

Epithelial–mesenchymal transition in oral squamous cell carcinoma: An insight into molecular mechanisms and clinical implications

P Jayanthi¹, B R Varun², J Selvaraj³

¹Department of Oral and Maxillofacial Pathology, Azeezia College of Dental Sciences and Research, Kollam, ²Department of Oral and Maxillofacial Pathology, PMS College of Dental Sciences and Research, Thiruvananthapuram, Kerala, ³Department of Biochemistry, Saveetha Dental College, Chennai, Tamil Nadu, India

Abstract

Epithelial–mesenchymal transition (EMT) is an important event in embryonic development, fibrosis and cancer invasion. During cancer progression, the activation of EMT permits cancer cells to acquire migratory, invasive and stem-like properties. Despite recent advances in treatment, there is no improvement in the 5-year overall survival rate of oral squamous cell carcinoma (OSCC). Local recurrence and lymph node metastasis are considered to be mainly responsible for the low survival rate in OSCC. EMT plays a major role in local recurrence and lymph node metastasis of oral cancer. This review article addresses the clinical implications of EMT in OSCC and explains the molecular mechanisms of EMT, highlighting the cadherin switching and signaling pathways involved.

Keywords: Invasion, migratory, oral carcinoma, transition

Address for correspondence: Dr. P Jayanthi, Department of Oral and Maxillofacial Pathology, Azeezia College of Dental Sciences and Research, Kollam, Kerala, India.

E-mail: drjayanthip@gmail.com

Submitted: 25-Nov-2019, **Accepted:** 15-Dec-2019, **Published:** 08-May-2020

INTRODUCTION

Epithelial–mesenchymal transition (EMT) is a biological process where the epithelial cells acquire mesenchymal traits. The epithelial cells are characterized by tight cell–cell adhesions and apical–basal polarity, whereas the mesenchymal cells are generally elongated in appearance with loose cell–cell interactions, resulting in increased cell migration.^[1] The conversion of epithelial cells to mesenchymal cells is important for normal embryogenesis and organ development, especially during gastrulation and neural crest cell migration. In addition, EMT has been observed in several pathophysiological conditions in human

adults, including inflammation and wound healing. The process of EMT has been linked to cancer cell metastasis and invasion, leading to increased risk of recurrence and decreased survival rate in various types of cancers such as colorectal cancer, breast cancer, lung cancer and oral cancer.^[2]

MECHANISM OF EPITHELIAL–MESENCHYMAL TRANSITION

The process of EMT is divided into three distinct types, namely EMT Type 1, 2 and 3. EMT-1 occurs

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Jayanthi P, Varun BR, Selvaraj J. Epithelial–mesenchymal transition in oral squamous cell carcinoma: An insight into molecular mechanisms and clinical implications. *J Oral Maxillofac Pathol* 2020;24:189.

Access this article online

Quick Response Code:



Website:

www.jomfp.in

DOI:

10.4103/jomfp.JOMFP_334_19

in normal embryogenesis; EMT-2 in wound healing and tissue regeneration; and EMT-3 is associated with cancer progression.^[3] At the molecular level, three important mechanisms contribute to the changes in type 3 EMT.^[4]

1. Downregulation of E-cadherin
2. Changes in the expression of microRNA (miRNA)
3. Reorganization of actin and formation of invadopodia.

Downregulation of E-cadherin

The hallmark of EMT process is loss of cell–cell adhesion and increased cell motility by the downregulation of E-cadherin (epithelial cadherin) and upregulation of mesenchymal type N-cadherin. This process is known as “cadherin switching” appears to provoke cancer cell migration and invasion. The functional loss of E-cadherin may be due to germ line or somatic mutation in E-cadherin gene, DNA hypermethylation of E-cadherin gene, proteolytic cleavage of E-cadherin or transcriptional suppression of E-cadherin gene. Genetic mutation of E-cadherin gene is extremely rare, whereas epigenetic modification in the form of DNA hypermethylation in the promoter region of E-cadherin gene has been frequently found in cancer cells.^[5]

Proteolytic cleavage of E-cadherin by γ -secretase and a disintegrin and metalloproteinase 10 results in the generation of E-cadherin fragments both inside and outside the cell. Studies have shown that the shed extracellular domain of E-cadherin interferes with cell–cell adhesion, thereby enhancing cell detachment and migration. The intracellular fragment translocates from the cytoplasm into the nucleus where it activates the transcriptional repressor called “Kaiso.” Kaiso exerts its suppressive activity on the promoter region of target genes, which are yet to be identified.^[6,7]

