Epithelial-Mesenchymal Transition in Normal Development and Carcinogenesis

Normal Gelişim Süreci ve Karsinogenezde Epitel-Mezenkimal Dönüşüm

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ABSTRACT

Epithelial-mesenchymal transition (EMT) is an important developmental process allowing epithelial cells to loose their epithelial properties and gain mesenchymal characteristics through a series of morphological and biochemical changes. As a result of EMT, compact and ordered epithelial cells become less compact and motile, enabling them to migrate to different sites and generate different tissues during normal development. EMT also contributes to metastasis during carcinogenesis, as the epithelial cells of primary tumors undergoing EMT become invasive and migratory. Understanding the EMT process and its regulation during embryonic development may advance our knowledge on carcinogenesis based on the molecular and cellular similarities between pathological and developmental EMT.

Keywords : Epithelial-mesenchymal transition, TGF-ß, carcinogenesis, embryonic development.

ÖZET

Epitel-mezenkimal değişim (EMD), bir çok morfolojik ve biyokimyasal değişiklikler sonucu epitel hücrelerinin epitel özelliklerini kaybederek mezenkimal özellikler kazanmalarına sebep olan önemli bir gelişim sürecidir. EMD'nin sonucunda kompakt ve düzenli olan epitel hücreleri normal gelişim sürecinde organizmanın farklı kısımlarına göç ederek bu kısımlarda farklı dokuları oluşturabilmelerini olanaklı kılan daha az kompakt ve hareketli bir yapı kazanırlar. Birincil tümörlerin EMD geçiren epitel hücreleri istilaci ve göç edici özellikler kazandığından, EMD karsinogenez sırasındaki metastaz oluşumuna da katkı sağlamış olur. Patolojik ve normal EMD arasındaki moleküler ve hücresel benzerlikler nedeniyle, normal gelişim sırasında gerçekleşen EMD sürecini ve regülasyonu anlamak karsinogenezi daha iyi anlamamızı sağlayabilir.

Anahtar kelimeler: epitel-mezenkimal değişim, TGF-ß, karsinogenez, embrionik gelişim.

Epithelial tissue is composed of tightly packed polarized epithelial cells (apical-basal orientation) and it serves as a protective barrier against environmental hazards. Structural rigidity, which is maintained mainly by strong cell-cell and cell-matrix interactions, is fundamental for the epithelial tissue to carry out its function. Mesenchymal tissue, on the other hand, is an undifferentiated loose connective tissue found in early embryo. Unlike epithelial cells, mesenchymal cells are fibroblast-like shaped cells that mostly lack direct cell-cell contact and polarity, providing them with high mobility (14). Mesenchymal cells also display stem cell properties enabling their differentiation into other cell types including adipocytes, chondroblasts and osteoblasts (14).

All types of cells in an organism are derived from a single cell, the fertilized egg. This single cell has to differentiate into different cell types generating three distinct tissue types; endoderm, ectoderm and mesoderm in a highly regulated manner. It has been believed that differentiated epithelial cells reach a terminal stage where they are no longer able to differentiate into other cell types. However, this notion has been challenged by recent observations where terminally differentiated epithelial cells undergo phenotypic changes in response to tissue damage or pathological stress (2,14,17). Epithelial-mesenchymal transition (EMT) is a biological process that allows polarized epithelial cells to gain mesenchymal properties through undergoing several morphological and biochemical changes. Therefore, EMT is accepted as the mechanism for cellular diversity during embryogenesis and adulthood. As a result of EMT, epithe-

lial cells down-regulate epithelial markers and loose their epithelial properties such as polarity, rigid architecture and up-regulate mesenchymal markers, thus gain mesenchymal features including a fibroblast-like shape, mobility and resistance to apoptosis. All these changes alter the cell-cell and cell-matrix connections in the epithelial tissue, allowing the epithelial cells to release from their original epithelial layer and migrate to the other parts of the body during normal development, tissue reconstruction and repair (6). After migrating to their new destinations mesenchymal cells can regain their original epithelial features through a reverse biological process called mesenchymal-epithelial transition (MET) (7,16). Formation of the nephrons from the metanephric mesenchyme, coelomic-cavity formation and somitogenesis occur via MET (9).

