The Role and Molecular Mechanisms of Epithelial-Mesenchymal Transition (EMT) in the Progression of Esophageal Squamous Cell Carcinoma

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Abstract: This paper systematically explored the critical role and associated molecular mechanisms of epithelial-mesenchymal transition (EMT) in the progression of esophageal squamous cell carcinoma (ESCC). Through a comprehensive literature review, the major molecular markers of EMT in ESCC and its mechanisms in promoting tumor invasion and metastasis were analyzed. These mechanisms include the reduction of cell adhesion, enhancement of mesenchymal traits and motility, and facilitation of extracellular matrix degradation. Studies have shown that during EMT, the expression of epithelial markers such as E-cadherin is downregulated, while mesenchymal markers such as N-cadherin and vimentin are upregulated. These changes significantly enhance the invasive and metastatic potential of tumor cells. As a key driver of ESCC progression, EMT not only sheds light on the biological characteristics of this disease but also provides a theoretical basis for developing novel therapeutic strategies and improving patient prognosis.
Keywords: EMT; esophageal squamous cell carcinoma; invasion; metastasis

I. Introduction

A. Current Status and Challenges of ESCC

Esophageal cancer (EC) ranked sixth globally in cancer-related mortality, with esophageal squamous cell carcinoma (ESCC) being the most common histological subtype, accounting for approximately 90% of all EC cases worldwide. The incidence of ESCC exhibits notable geographic variation, predominantly concentrates in regions such as East to Central Asia, the East African Rift Valley, and parts of Southern Africa. Among these, China reported the highest incidence and mortality rates globally, contributing to more than half of the newly diagnosed cases and deaths from ESCC.

The primary risk factors for ESCC includes prolonged smoking, excessive alcohol consumption, unhealthy dietary habits (e.g., consuming hot or pickled foods), chronic esophageal inflammation, exposure to carcinogens (such as nitrosamines and mold-contaminated foods), and genetic susceptibility. Despite the critical importance of early detection for improving prognosis, the majority of patients were diagnosed at advanced stages due to nonspecific early symptoms, such as mild dysphagia or chest discomfort, resulting in limited treatment efficacy.

Additionally, the treatment of ESCC faces significant challenges, including resistance to chemotherapy and radiotherapy, tumor heterogeneity impeding targeted therapies, and high rates of postoperative recurrence and distant metastasis. The five-year survival rate for advanced ESCC remains below 20%. Therefore, improving ESCC diagnosis and treatment through molecular

mechanism research, early screening technologies, and optimized personalized therapeutic strategies continues to pose a major challenge in clinical and research domains.

B. Definition and Biological Characteristics of EMT

Epithelial-mesenchymal transition (EMT) refers to a dynamic process during which epithelial cells underwent a series of biological changes to acquire mesenchymal traits. During EMT, epithelial cells lose their polarity and intercellular tight junctions, accompanied by the downregulation of epithelial markers such as E-cadherin. Simultaneously, cells gain mesenchymal characteristics, including enhanced motility and invasiveness, along with the upregulation of mesenchymal markers such as N-cadherin and vimentin.

EMT can be categorized into three main types: development-associated EMT (e.g., during embryogenesis), fibrosis-associated EMT (e.g., during chronic inflammation-induced fibrosis), and tumor-associated EMT (e.g., during cancer cell invasion and metastasis).

Biologically, EMT represents a highly plastic process regulated by multiple signaling pathways and transcription factors, including Snail, Twist, and ZEB. External stimuli, such as the activation of TGF-β, Wnt, and Notch signaling, as well as hypoxia, inflammation, or metabolic stress in the tumor microenvironment, are known to induce EMT.

EMT endows cells with enhanced adaptability, enabling them to breach the basement membrane, invade surrounding tissues, and establish metastatic lesions. Furthermore, EMT are closely associated with the acquisition of cancer stem cell-like properties, therapy resistance, and immune evasion, underscoring its significance as a key focus in studying tumor progression and therapeutic strategies.

C. Research Objectives and Significance

This review aims to systematically explore the critical roles of EMT in the progression of ESCC and its associated molecular mechanisms. By synthesizing current research on EMT-related signaling pathways, transcription factors, and non-coding RNAs, this study seeks to elucidate their central regulatory functions in ESCC invasion, metastasis, therapeutic resistance, and prognosis.