One of the major events contributing to EMT is the activation of transcription factors (EMT-TFs), which function as the repressors of E-cadherin gene. EMT-TFs are usually activated by several growth factors, such as transforming growth factor-beta (TGF- β), epidermal growth factor and fibroblast growth factor, probably emanating from tumor-associated stromal cells.^[4] TGF- β -mediated signaling pathway is the widely accepted mechanism that regulates the EMT process. Upon binding to the putative receptors, TGF- β activates intracellular signaling molecules such as SMAD-2 and SMAD-4, which, in turn, activate the transcriptional repressors of E-cadherin gene.^[8] Five types of EMT-TFs have been described in the literature, namely SNAIL1, SNAIL2, ZEB1, ZEB2 and TWIST1.

SNAIL1

Snail1, a member in the family of zinc-finger transcription factor, is considered to be the master gene for EMT. Snail1 binds to E-box in the promoter region of E-cadherin, occludin and claudin genes, thereby repressing the transcription of these genes. Snail1 induces the activity of histone deacetylase (HDAC1) genes to remove the acetyl groups from the histone proteins, resulting in a high-affinity binding between histones and DNA, which prevents the transcription of E-cadherin gene.^[9]

SNAIL2

Snail2, also known as slug, belongs to the family of zinc-finger transcription factor and functions similar to that of Snail1. Slug binds to *cis*-elements in the promoter of E-cadherin gene through its C-terminal “zinc-finger” domain and represses the gene expression by recruiting chromatin-modifying proteins through its N-terminal “SNAG” domain. Villarejo *et al.* have shown that the overexpression of slug not only reduces E-cadherin expression but also increases the expression of MMP2, resulting in tumor metastasis *in vivo*.^[4,9]

ZEB1

Zinc finger E-box-binding homeobox 1 (ZEB1) binds to E-boxes and represses the expression of E-cadherin to induce EMT. ZEB1 can function as an activator by interacting with Smads, the signaling mediators of the TGF- β pathway. Dave *et al.* have demonstrated that Snail1 acts cooperatively with Twist1 to control the expression of ZEB1.^[10]

ZEB2

Zinc finger E-box-binding homeobox 2 (ZEB2) induces EMT by binding to the E-cadherin promoter and repressing the transcription of E-cadherin gene. ZEB2 has been shown to repress the expression of several genes encoding junctional proteins, including desmosomal proteins desmoplakin and plakophilin 2 and tight junction protein claudin 4.^[11]

TWIST1

Twist1, a basic helix-loop-helix transcriptional factor, is a master regulator of gastrulation and mesoderm specification. The ectopic expression of Twist1 upregulates mesenchymal cell markers (fibronectin, vimentin, smooth muscle actin and N-cadherin) and loss of epithelial markers (E-cadherin). Twist1 has been shown to play a vital role in the intravasation step of metastasis, angiogenesis and chromosomal instability. Under hypoxic conditions, hypoxia-inducible factor-1 promotes EMT through the induction of Twist1. Twist1, in turn, activates Bmi1,

both of which are essential for promoting EMT and tumor-initiating capacity.^[12]

Changes in the expression of microRNA

miRNA is small, noncoding RNAs that are between 21 and 25 nucleotides in length. They bind to the target mRNA and cause either degradation of target mRNA or may induce transcriptional repression of mRNA. Dysregulation of certain types of miRNA has been associated with tumor metastasis and resistance to therapy by modulating the EMT process. For example, mi-RNA 200 family suppresses the activity of EMT transcription factors ZEB1 and ZEB2, thereby retaining the epithelial characteristics of cancer cells, while miRNA-21 and miRNA-181 enhance TGF- β -induced EMT process.^[13]

Reorganization of actin and formation of invadopodia

In the normal epithelial cells, E-cadherin in the adherens junction usually co-localizes with β -catenin and p120-catenin in the cytoplasmic membrane. This co-localization is critical for linking the intercellular adherens junction to the intracellular actin filament. Following the loss of E-cadherin function and disruption of adherens junction, β -catenin and p120-catenin located in the E-cadherin complex get dissociated and are accumulated within the cytoplasm. The excessive β -catenin in the cytoplasm can migrate to the nucleus, where it activates a transcription factor, called T-cell factor (TCF). TCF, along with activated SMAD 2 and SMAD3, triggered by TGF- β signaling pathway upregulates the expression of target genes (c-myc, cyclin D1 and slug) necessary for tumor cell proliferation, migration and invasion.^[14]