Gastrulation and formation of peripheral nervous system components by neural crest (NC) cells are two striking examples for involvement of EMT in normal developmental processes. Gastrulation is an embryonic phase of development during which mesoderm and endoderm are generated from ectoderm. A subset of epiblast (initial epithelial embryonic layer) cells of the primitive streak undergo the EMT process, become mobile and generate mesoderm and endoderm, while the cells remaining in the epiblast form ectoderm. Therefore, EMT in gastrulation leads to formation of three germ layers from a single layer (1). Epithelial cells of the embryonal neural tube undergo EMT and become mobile neural crest (NC) cells. NC cells migrate away from the neural tube and differentiate into several distinct cell types including ganglia, glial cells and melanocytes (1).

In addition to being fundamental for embryonic development, several animal tumor models suggested that EMT is a key player also in tumorigenesis as it promotes invasive and metastatic behavior of epithelial cancer cells (5). Several genes and signaling pathways involved in EMT in embryonic development are also observed in EMT during carcinogenesis. It has been argued that the epithelial cells of the primary tumors disseminate from the primary tumor site to the sites of metastasis by losing contact with each other and becoming motile by EMT. EMT-derived invasive and migratory cancer cells typically generate secondary tumors at distant sites where they have metastasized. At these secondary sites, cancer cells no longer exhibit the mesenchymal phenotypes and regain epithelial properties (establishing secondary tumors) through MET (7). In breast, ovarian, colon and esophageal cancer models characteristics of EMT have been observed (10).

Subtypes of EMT

EMTs can be classified into three functionally distinct subtypes. Type I EMT is associated with implantation, embryo formation and organ development and it is involved in differentiation of cell types. It does not cause either fibrosis or induce invasive behavior. Type I EMTs are able to undergo MET and form secondary epithelia. Type II EMT generates fibroblasts for tissue reconstruction and is related to wound healing, tissue regeneration and organ fibrosis. Unlike type I EMT, type II EMT occurs in response to inflammation and stops once the inflammation is attenuated during tissue regeneration and wound healing. Type III EMT is encountered in epithelial cancer cells of solid tumors. Carcinoma cells undergoing type III EMT gain the ability to invade and migrate to other sites through circulation, thus generate secondary tumors at the secondary sites where they have metastasized. Although the specific signals delineate the three subtypes, it is clear that each EMT type leads to different consequences.

Regulation of EMT

Changes in gene expression levels occur during EMT including down-regulation of epithelial markers such as E-cadherin and up-regulation of mesencyhmal markers such as vimentin and N-cadherin (17). The hallmark for EMT is the loss of an epithelial marker, E-cadherin, which is central to cell-cell adhesion and acts as an invasion suppressor molecule (16). Loss of E-cadherin expression in carcinoma has been proposed to be related to the increased invasiveness and transition of adenoma to carcinoma and consequently decreased patient survival. Consistent with this, expression level of E-cadherin is usually inversely correlated with tumor grade and stage (13,18). E-cadherin expression can be regulated at the genomic and transcriptional level. Activation of the E-cadherin gene CDH1 can be prevented by mutations as observed in diffuse gastric carcinoma, lobular breast carcinoma, endometrial, and thyroid carcinoma. Loss of CDH1 heterozygosity and hypermethylation of the CDH1 promoter are other mechanisms described in tumors for loosing E-cadherin expression (16). EMT-inducing signaling pathways can be activated via several growth factors including fibroblast growth factor (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF) and transforming growth factor (TGF)-ß (9).

