Review Article

Epithelial–mesenchymal transition in cancer stem cells: Therapeutic implications

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Abstract

Cancer stem cells (CSCs) are cancer cells that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs may generate tumors through the processes of self-renewal and differentiation into multiple cell types. CSCs present in tumors are normally resistant to conventional therapy and may contribute to tumor recurrence. Tumor residuals present after therapy, with CSCs enrichment, have all the hallmarks of epithelial–mesenchymal transition (EMT). In this review, we discuss the relationship between EMT and CSCs in cancer progression and its therapeutic implications in oral squamous cell carcinoma.

Keywords: Cancer stem cells, cancer therapeutics, epithelial–mesenchymal transition, oral squamous cell carcinoma

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INTRODUCTION

The epithelial-to-mesenchymal transition (EMT) is a process in which epithelial cells change their morphology, lose their polarity, and acquire the migratory properties of mesenchymal cells. It was first recognized as a feature of embryogenesis, but it is also activated during wound healing, organ fibrosis, and tumorigenesis. Cancer stem cells (CSCs) are a heterogeneous population of cells within cancer, have similar characteristics to normal stem cells, including the ability to self-renewal and differentiation. These cells can differentiate into various cells to support tumors and are a source of tumorigenesis, metastasis, and relapse. Many reports have shown that cells undergoing EMT can acquire stem cell-like characteristics and express markers of the EMT, this demonstrates good conjunction between EMT and stem cells.[1] However, the correlation between stemness and EMT is still unclear. Recent studies have linked the epithelial–mesenchymal plasticity and stem cell-like traits, which provides new insights into the conflicting relationship between EMT and CSCs.[2] In this review, we discuss the current knowledge about the link between EMT and CSCs in cancer progression and evaluate the controversies and future perspectives.

EPITHELIAL–MESENCHYMAL TRANSITION

EMT is a highly dynamic process, in which epithelial cells can convert into a mesenchymal phenotype. EMT

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usually occurs in normal physiological conditions like tissue morphogenesis and repair, tissue reconstruction, and fibrogenesis.[3] In embryogenesis, the neural crest cells will change their epithelial phenotype to the fibroblast-like morphology and migrate to a distant site to form new organs by acquiring the complex process of EMT. Most studies show that EMT is the process of breakdown of intercellular adhesion between epithelial cells by inhibiting expression of E-cadherin and expression of higher levels of mesenchymal cadherin, this process is known as cadherin switching. In addition to the normal physiological process, EMT also exploits some role in certain pathological conditions like tumorigenesis and metastasis, resulting in poor tumor staging, increased recurrence, and a decreased survival rate in several cancers.[4]

EMT phenomenon can be divided into three types, type one seen in embryogenesis, type two in wound healing, tissue regeneration, and organ fibrosis, and type three in cancer cell metastasis and invasion. Loss of cell-to-cell adhesion by activation of transcriptional repressors, actin reorganization and formation of invadopodia, expression of relevant matrix metalloproteinases (MMPs), and changes in the expression of microRNAs are the four important molecular mechanisms that make a change in the type three EMT.[4]

The first step of cancer metastasis and invasion is the loss of cell-to-cell adhesion and loss of cell to ECM adhesion, which is triggered by the activation of the EMT pathway. Following the EMT process, the tumor cells express several mesenchymal markers like vimentin, N-cadherin, and fibronectin concomitant with the loss of E-cadherin. Loss of E-cadherin expression is induced by several growth factors like transforming growth factor β (TGFβ), epidermal growth factor, interleukin-6, and fibroblast growth factor (FGF). After binding to their corresponding receptors, these growth factors can activate the intracellular signaling pathways that activate several transcriptional repressors of E-cadherin gene transcription, such as Snail, Slug, zinc finger E-box binding homeobox (ZEB) 1, ZEB2, and Twist.[4]

Reorganization of actin and formation of invadopodia are the other two mechanisms contributing to the EMT progression. β-catenin and p120-catenin present in the adherens junction of normal epithelium plays a critical role in linking the intercellular adherens junction to the intracellular actin filament. Adherens junction disruption results in β-catenin and p120-catenin dissociation and its cytoplasmic accumulation. Excess β-catenin present in the cytoplasm migrates into the nucleus and activates transcription factor T-cell factor (TCF), SMAD2, and SMAD3 necessary for tumor cell proliferation, migration, and invasion. Excessive p120-catenin accumulated in the cytoplasm also downregulates the activity of the GTPase enzyme required for actin assembly and upregulates the activity of Rac and Cdc-42 necessary for the formation of invadopodia.[5] Invadopodia, containing MMPs (membrane type I-MMP or MMP14) plays the main role in ECM degradation and cell migration. Different types of noncoding microRNA expression have been found to act both as facilitators and as an inhibitor of the EMT program.[5]