In addition to β -catenin, p120-catenin is also accumulated in the cytoplasm, following the dissociation of E-cadherin complex. P120-catenin is found to downregulate the activity of GTPase enzyme, Rho-A that is necessary for the regulation of actin assembly and thereby the stability of cell–cell adhesion. Excessive p120-catenin in the cytoplasm can upregulate the activity of two other enzymes, Rac and Cdc-42, which help in the formation of migratory membrane protrusions called lamellipodia and filopodia.^[14]

The lamellipodia and filopodia, collectively known as invadopodia, contain MMP, specifically membrane type I-MMP (MT-1 MMP or MMP14). The membrane type I-MMP is involved in extracellular matrix degradation and cell migration. The expression of MT-1 MMP was originally reported in lung carcinomas and stromal fibroblasts near the advancing tumor front. Subsequently, increased expression of MT-1 MMP was found in other cancers, including colon, breast and head-and-neck cancers,

where the expression directly correlates with poor patient prognosis.^[15]

EPITHELIAL–MESENCHYMAL TRANSITION AND CANCER STEMNESS

Cancer stem cells (CSCs) are cancer cells that have characteristic features similar to that of 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 stem cell properties of self-renewal and differentiation into multiple cell types. Such cells are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. CSCs are located in the invasive fronts of head-and-neck squamous cell carcinomas (HNSCCs) close to blood vessels (perivascular niche). Endothelial cell-initiated signaling events are critical for the survival and self-renewal of these stem cells. Markers such as aldehyde dehydrogenase, CD133 and CD44 have been successfully used to identify highly tumorigenic CSCs in HNSCC.^[16]

Brabletz hypothesized that there are two types of CSCs: stationary CSCs (sCSCs) and migrating CSCs (mCSCs). sCSCs are embedded in the epithelia and are nonmobile, whereas mCSCs mediate tumor cell metastasis. They proposed that mCSCs were derived from sCSCs that underwent the process of EMT. In other words, EMT was an essential component involved in promoting metastasis from the CSCs protected within the niche.^[17]

The human mammary epithelial cells that are exposed to TGF- β or the ectopic expression of Snail1/Twist1 in these cells induce a cell population with stem cell characteristics, including enhanced expression of CD44 and low expression of CD24.^[18] Prostate cancer cells with the mesenchymal phenotype display stem-like properties, including increased expression of the pluripotency genes such as Sox2, Nanog and Oct4.^[19] In pancreatic cancer, ZEB1 is the critical link between the activation of EMT and the acquisition of stem-like properties and functions by suppressing miR-200 family members, which are the strong inducers of epithelial differentiation. Activation of ZEB1 promotes EMT and the expression of stem cell factors such as Sox2 and Klf4.^[20]

CLINICAL IMPLICATIONS OF EPITHELIAL–MESENCHYMAL TRANSITION

EMT plays a major role in local recurrence and lymph node metastasis and is associated with a low survival rate in patients with oral squamous cell carcinoma (OSCC). Krisanaprakornkit and Iamaroon have demonstrated the

downregulation of E-cadherin and MMP-9, the epithelial phenotypes and upregulation of vimentin and MMP-2, the mesenchymal phenotypes, in OSCC cell lines. Vimentin, in particular, was immunolocalized in the cytoplasm of OSCC cells at the invasive tumor fronts in their study.^[4]

Snail1 and slug are the master genes in regulating E-cadherin expression during the process of EMT. In an OSCC model, Snail-transfected cells showed complete EMT phenotypes with a fibroblast-like appearance, vimentin filaments, E-cadherin/N-cadherin switching and lack of hemidesmosomes. In addition, ZEB-1 and ZEB-2 were upregulated in these cells.^[21]

In a HNSCC model, the immunohistochemical results revealed a high expression of N-cadherin in the majority of HNSCC cases and the expression of N-cadherin significantly correlated with malignant behaviors. Cadherin switching was found in 30 of 80 HNSCC cases and correlated with histologic differentiation, pattern of invasion and lymph nodes metastasis.^[22]

CONCLUSION

OSCC is a devastating disease and remains a major threat to global public health. Extensive studies have been performed and elucidated the complex nature of OSCC carcinogenesis. Emerging knowledge from these studies on EMT in the last decade has provided a better understanding of the mechanisms of EMT in human cancers, including OSCC. Undoubtedly, this knowledge will contribute significant advances to the biology of carcinogenesis, leading to the development of new biomarkers for the diagnosis and prognosis and targeted therapeutics for patients with OSCC.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Nieto MA, Huang RY, Jackson RA, Thiery JP. EMT: 2016. *Cell* 2016;166:21-45.
2. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: New insights in signaling, development, and disease. *J Cell Biol* 2006;172:973-81.
3. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009;119:1420-8.