II. Key Molecular Markers of EMT

A. Epithelial Markers

The expression of epithelial-specific markers is downregulated during EMT, leading to the disruption of intercellular adhesion and the loss of polarity. These markers primarily include the following:

1. E-cadherin

E-cadherin, a member of the cadherin superfamily, is a transmembrane protein with a molecular weight of 120 kDa. It plays a critical role in forming adherens junctions between epithelial cells, mediating cell-cell adhesion through its extracellular domain while interacting with catenin family proteins (e.g., α-catenin, β-catenin, and p120-catenin) via its cytoplasmic domain to link to the actin cytoskeleton. These interactions maintain the integrity and stability of epithelial cell layers. E-cadherin is essential for preserving epithelial polarity and tissue architecture, influencing cellular morphology and function. Additionally, E-cadherin participates in cellular signaling, particularly in contact inhibition of proliferation, thereby regulating the cell cycle and proliferation.

During tumor-associated EMT, the downregulation of E-cadherin expression is a hallmark event closely associated with the acquisition of invasive and metastatic abilities by tumor cells. The reduction in E-cadherin weaken

intercellular adhesion, enabling tumor cells to detach from the primary tumor and disseminate. Moreover, the expression of E-cadherin is influenced by the tumor microenvironment, including 3D culture conditions and extracellular matrix components, which modulate E-cadherin levels and affected the EMT state and behavior of tumor cells.

2. Claudins and Occludins

Claudins and Occludins are the key proteins constituting tight junctions, located at the apical-basal regions of epithelial cell membranes. They regulate solute and ion permeability across cell layers, maintain cell polarity, and form intercellular barriers to separate the apical and lateral membrane regions. Claudins comprise a diverse group of proteins (27 types in humans) that determine the solute and electrolyte permeability of tight junctions, with tissue-specific expression patterns. Occludin, the first tight junction protein identified, contains two extracellular loops and two splice variants. While it regulates the permeability of intercellular connections, embryonic stem cells lacking Occludin still form tight junction structures, suggesting it is not essential for junction formation. However, Occludin-knockout mice exhibited multiple physiological defects, highlighting its importance in maintaining normal functions.

In tumor-associated EMT, the downregulation of Claudins and loss of Occludin function lead to the disassembly of tight junctions, promoting the transition from an epithelial to a mesenchymal phenotype and enhancing cellular migratory capacity. These changes are particularly significant in tumor invasion and metastasis.

3. Cytokeratins

Cytokeratins, epithelial-specific intermediate filament proteins, form the basis of the cytoskeleton and are essential for maintaining cell shape and structural integrity. They also support intercellular adhesion and cell polarity, which are critical for epithelial tissue architecture and function. Additionally, cytokeratins participates in cell signaling, affecting processes such as growth, differentiation, and apoptosis. During tumor-associated EMT, cytokeratin expression is significantly reduced, representing a critical step in the acquisition of mesenchymal traits, enhanced migration, and invasion. Changes in cytokeratin expression serve not only as biomarkers to monitor EMT but also facilitate the detection of circulating tumor cells (CTCs) in liquid biopsies, aiding in cancer diagnosis, prognosis, and therapeutic response monitoring.

B. Mesenchymal Markers (e.g., N-cadherin, Vimentin)

The expression of mesenchymal markers is significantly upregulated during tumor-associated EMT, enabling cells to acquire migratory and invasive capabilities. These markers include the following:

1. N-cadherin

N-cadherin, also known as neural cadherin, is an adhesion molecule primarily expressed in mesenchymal cells. It mediates loose cell-cell connections, facilitating cell motility.

During tumor-associated EMT, N-cadherin expression is closely associated with the invasiveness and metastatic potential of tumor cells. EMT involves the loss of epithelial characteristics and the acquisition of mesenchymal traits, characterized by the downregulation of E-cadherin and the upregulation of N-cadherin. Elevated N-cadherin levels are correlated with enhanced migratory and invasive abilities of tumor cells, playing a pivotal role in cancer progression and metastasis. Thus, N-cadherin not only serves physiological functions but also plays an essential

role in tumor development and EMT.

2. Vimentin

Vimentin, an intermediate filament protein with head, rod, and tail domains, is predominantly expressed in mesenchymal cells. It forms a highly elastic network that enhances cellular tensile strength, resilience, and elasticity, particularly during migration and mechanical stress. Vimentin also participates in cell signaling by recruiting and localizing proteins such as ERK and Slug, thereby regulating migration and EMT. During cell division, Vimentin is dynamically reorganized through phosphorylation events, which are critical for maintaining cytoskeletal integrity and function.

In tumor-associated EMT, epithelial cells originally expressing cytokeratins begin expressing Vimentin. This switch is a key hallmark of EMT and is associated with the acquisition of stem-like traits, invasiveness, chemoresistance, and tumor recurrence.

3. Fibronectin

Fibronectin, a critical extracellular matrix (ECM) protein, plays a central role in cell adhesion, migration, proliferation, and tissue structure maintenance. As a major ECM component, it is involved in wound healing and tissue repair, promoting interactions between cells and the matrix. Additionally, fibronectin participates in embryonic development, immune regulation, and blood clotting.