CANCER STEM CELLS

American Association of Cancer Research defined CSCs as a subset of cells with the capability of self-renewal and differentiation into the heterogeneous lineages that constitute the tumor mass. Francesco Durante in 1874 proposed that cancer can originate from a small population of cells with stem cell-like properties.[6] The first evidence of the role of CSCs was documented in human acute myeloid leukemia by Bonet and Dick in 1997. Prince *et al*. in 2007 first identified CSCs in head and neck carcinoma through the expression of CD44 by a few subpopulations of cells.[6] CSCs interact with transformed cells and other stromal cells within the tumor microenvironment through adhesion molecules and paracrine factors and promote the differentiation of CSCs, enhance angiogenesis, recruit immune and stromal cells, and promote tumor invasion and metastasis.[7] The expression of stem cell markers like CD44, CD133, CD117, OCT-4, and aldehyde dehydrogenase (ALDH) are used to isolate CSCs from oral squamous carcinomas (OSCC). In OSCC, CSCs express many of the same proteins involved in the core network that regulates ESCs like OCT4, SOX2, and NANOG. Immunohistochemical staining for OCT4, SOX2, and NANOG in OCSCC demonstrate that OCT4 and SOX2 are expressed significantly higher in tumor-adjacent tissue compared to both normal tissue and the tumor. However, NANOG is highly expressed in both tumor tissue and peritumoral tissue, compared to normal tissue.[8]

THE LINK BETWEEN EMT AND CSCS

The relationship between EMT and CSCs in the field of carcinogenesis was established in recent years, both factors contributing to tumor recurrence, metastasis, and drug resistance. Many reports have shown that cells undergoing EMT can acquire stem cell-like characteristics and express markers of the EMT, this demonstrates a good link between EMT and stem cells. Based on Brabletz hypothesis,

there are two types of CSCs present in tumor: Stationary CSCs (sCSCs) and migrating CSCs (mCSCs). sCSCs are nonmobile and present in the epithelium, whereas mCSCs mediate tumor cell metastasis. They suggested that mCSCs were derived from sCSCs that went through the process of EMT, that is, EMT was an essential component involved in promoting metastasis from the CSCs protected within the niche.[9]

Important signaling pathways linking EMT and cancer stem cells

The formation of EMT and CSCs is a dynamic process, and this process is triggered by multiple cellular signaling pathways, such as TGFβ, Wnt/β-catenin, Hedgehog and Notch pathways.[10]

TGFβ signaling

Multifunctional cytokine TGFβ is one of the major EMT inducers. Recent studies show the induction of EMT by TGFβ has been linked to the acquisition of tumor-initiating stem cells (TISCs) in breast cancer.[10] Van der Horst *et al*.[11] found that mesenchymal liver cancer with EMT demonstrated TISC characteristics, such as tumor-sphere formation. They also found that TGFβ induced EMT and TISC characteristics through the up regulation of SNAIL and Nanog. Muraoka-Cook *et al*.[12] has shown that the gene-expression profile of the human mammary epithelial cell line introduced by EMT inducers, including TGFβ, closely aligns with a stem cell-like expression profile. TGFβ usually works together with Wnt, Hedgehog, Notch, and Ras signaling pathways for complete induction of EMT. However, Tang *et al*.[13] reported that in transformed human breast epithelial cells, TGFβ stimulation reduced the stem cell-like properties and TGFβ inhibition increased the size of the CSC population and promoted tumorigenesis by another mechanism that was independent of direct effects on proliferation.

Wnt/β-Catenin signaling

The Wnt/β-catenin signaling pathway can adjust stem cell renewal and also it is involved in EMT induction in cancer. Overexpression of the homeobox protein Six1 in the mouse mammary gland produces highly aggressive tumors with an EMT phenotype, stem cell features, and activated Wnt signaling, providing *in vivo* evidence for the emergence of cells with combined EMT–CSC phenotypes. Inhibition of Wnt signaling could reduce the self-renewal capacity of cancer cells, and downregulated the expression of SLUG and TWIST, transcriptional factors regulating the expression of genes responsible for the EMT, but activation of β-catenin signaling leads to stem cell proliferation and tumorigenesis. Stem cell-like properties of EMT can be inhibited by treatment targeting the Wnt/β-catenin pathway.[10]

Hedgehog signaling

The formation of CSCs and EMT is related to Hedgehog signaling. Reports showed that Hedgehog signaling played a critical role in the maintenance of TISCs and Bmi-1, which may directly mediate Hedgehog signaling to confer a self-renewal capacity in TISCs. The downregulation of Hedgehog signaling inhibits CSCs and EMT, accompanied by down-regulation of SNAIL and upregulation of E-cadherin.[10]

