Epithelial Mesenchymal Transition in Oral Squamous Cell Carcinoma

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***Abstract*: During embryonic development Epithelial mesenchymal transition is an important process which enables the epithelial cells to acquire mesenchymal fibroblast like properties and show reduced intercellular adhesion and increased motility. During cancer progression the activation of EMT permits cancer cells to acquire migratory invasive and stem like properties. Various Neoplasias and Chronic inflammation of tissues appropriate and subvert similar processes as Epithelial Mesenchymal Transition associated with embryo implantation embryogenesis and organ development. Recent advances have shown an enormous amount of evidence pointing towards a critical role of EMT like events during tumor progression and malignant transformation providing the nascent cancer cells with invasive and metastatic properties. Activation of the phosphatidylinositol kinase PK AKT axis has recently been emerging as a central feature of EMT and their role in EMT during development of cancer will be discussed in the article with a focus on E cadherin regulation along with several other oncogenic pathways like peptide growth factors Src Ras Ets integrin, Wnt b catenin and Notch which induce EMT and downregulates the cell adhesion molecule E-Cadherin.**

The article also aims to discuss the Biomarkers for EMT, such as Epithelial Plasticity which can help identify at least three types of EMT, Cell surface markers, Cytoskeletal Markers and how they can be used as a screening tool for early diagnosis and better prognosis of Oral Squamous Cell Carcinoma.

***Index Terms*: Epithelial mesenchymal transition, E cadherin, oral carcinoma, metastasis, biomarkers.**

I. INTRODUCTION

Across the globe, oral cancer accounts for 2%–4% of all cancer cases. Oral squamous cell carcinoma is the most common malignant epithelial neoplasm affecting the oral cavity. It has a lofty prevalence in certain parts of the world. In India, mostly commonly diagnosed in male patients and has relatively low survival rates. More than 90% of cancer cases in head and neck region are OSCC. In South Asian countries, consumption of smokeless tobacco and areca nut products are the main etiological factors associated with OSCC. The 5-year survival rate of OSCC patients is no more than 60% due to tumour metastasis and subsequent recurrence. The metastasis from the primary site is due to a complex process known as epithelial-to-mesenchymal transition (EMT).

II. WHAT IS EMT?

Epithelial–mesenchymal transition (EMT) is a reversible biological process where the epithelial cells acquire mesenchymal traits or fibroblasts like properties. The role of EMT in OSCC is metastasis, including diverse EMT markers, regulatory networks and crucial EMT-inducing transcription factors. During the EMT, epithelial cells gradually acquire the structural and functional characteristics of mesenchymal cells [1], leading to the upregulation of cell migration and the promotion of tumour cell dissemination.

The phenomenon of EMT has 3 distinct types [2] –

(i) TYPE 1 - observed in embryogenesis

(ii) TYPE 2- seen in the wound healing, tissue regeneration, and organ fibrosis

(iii) TYPE 3 – seen in cancer cell metastasis and invasion.

Therefore, EMT attracted broad attention due to its closely-knit relationship with cancer invasion and metastasis in this article we have delineated the general molecular mechanisms of EMT, evidenced by alterations of cell morphology during EMT, the activation of signaling pathways, reorganization of actin and formation of invadopodia, and the presence of cadherin switching.

III. MECHANISM OF EMT

At the molecular level the process of Epithelial Mesenchymal transformation is contributed by three important mechanisms :-

1. Down regulation of E- cadherin
2. Significance of microRNA in EMT
3. Reorganization of actin and formation of invadopodia

DOWN REGULATION OF E-CADHERIN

The loss of cell to cell adhesion, triggered by an EMT program is among the first critical steps of cancer metastasis, invasion, and progression. The downregulation of E-cadherin (epithelial cadherin) and upregulation of mesenchymal type N-cadherin is an important step in EMT. This process is known as “cadherin switching”. Several reports have shown the acquisition of mesenchymal markers in carcinoma cells, such as vimentin, N-cadherin, and fibronectin, with the concomitant loss of epithelial E-cadherin in the processes of EMT. Consequently, E-cadherin is considered to be a “master regulator” of EMT. The functional loss of EMT may be due to mutations of E-cadherin in germ-line and somatic cells; which is rare, chromosomal aberrations, the cleavage of E-cadherin

by proteinases, DNA hypermethylation of the E-cadherin gene and transcriptional suppression of E-cadherin; which is more commonly found in cancer cells [3].