TGF-ß is a cytokine family with 33 members in humans. TGF-ß does not only play an important role in regulating growth, differentiation and migration of cells during development, it is also involved in cancer progression and metastasis. In carcinogenesis, TGF-ß initially suppresses tumorigenesis through inhibiting cell growth and promoting apoptosis. However, in advanced cancers TGF-ß enhances invasiveness and metastasis (thus, promotes tumorigenesis) via inducing EMT (5).

Key regulators of EMT function downstream of the various EMT pathways and several of them have been shown to be E-cadherin repressors. One of them is a zinc finger

DNA binding protein Snail, which is involved in both normal growth and tumorigenesis. Snail represses the expression of E-cadherin and other target genes through recognizing E box motifs in the promoter regions. Severe defects have been observed in mesoderm formation in Snail null Drosophila. In vertebrates, Snail and a closely related gene Slug are fundamental in the migration of neural crest cells. Snail has also been shown to be a key mediator of EMT in human and mouse invasive carcinoma cells (9). Twist, a helix-loop-helix transcription factor, is another important EMT inducer. From Drosophila to vertebrates, Twist represses E-cadherin via binding to the E box motifs that are also targeted by Snail. Twist and Snail overexpression have been shown to be associated with loss of E-cadherin expression and gain of N-cadherin in human gastric cancer (8). Many EMT-inducing transcription factors are often activated simultaneously and they regulate each other. For example, Twist1 directly induces Snail1 expression to induce EMT during Drosophila mesoderm formation. In human cells, Twist1 activates Snail2 transcription thorough directly binding to its promoter region. The direct association between Twist1 and Snail2 is well conserved through evolution and plays a critical role during embryogenesis and tumor metastasis (3). Upon binding to its receptors, TGF-ß promotes binding of SMAD4-SMAD2/3 transcription factor complex to the promoter regions of Snail and Slug. Twist1 expression is also induced by TGF-ß (17).

Several studies from different groups suggest epigenetic modifications play an important role in up-regulation of epithelial genes and down-regulation of mesenchymal

genes during EMT (9). TGF-ß contributes to the epigenetic control of EMT through inducing a DNA methyltransferase, DNMT1. Furthermore, TGF-ß stimulation reduces the heterochromatic marker, di-methylation of Lys9, and increases in the euchromatin marker (tri-methlylation of Lys4) (5). Snail expression has been shown to be correlated with E-cadherin promoter hyper-methylation in various types of carcinoma. After binding to the promoter region of E-cadherin, Snail recruits a repressor complex involving histone deacetyltransferases 1 and 2 (HDAC1 and 2). Recruitment of this complex results in a decrease in histone H3/H4 acetylation and an increase in histone H3 methylation, which leads to a heterochromatic structure and represses E-cadherin expression (9).

Hypoxia (pO2 level <10 mmHg) is a microenvironmental factor associated with normal development and tumor progression. Low oxygen condition up-regulates hypoxia inducible factor (HIF-1). HIF-1 mediates the hypoxic response by forming a functional transcription factor, which activates transcription of target genes (9,17). Hypoxia/HIF up-regulates EMT-inducing transcription factors, Snail and Twist, possibly through TGF-ß stimulation (5). Under hypoxic conditions, HIF-1 induces Snail and Twist expression, which in turn weakens the expression of E-cadherin (9).

CONCLUSION

EMT is a biological process involved in both normal development and carcinogenesis. The outcome of the process is the generation of motile, mesenchymal cells. Different types of EMTs are associated with implantation, embryo formation, organ development, and cell differentiation (Type I), tissue regeneration and fibrosis (Type II) and tumor growth and cancer progression (Type III). Common key mediators can explain the similarities between the developmental and oncogenic EMT processes. Despite the common contributing pathways, the induction and progression of EMT can vary significantly among different EMT types. Although steps of developmental EMT process are well-defined, cancer-associated EMT is less clearly defined and vary among different pathologies. Inspecting the signals activating EMT and the responsiveness of the cells to these signals may advance our understanding of tumorigenesis.

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