Understanding epithelial-mesenchymal transition in oral cancer: Made easy

R. Shesha Prasad¹, Anuradha Pai¹, K. Shyamala²

¹Department of Oral Medicine and Radiology, The Oxford Dental College and Hospital, Bengaluru, Karnataka, India, ²Department of Oral and Maxillofacial Pathology, Raja Rajeswari Dental College & Hospital, Bengaluru, Karnataka, India

Keywords: Epithelial-mesenchymal transition, oral cancer, potentially malignant disorders

Abstract
Epithelial-mesenchymal transition (EMT) has a key role in embryogenesis. Formation of mesenchymal cells that can move, occurs by a process of differentiation from epithelial cells, through a process known as EMT, which is pivotal in various developmental processes, wound healing and behavior of stem cells. It also contributes pathologically to fibrosis and progression of metastatic disease. A metamorphosis of epithelia to mesenchymal cells is seen in tumourogenesis via EMT through which cancer cells acquire invasiveness to enter its surrounding stroma, creating a favorable microenvironment for cancer progression, metastasis, and chemoresistance. EMT pathway is of great therapeutic interest in the treatment of cancer and could potentially be targeted either to prevent tumor dissemination in patients at high risk of developing metastatic lesions or to eradicate existing metastatic cancer cells in patients with more advanced disease. This review aims to understand the importance of EMT in oral cancer.

Introduction
Tissue repair and various pathological processes including fibrosis, invasiveness of tumor, metastasis involves origin of mesenchymal cells which is poorly understood. The transition of the epithelium to mesenchymal cells was considered to be one such source of mesenchymal cells. It was also observed that embryogenesis, tissue repair, organ development and numerous cancers involved similar transitions. It was noted that epithelial cells could acquire characteristics by down regulation of epithelial cells in chick embryos. This process was called epithelial-mesenchymal transition (EMT) phenotype which has an inherent plasticity of transforming partially or fully into mesenchymal cells.

Cell Basics
Epithelia have various functions and are arranged in single or multiple layers in various tissues. The apico basal polarity of epithelial cells is established through the sequential arrangement of adherence junctions, desmosomes. The epithelial cell layer communicates with each other through specialized intercellular junctions. The adjacent connective tissue is separated by a basement membrane. Mesenchymal cells are organized loosely in a three dimensional extracellular matrix made up of connective tissue separated from the epithelia above by a basement membrane.

The Need for EMT - Why does it Happen?
The core of cell division is to generate more cells needed for growth and development. All cells in a body are derived from a single zygote. Cells undergo a complex process of differentiation assuming various phenotypic states during development and certain epithelia are more plastic allowing them to move back and forth from epithelial to mesenchymal states via EMT and MET. Most importantly after completion of development, these cells remain permanently in the state of differentiation. However, literature suggests that a transdifferentiation does occur during tissue repair, healing, fibrosis, and cancers that lead to EMT.
Cellular Events during EMT

Figure 1 depicts the summary of cellular events during EMT.\(^1\)

Classifying EMTs

EMTs are known to occur in physiology, repair and fibrosis importantly in cancer.\(^{1,4}\) Classifying EMTs based on these different scenarios delineated the functional and pathological consequences. Based on biological and biomarker context they were classified into three subtypes.\(^4\)

Type 1 EMT: EMT during implantation, embryogenesis, and development

Type 2 EMT: EMT associated with tissue regeneration and organ fibrosis

Type 3 EMT: EMT associated in cancer progression and metastasis.

Type 3 EMT is considered in this review as it is mainly involved in oral cancer progression and metastasis.

EMT and Cancer

Progression of cancer from precancer involves the destruction of the basement membrane by epithelial dysplastic cells. After crossing the basement membrane, these tumor cells metastasize to different sites which dictate the prognosis of oral cancer.\(^5\) Literature review suggests that EMT plays an important role in invasion and metastasis.\(^{5,6}\) Acquisition of malignant phenotypes by epithelial cells involve activation of a complex EMT event. Animal studies have demonstrated mesenchymal characteristics in epithelial cells that have become carcinogenic.\(^1\) Numerous EMT markers are overexpressed in cancer and oral cancer, in particular, some of the important markers are enumerated below.

EMT Biomarkers and their Prognostic Implications in Cancer

EMT markers and their role in physiology and cancer are briefed in Table 1.\(^{5,6}\)

Other transcriptional factors such as zinc finger proteins (ZEB1, ZEB2), bHLH protein (TWIST), and the snail family of zinc finger proteins (snail, slug) are associated with EMT in progression. Various signaling pathways involving transforming growth factor-β (TGF-β), Wnt cascade in carcinogenesis and metastasis.\(^{5,6}\) However, a detailed description of these complex mechanisms is beyond the scope of this article.