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. 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" [4].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. 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.

5 types of EMT-TFs

-SNAIL1 :- It is a member in the family of zinc-finger transcription factor and it is considered to be the master gene of EMT. It binds to E-box in the promoter region of E-cadherin, thereby repressing its transcription. It functions by 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 [5].

-SNAIL2:- Also known as slug, it function is similar to that of SNAIL1. 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 [5].

- 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 [6].

-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.

-TWIST1:- It is a master regulator of gastrulation and mesoderm specification however its ectopic expression Twist1 upregulates mesenchymal cell markers (fibronectin, vimentin, smooth muscle actin and N-cadherin) and loss of epithelial markers (E-cadherin).

SIGNIFICANCE OF MicroRNAs IN EMT

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. MicroRNAs have been reported to act both as a facilitator and as an inhibitor of the EMT program. Eg- microRNA 200 and microRNA 205 can block the suppressive activity of transcriptional repressors of E-cadherin expression, including ZEB1 and ZEB2, whereas lack of microRNA 200 is implicated in the promotion of EMT by up-regulating vimentin expression and diminishing E-cadherin expression [7].

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 and is important 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 signalling pathway upregulates the expression of target genes necessary for tumour cell proliferation, migration and invasion [8].

The lamellipodia and filopodia, collectively known as invadopodia are migratory membrane protrusions that contain MMP, specifically membrane type I-MMP (MT-1 MMP or MMP14).They are found in cancer cells of head and neck squamous cell carcinoma that undergo EMT. The membrane type I-MMP is involved in extracellular matrix degradation and cell migration. MT1-MMP expression have also been described in several other carcinomas, such as colon, breast, bladder, head and neck, and cervical cancers.

IV. EMT IN OSCC

The mortality rate is still high in OSCC due to the failure to control tumour recurrence and metastasis. EMT is the process by which epithelial cells having non-invasive characteristics adopt a mesenchymal phenotype like invasive characteristics. The epithelial cells undergoing EMT involve reorganizing their cytoskeleton. After the transition, those cells dissolve the extracellular matrix that restrains them and start spreading to the surrounding tissue.

Cells proceeding EMT exhibit down-regulation of, MMP-9, and epithelial markers including E-cadherin, and beta-catenin, and up-regulation of MMP-2 and mesenchymal markers including N-cadherin, vimentin, fibronectin, and Snail-1/2 [9]. In Cadherin switching, the loss of E-cadherin expression and the gain of N-cadherin expression, is a crucial event of EMT in human cancers. Snail is a master gene in regulating E-cadherin during the process of EMT. In an OSCC model, Snail-transfected cells showed complete EMT phenotypes with a fibroblast-like appearance, vimentin filaments, cadherin switching, and lack of hemidesmosomes. Zinc-finger E-box binding homeobox 1 (ZEB-1) plays crucial roles in epithelial-to-mesenchymal transition during tumour carcinogenesis. ZEB-1 is a biomarker for the prognosis of cancer. ZEB-1 and ZEB-2 were upregulated in these cells. The integrin αVβ6 has been found to play an important role in EMT [10]. When the full length β6 integrin was expressed in poorly invasive OSCC cells, OSCC cells increased expression of vimentin and reduced expression of keratin and E-cadherin, while OSCC cells with the expression of the truncated form of β6 subunit retained their epithelial characteristics and did not alter vimentin or E-cadherin expression.

WHAT SIGNALS EMT IN OSCC ?