4. Krisanaprakornkit S, Iamaroon A. Epithelial-mesenchymal transition in oral squamous cell carcinoma. *ISRN Oncol* 2012;2012:681469.
5. Skrypek N, Goossens S, De Smedt E, Vandamme N, Berx G. Epithelial-to-mesenchymal transition: Epigenetic reprogramming driving cellular plasticity. *Trends Genet* 2017;33:943-59.
6. Maretzky T, Reiss K, Ludwig A, Buchholz J, Scholz F, Proksch E, *et al.* ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration, and beta-catenin translocation. *Proc Natl Acad Sci U S A* 2005;102:9182-7.
7. Pierre CC, Hercules SM, Yates C, Daniel JM. Dancing from bottoms up-Roles of the POZ-ZF transcription factor Kaiso in Cancer. *Biochim Biophys Acta Rev Cancer* 2019;1871:64-74.
8. Saitoh M. Epithelial-mesenchymal transition is regulated at post-transcriptional levels by transforming growth factor- β signaling during tumor progression. *Cancer Sci* 2015;106:481-8.
9. Villarejo A, Cortés-Cabrera A, Molina-Ortíz P, Portillo F, Cano A. Differential role of Snail1 and Snail2 zinc fingers in E-cadherin repression and epithelial to mesenchymal transition. *J Biol Chem* 2014;289:930-41.
10. Dave N, Guaita-Esteruelas S, Gutarra S, Frias À, Beltran M, Peiró S, *et al.* Functional cooperation between Snail1 and twist in the regulation of ZEB1 expression during epithelial to mesenchymal transition. *J Biol Chem* 2011;286:12024-32.
11. Vandewalle C, Comijn J, De Craene B, Vermassen P, Bruyneel E, Andersen H, *et al.* SIP1/ZEB2 induces EMT by repressing genes of different epithelial cell-cell junctions. *Nucleic Acids Res* 2005;33:6566-78.
12. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, *et al.* Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004;117:927-39.
13. Abba ML, Patil N, Leupold JH, Allgayer H. MicroRNA regulation of epithelial to mesenchymal transition. *J Clin Med* 2016;5:1-20.
14. Wildenberg GA, Dohn MR, Carnahan RH, Davis MA, Lobdell NA, Settleman J, *et al.* p120-catenin and p190RhoGAP regulate cell-cell adhesion by coordinating antagonism between Rac and Rho. *Cell* 2006;127:1027-39.
15. Castro-Castro A, Marchesin V, Monteiro P, Lodillinsky C, Rossé C, Chavrier P. Cellular and Molecular Mechanisms of MT1-MMP-Dependent Cancer Cell Invasion. *Annu Rev Cell Dev Biol* 2016;32:555-76.
16. Atena M, Reza AM, Mehran G. A review on the biology of cancer stem cells. *Stem Cell Discov* 2014;4:83-9.
17. Brabletz T. EMT and MET in metastasis: Where are the cancer stem cells? *Cancer Cell* 2012;22:699-701.
18. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, *et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;133:704-15.
19. Kong D, Banerjee S, Ahmad A, Li Y, Wang Z, Sethi S, *et al.* Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells. *PLoS One* 2010;5:e12445.
20. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, *et al.* The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. *Nat Cell Biol* 2009;11:1487-95.
21. Takkunen M, Grenman R, Hukkanen M, Korhonen M, García de Herreros A, Virtanen I. Snail-dependent and -independent epithelial-mesenchymal transition in oral squamous carcinoma cells. *J Histochem Cytochem* 2006;54:1263-75.
22. Nguyen PT, Kudo Y, Yoshida M, Kamata N, Ogawa I, Takata T. N-cadherin expression is involved in malignant behavior of head and neck cancer in relation to epithelial-mesenchymal transition. *Histol Histopathol* 2011;26:147-56.