 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 TNFα signaling | Bortezomib, a proteasome inhibitor suppresses NFκB activation | Phase II trial | [50, 51] |
| Advanced solid tumors; advanced lung cancer | Increased expression of HIF1α | Drug, PX-478 inhibits  HIF1α expression  Topotecan along with conventional chemotherapies such as cisplatin or bevacizumab inhibit HIF1α expression | Phase I trial  Phase I/II trial | [52,53] |

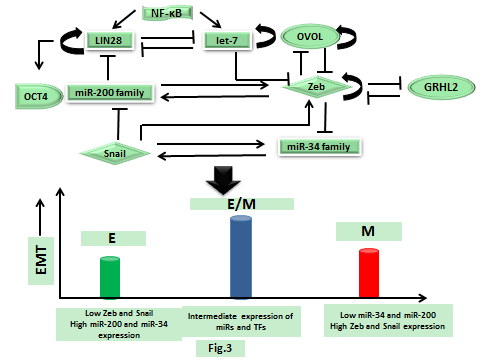
CRPC: Castration-resistant prostate cancer; Skp2: S-phase protein kinase 2; EMT: Epithelial-mesenchymal transition; CSCs: Cancer stem cells; IGF1: Insulin-like growth factor 1; FAK: Focal adhesion kinase; TGF-beta: Transforming growth factor-beta; ABC: ATP-binding cassette; siRNA: Small interfering RNA; TNF-α: Tumor necrosis factor-α; IL6: Interleukin 6; NF-κB: Nuclear factor-κB; CCL2: C-C motif chemokine ligand 2; HIF1α: Hypoxia inducible factor 1-alpha; PX-478: S-2-amino-3-[4’-N,N,-bis(chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride.



# Figure 1 Epithelial-mesenchymal transition progression in epithelial cancer cells: Cancer cells with E phenotype exhibit epithelial-mesenchymal transition at primary tumor site, loose cell-cell contacts, gain migratory abilities, undergo morphological change and acquire M phenotype. Co-expression of epithelial and mesenchymal marker proteins in cancer cells with partial E/M hybrid phenotype is associated with increased cellular plasticity and stemness. Cancer stem cells with hybrid E/M phenotype undergoing partial EMT and not complete EMT gain self-renewability, migratory and invasive traits during cancer metastasis. EMT: Epithelial-mesenchymal transition; E: Epithelial; M: Mesenchymal.

# 

**Figure 2 Signaling pathways regulating epithelial-mesenchymal transition and mesenchymal-epithelial transition: Aberrant activation of signaling pathways including Notch, Wnt, Hedgehog, Receptor tyrosine kinase, Transforming growth factor-beta, tumor necrosis factor-alpha regulate the expression of epithelial-mesenchymal transition-activating transcription factors.** EMT-ATFs induce EMT by repressing and activating the expression of epithelial and mesenchymal genes respectively. Epithelial plasticity confers long term survival advantages to the disseminated cancer stem cells at distant sites, makesthemresistant to conventional therapies and allows the cancer to relapse. EMT: Epithelial-mesenchymal transition; MET: Mesenchymal-epithelial transition; TGF-β: Transforming growth factor-beta; TNF-α: Tumor necrosis factor-alpha; EMT-ATFs: EMT-activating transcription factors.



# Figure 3 Epithelial-mesenchymal transition regulatory network: Mutually exclusive inhibitory loops including miR-200family/Zeb; miR-34family/Snail; LIN28/let-7 bring about bistable switch between epithelial (E) and mesenchymal (M) phenotypes, control Epithelial-mesenchymal transition/mesenchymal-epithelial transition and stemness. Phenotypic stability factors like OVOL and GRHL2 couple to core-EMT decision making circuits and stabilize hybrid E/M phenotype. NF-κB controls LIN28/let-7 regulation and elevates the likelihood of hybrid E/M phenotype. Solid arrows represent the activation; solid lines represent the repression and circular loops represent the self-activation. Hybrid E/M: Hybrid epithelial/mesenchymal; NF-κB: Nuclear factor kappa B; miR: MicroRNA; EMT: Epithelial-mesenchymal transition.

# 

**Figure 4 Cancer stem cells, epithelial plasticity and therapeutic strategies.** A: Existence of quiescent CSCs that possess the potential to self-renew, ability to proliferate and aberrantly differentiate into heterogeneous lineages of cancer cells and tumor microenvironment by creating immunosuppressive environment regulate epithelial plasticity and enable CSCs to survive, exhibit resistance to growth inhibitory drugs and cause tumor to progress; B: Therapeutic strategies including delivery of miRNA mimics to enforce the expression of tumor suppressor genes, administration of anti-miRNAs to downregulate the expression of oncogenes, shRNA mediated knockdown of oncogenic factors to revert the mesenchymal/CSC phenotype to epithelial non-CSC phenotype and creating inhospitable tumor microenvironment not only confer therapeutic check on epithelial plasticity but also sensitize cancer stem cell populations to the killing effects of therapeutic drugs. CSC: Cancer stem cell; miRNAs: MicroRNAs; ShRNA: Short hairpin RNA.