Protein kinase B (PKB, or Akt) plays a role in cell metabolism, growth, proliferation, and survival. Its activation is controlled by a multi-step process that involves phosphoinositide-3-kinase (PI3K). Activation of the PI3K/Akt signaling pathway is a frequent event in human cancers, including OSCC. The role of Akt in the biology of OSCC was investigated by employing OSCC lines engineered to express constitutively active Akt [11]. The results revealed that OSCC cells underwent EMT characterized by down-regulation of E-cadherin, desmoplakin, and beta-catenin and up-regulation of vimentin. Morphologically, OSCC cells lost epithelial cell characteristics and acquired fibroblast-like properties. Interestingly, in a study by Hong KO et al, when Akt activity was inhibited, OSCC cells acquired the mesenchymal-epithelial reverting transition and re-expressed E-cadherin; hence, the epithelial characteristics were restored. This phenomenon is an important step of cancer cells to dwell in the metastatic sites and adapt to their new microenvironment. It was suggested that a strategy involving Akt inhibition be a promising therapeutic approach in controlling cancer invasion and metastasis in OSCC patients.

The Wnt/β-catenin pathway is one of the major signaling pathways in cell proliferation, oncogenesis, and EMT. A recent study [12], showed that aberrant cytoplasmic accumulation of β-catenin induced TCF/LEF-mediated transcriptional activity, up-regulation of MMP-7, and induced EMT in OSCC cells, hence, enhancing invasion and migration of OSCC cells.

Hypoxia has been found to play a critic role in EMT [13]. The hypoxic microenvironment is common to any cancer cells and can trigger EMT via regulating the expression and activity levels of major transcriptional repressors. In tongue SCC tissues, hypoxia inducible factor (HIF)-1α, HIF-2α, and Twist-2 were overexpressed and the overexpression of these molecules, except HIF-2α, was associated with a shorter disease-free survival. The co-expression of more than two markers from HIF-1α and Twist2 be a significant prognostic predictor in patients with tongue SCC.

TGFβ acts as a tumor suppressor in normal epithelial cells and cancer cells at their early stages of carcinogenesis. As the carcinogenesis progresses, cancer cells can switch their responses to TGFβ and utilize TGFβ as a potent oncogenic activator upon stimulation with recombinant TGFβ1, Slug and MMP-9 were upregulated, while Snail expression increased and fell, in concert with the expression of MMP-2 in OSCC cells, suggesting that both Snail and Slug act as regulators of TGFβ1-triggered EMT [14]. Collectively, TGFβ and EGF may play a complex role in EMT as well as modulating ECM degradation and facilitating the progression of OSCC carcinogenesis.

V. BIOMARKERS FOR EPITHELIAL MESENCHYMAL TRANSITION

Biological markers are a measurable indicator of physiological state or condition. They are the tools that help in diagnosis, prevention, or retrogression of disease.

Metastatic Epithelial Mesenchymal Transition is categorized as Type III EMT under Epithelial Cell Plasticity. Type III EMT involves epithelial carcinoma cells in primary nodules transitioning to metastatic tumor cells in order to migrate through the blood stream and, in some cases, form secondary nodules in distant metastatic sites by MET (Mesenchymal-Epithelial Transition).

A variety of biomarkers have been used to demonstrate all three subtypes of EMT, some are acquired and some are attenuated.

Acquired biomarkers for Type III EMT:

1. Cell surface proteins:
a. OB-Cadherin
b. Alpha-5-beta-1 Integrin
c. AlphaV-5-beta-6 Integrin
d. Syndecan-1
2. Cytoskeletal markers:
a. FSP-1
b. Alpha-SMA
c. Beta-Catenin
3. ECM Proteins:
a. α1(I) collagen
b. α1(III) collagen
4. Transcription Factors:
a. Snail1 (Snail)
b. Snail2 (Slug)
c. ZEB1
d. CBF-A/KAP-1 complex
e. Twist
f. LEF-1
g. Ets-1
5. mRNAs:
a. miR-21

The concept that EMT involves the formation of metastatic cancer cells is based on the observation that acquisition of mesenchymal markers such as FSP1 (fibroblast-specific protein 1) by epithelial carcinoma cells is associated with increased metastatic potential, as is nuclear overexpression of β-catenin and loss of epithelial cell adhesion molecules such as E-cadherin [15].

VI. 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.

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