Notch signaling

In human mammary stem cells, the Notch signaling pathway has been shown to contribute to EMT induction and regulate asymmetric cell-fate decisions. Many reports have described the close connections between Notch signaling pathways stem cell function and EMT.[10]

CLINICAL IMPLICATIONS OF EMT AND CSCS

Early clinical trials showed that mono-therapy had a limited role in head and neck squamous cell carcinoma (HNSCC) compared to traditional chemotherapy. One of the most effective methods for HNSCC is targeting CSCs because CSCs can resist chemotherapy and radiotherapy and are left behind to continue to grow and spread. There are so many efforts that have been made towards attacking CSCs in different tumor types [Table 1], but most of them showed limited effect because of the failure to identify the real CSCs populations. However, some recent preclinical and clinical progress in targeting HNSCC CSCs is going on by using cell surface markers, tyrosine kinase, immune checkpoint inhibitors, and stem cell factors. Certain natural products like cyclic depsipeptides, BE-43547A2 identified as a useful drug to target CSCs in different tumors. The stemness of the OSCC CSCs can be suppressed by using Curcumin, a natural product extracted from Curcuma longa plants through the inhibition of RXRα.[14]

EMT and CSCs have a strong correlation for cancer progression and metastasis, also to the acquisition of chemoresistance of tumor cells. Some studies have tried to apply the molecular mechanism of the link between CSC and EMT to carry on the initial application of cancer treatment. For example, tumor suppressor p53 loss in mammary epithelial cells induces EMT and enriches CSCs through repression of miR200c. Therefore, activation of the p53–miR200c pathway can be used to suppress EMT-associated CSCs to treat cancer. Genistein, a natural chemo preventive agent can be used for the overexpression

Table 1: Emerging Therapeutic agents targeting cancer stem cells

Agents	Suggested Patient Population
TGF*-β inhibitors[15,16]	
TGF-ßR1 inhibitors	Glioblastoma, unresectable HCC
Galunisertib (LY2157299)	R/R† mesothelioma, melanoma, and RCC
Anti-TGF-β antibodies	
Fresolimumab (GC-1008)	R/R high-grade glioma, pancreatic cancer, melanoma
Trabedersen (AP 12009)	
Wnt inhibitors[17-19]	
Ligand sequestration	Various R/R solid tumors
OMP-54F28	R/R pancreatic cancer
Inhibitors of b-catenin activity	R/R AML‡, myeloma
PRI-724	
CWP232291	
Hedgehog inhibitors[20,21]	
SMO antagonists	Adult R/R SHH§ medulloblastoma
Vismodegib (GDC-0449)	R/R hedgehog-activated medulloblastoma
Sonidegib (LDE225)	Myeloid neoplasms
Glasdegib (PF-04449913)	
Notch inhibitors[22-26]	
γ-secretase inhibitors	Adult R/R high-grade glioma, R/R breast cancer
MK-0752	Various solid tumors
RO4929097	R/R desmoid tumors not amenable to surgery, T-ALL‖
PF-03084014	1. Pancreatic cancer, NSCLC¶
DLL4 inhibitors	
Demcizumab (OMP-21M18)	

*TGF, transforming growth factor; †R/R, relapsed or recurrent; ‡AML, acute myeloid leukemia; §SHH, sonic hedgehog; ‖ALL, acute lymphoblastic leukemia; ¶NSCLC, non-small cell lung cancer

of FoxM1 in pancreatic cancer which is responsible for the acquisition of EMT and CSC phenotypes.[27] The G9a is a protein, it can induce EMT and CSC-like properties in HNSCC. Hence, chemotherapeutic agents which act on the G9a–Snail axis can be used for the treatment of HNSCC.[28]

CONCLUSION

The highly dynamic process, EMT is crucial for the normal pathophysiological process, but it has a deleterious consequence in enhancing tumor progression and metastasis. CSCs, a small population among tumor cells, can self-renew and differentiate into different tumor cell types. They are one of the very important players in the initiation and progression of cancer. The relationship between EMT and CSCs in the field of carcinogenesis was established in recent years, both factors contributing to tumor recurrence, metastasis, and drug resistance. Cancer therapy targeting the CSCs and EMT would be one of the encouraging as well as evasive treatment options, but they are still in their infancy. The ongoing discoveries of more molecular mechanisms behind the EMT and CSCs in tumor progression may bring new therapeutic platforms for cancer therapy.

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Conflicts of interest
There are no conflicts of interest.

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