In tumor-associated EMT, fibronectin serves as both a biomarker and a functional regulator, playing diverse roles in tumor invasion, metastasis, fibrotic diseases, and inflammatory responses. Its upregulation is linked to ECM remodeling, mesenchymal transition, and changes in the tumor microenvironment, making it a potential therapeutic target for related diseases.

4. α-SMA (α-smooth muscle actin)

α-SMA, an isoform of smooth muscle actin, is expressed in smooth muscle cells and certain non-muscle cells. Under normal physiological conditions, α-SMA is predominantly found in smooth muscle cells, contributing to vascular and tissue tension and contractility. It is also present in certain epithelial cells and stem cells, playing a role in cytoskeletal construction and cell motility.

During tumor-associated EMT, α-SMA expression is elevated, conferring enhanced motility to tumor cells. This enables tumor cells to penetrate the basement membrane, enter the bloodstream, and form distant metastatic foci.

III. The Role of EMT in ESCC Progression

EMT promotes invasion and migration in ESCC through various mechanisms:

A. Reduced Cell Adhesion

EMT plays a critical role in ESCC invasion and migration by suppressing the expression of E-cadherin, thereby weakening intercellular adhesion.

The downregulation of E-cadherin is typically mediated by specific transcription factors such as Snail, Slug, and Twist. These transcription factors inhibit E-cadherin transcription by binding to the promoter region of the E-cadherin gene, leading to its reduced expression in ESCC cells. The decrease in E-cadherin not only disrupts intercellular adhesion but also induces cytoskeletal reorganization, conferring enhanced motility to tumor cells. Furthermore, the loss of E-cadherin activates signaling molecules such as β-catenin, which further promote the expression of invasion- and migration-related genes.

As E-cadherin levels decrease, tumor cells become more dissociated, enabling them to traverse the extracellular matrix (ECM) and invade the basement membrane. This process is often accompanied by the upregulation of N-cadherin

and vimentin, facilitating the transformation of tumor cells into motile, mesenchymal-like cells. Such transformation paves the way for tumor cells to penetrate the basement membrane, enter the vasculature or lymphatic system, and ultimately metastasize to distant sites.

B. Enhanced Mesenchymal Traits and Motility

During EMT, the enhancement of mesenchymal traits endows ESCC cells with greater motility, serving as a central mechanism of invasion and migration. This process is characterized by the upregulation of mesenchymal markers such as N-cadherin. The expression changes of these molecules confers significant motility, plasticity, and invasiveness to cancer cells. Unlike the epithelial-specific E-cadherin, N-cadherin promotes weak intercellular adhesion, allowing cells to detach more easily from the primary tumor site.

Moreover, N-cadherin interacts with the actin cytoskeleton to enhance the driving force behind cell migration. The upregulation of mesenchymal markers further augments the migratory capacity of ESCC cells, driving cancer cell invasion and metastasis.

C. Matrix Degradation

During EMT, the elevated expression of matrix metalloproteinases (MMPs) such as MMP2, MMP7, and MMP9 play a pivotal role in ESCC progression. These MMPs, as major enzymes responsible for ECM degradation, break down key ECM components such as collagen, elastin, and glycosaminoglycans. This process enable cancer cells to breach tissue barriers, thereby facilitating their migration and invasion.

IV. Future Research Directions and Challenges

In the study of esophageal squamous cell carcinoma (ESCC), elucidating the regulatory networks of EMT, advancing clinical translation, and leveraging multi-omics technologies represent critical future directions: investigating the roles of transcription factors such as Snail and Twist, as well as non-coding RNAs, in EMT; exploring post-translational modifications of key proteins such as E-cadherin; developing EMT-targeting inhibitors, in combination with existing therapies; identifying EMT-related biomarkers to optimize patient stratification and predict therapeutic outcomes; employing genomics, proteomics, and other omics approaches to comprehensively understand the molecular mechanisms of EMT and identify novel targets.

These efforts collectively aim to advance precision medicine and personalized therapeutic strategies for ESCC.

V. Conclusion

EMT plays a pivotal role in the progression of esophageal squamous cell carcinoma (ESCC), facilitating its invasion and migration through multiple mechanisms, including the reduction of cell adhesion, enhancement of mesenchymal properties and motility, and promotion of matrix degradation. Investigating the molecular mechanisms underlying EMT not only provides deeper insights into the biological behavior of ESCC but also holds significant potential for developing novel therapeutic strategies and improving patient prognosis. Future research should focus on precisely elucidating the regulatory networks of EMT, exploring the clinical translation of EMT inhibition, and employing multi-omics technologies to comprehensively analyze molecular changes during EMT. Through these efforts, significant breakthroughs in the diagnosis and treatment of ESCC are expected to be anticipated.

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