EMT in Oral Cancer

Epithelial markers such as e-cadherin, cytokeratin, claudine, desmoplakin, beta keratin were down regulated in oral squamous cell carcinoma (OSCC), and mesenchymal markers such as N-cadherin, vimentin, fibronectin, and snail-1/2 were up regulated.\(^{5,6}\) Aberrancy in Wnt cascade is known to initiate EMT in OSCC.\(^9\) Change over in cadherin from E-cadherin to N-cadherin was found in 30 of 80 head and neck SCC (HNSCC) cases. Integrins particularly αVβ6 integrin which is cell surface associated with EMT in OSCC.\(^{10}\) Various growth factors like endodermal growth factor, TGF-β1 are known to play a crucial role in EMT involving OSCC.\(^7\)

Targeting EMT in Cancer

Numerous studies that investigate EMT in clinical and animal models are on the rise. Blocking induction of EMT and transcription factors (e.g. snail, slug, ZEB, and TWIST) can help decrease the chemotherapeutic resistance of cancer cells, shortening duration and preventing relapse.\(^{10}\) Targeting mesenchymal phenotype markers such as vimentin, N-cadherin, and fibronectin may decrease invasiveness thus slowing distant metastasis. Various medications, such as sorafinib, piperine, curcumin, salinomycin, and sulforaphane, have been under research to target EMT in cancer.\(^{10}\)

Future Directions

Newer proteins involved in EMT and HNSCC have been identified. To name a few are Forkhead box protein C 2, zona occludens 1, zinc finger E box binding homeobox 1 (ZEB 1). These are involved in angiogenesis, loss of cell adhesion and in various cancers. Its role in HNSCC is under research.\(^5\)

Studying EMT in precancer may throw light on the complex mechanisms that are initiated very early, much earlier to malignant transformation. For example, TGF-β is known to induce EMT and its role in cancer metastasis, and fibrosis is established.\(^{11}\) Its a known fact that TGF-β is the key regulator of fibrosis in oral submucous fibrosis (OSMF). Analyzing EMT signatures in OSMF could help researchers prevent the conversion of OSMF to malignancy. Similarly, role of EMT in other potentially malignant disorders like leukoplakia could be a major breakthrough in preventing frank cancer.
Conclusion

EMT is a complex process involved both in physiological and pathological entities. Its role in cancer progression and metastasis is established beyond doubt. Exploiting EMT to address early intervention of cancer and to decrease metastasis, have been constantly under research. As carcinogenesis is an extremely complicated multi step process involving numerous pathways, understanding the role of EMT cancer induction and progression could only be a baby step towards conquering cancer in terms of effective treatment and prevention of morbidity and mortality.

References


Table 1: EMT markers and their role in physiology and cancer

<table>
<thead>
<tr>
<th>Markers</th>
<th>Function</th>
<th>Significance in cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell surface proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Anchors epithelial cells to each other</td>
<td>Expression decreased in HNSCC as cell to cell adhesion decreased in cancer metastasis. Very low levels in poorly differentiated carcinomas. Predictive of diminished disease specific survival.</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>Cell to cell adhesion protein</td>
<td>E-cadherin to N-cadherin switch is used to monitor EMT. Highly malignant and poorly differentiated cancer cells have high N cadherin expression.</td>
</tr>
<tr>
<td>Integrins</td>
<td>Epithelial cells attached above the basement membrane by these proteins</td>
<td>Increased expression of specific subtypes seen in cisplatin drug resistance, and increased invasiveness of tumor cells.</td>
</tr>
<tr>
<td><strong>Cytoskeletal markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-SMA</td>
<td>Embryogenesis and wound healing</td>
<td>More expression in myofibroblasts seen in HNSCC.</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Intermediate filament mesenchymal marker expressed at sites of cellular elongation</td>
<td>Expression higher in metastatic cancer cells than primary HNSCC tumors. Reducing vimentin levels by RNA interference decreased proliferation, migration and metastasis of HNSCC.</td>
</tr>
<tr>
<td>β catenin</td>
<td>Plays a role in cell adhesion. E-cadherin is anchored to the cytoskeleton via β-catenin, a cytoplasmic protein</td>
<td>Plays a critical role in the invasion of HNSCC via the Wnt/β-catenin pathway. Nuclear β-catenin is correlated with a poor prognosis in patients with metastatic HNSCC.</td>
</tr>
<tr>
<td><strong>ECM proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>Major structural components of extra cellular matrix. 28 types</td>
<td>Collagen IV degradation and down regulation are represented as an early marker for cancer invasion beyond basement membrane. Collagen III promotes cancer progression. Collagen I overexpressed in HNSCC.</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Mediates cellular interactions with ECM and important in migration, growth, differentiation, and cell adhesion</td>
<td>Expression correlates to tumor aggression.</td>
</tr>
<tr>
<td>Laminin</td>
<td>Major component of basement membrane. 15 types</td>
<td>Expression of laminin 5 correlated with poor patient prognosis and increased tumor invasion.</td>
</tr>
<tr>
<td><strong>Transcription factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNAIL family</td>
<td>Regulate over expression of mesenchymal markers like fibronectin and suppress epithelial markers like E cadherin. 3 types</td>
<td>SNAIL 1 expression correlates to metastasis and poor prognosis in primary HNSCC. SNAIL 2 associated with tumor metastasis.</td>
</tr>
<tr>
<td>TWIST</td>
<td>Basic helix loop helix protein that modulates many target genes through E box responsive elements. 2 types</td>
<td>Up regulated in cancer metastasis. Repression of TWIST reverses EMT and metastatic phenotypes.</td>
</tr>
</tbody>
</table>

HNSCC: Head and neck squamous cell carcinoma, EMT: Epithelial-mesenchymal transition, ECM: Extracellular matrix.


