Epithelial mesenchymal transition and lung cancer

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ABSTRACT Despite the therapeutic advances, lung cancer remains the leading cause of cancer-related death in the United States and worldwide. Metastasis and recurrence are considered to be responsible for the failure of treatment. Recent studies indicate Epithelial mesenchymal transition, an evolutionarily conserved process, plays an important role in the embryonic development and cancer progression and is involved in the metastasis, drug resistance and correlated with progression of many tumors. Of importance, EMT is also involved in the acquisition of stemness phenotype of tumor cells. Although a growing body of evidence supports the role of EMT in the progression of many cancers, and a number of signal pathways, transcriptional factors and microRNAs involved in EMT process have been identified. However, the role of EMT in lung cancer is elusive. In this review, we present the recent findings in EMT including the molecular mechanisms of EMT, and the involvement of EMT in cancer progression, cancer stem cells and drug resistance, especially focusing on the correlation of EMT and lung cancer.

Key Words: epithelial mesenchymal transition; lung cancer; drug resistance; cancer stem cell

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Introduction

Despite the rapid advances in drug development and surgical procedure, lung cancer remains the leading cause of cancer-related death in the United States (1) and worldwide. The overall 5-year survival rate is approximately 15% (1). Surgery is still considered the best option for non small cell lung cancer (NSCLC) treatment. However, lung cancer is usually diagnosed until advanced stage, and fewer than 25% of NSCLC patients are considered as candidates for surgical therapy (2). Chemotherapy is another important therapeutic strategy for cancer treatment, but it fails to eliminate all the tumor cells due to drug resistance. Some patients are intrinsically resistant to chemotherapy referred as intrinsic resistance, whereas other patients eventually develop acquired resistance even after combination therapy, although they are initially sensitive to chemotherapy. Indeed, metastasis and therapeutic resistance are the major causes of failure in cancer treatment.

Epithelial mesenchymal transition (EMT) is an evolutionarily conserved process in which cells undergo conversion of epithelial cells to mesenchymal cells. EMT was originally defined in the study of embryonic development. EMT has been shown to be essential for embryonic development, gastrulation, neural nest and development of heart and other tissues and organs (3). Recent studies have extended the knowledge that EMT is also implicated in tissue repair, organ fibrosis, and cancer progression. Accumulating studies demonstrated that EMT is involved in the metastasis, treatment resistance and associated with the progression of many type of tumors. More important, recent studies in breast cancer suggested that EMT is also involved in the acquisition of characteristics of cancer stem-like cells (4), a rare subpopulation of cancer cells with capacity to self-renew, regeneration and differentiation, into a diverse type of cancer cells. The existence of cancer stem cells is thought to be crucial for initiation and maintenance of tumors as well as their metastasis.

The association of EMT and cancer progression has been revealed in several types of cancer, including breast cancer, prostate cancer, pancreatic cancer and hepatoma (5, 6). However, the role of EMT in lung cancer is less studied. In this review we highlight the recent findings in EMT and cancer, including the molecular mechanisms of EMT, and the roles of EMT in cancer progression, drug resistance and cancer stem-like cells, especially focusing on the lung cancer.

Molecular mechanisms of EMT

The major characteristic of EMT is the conversion from

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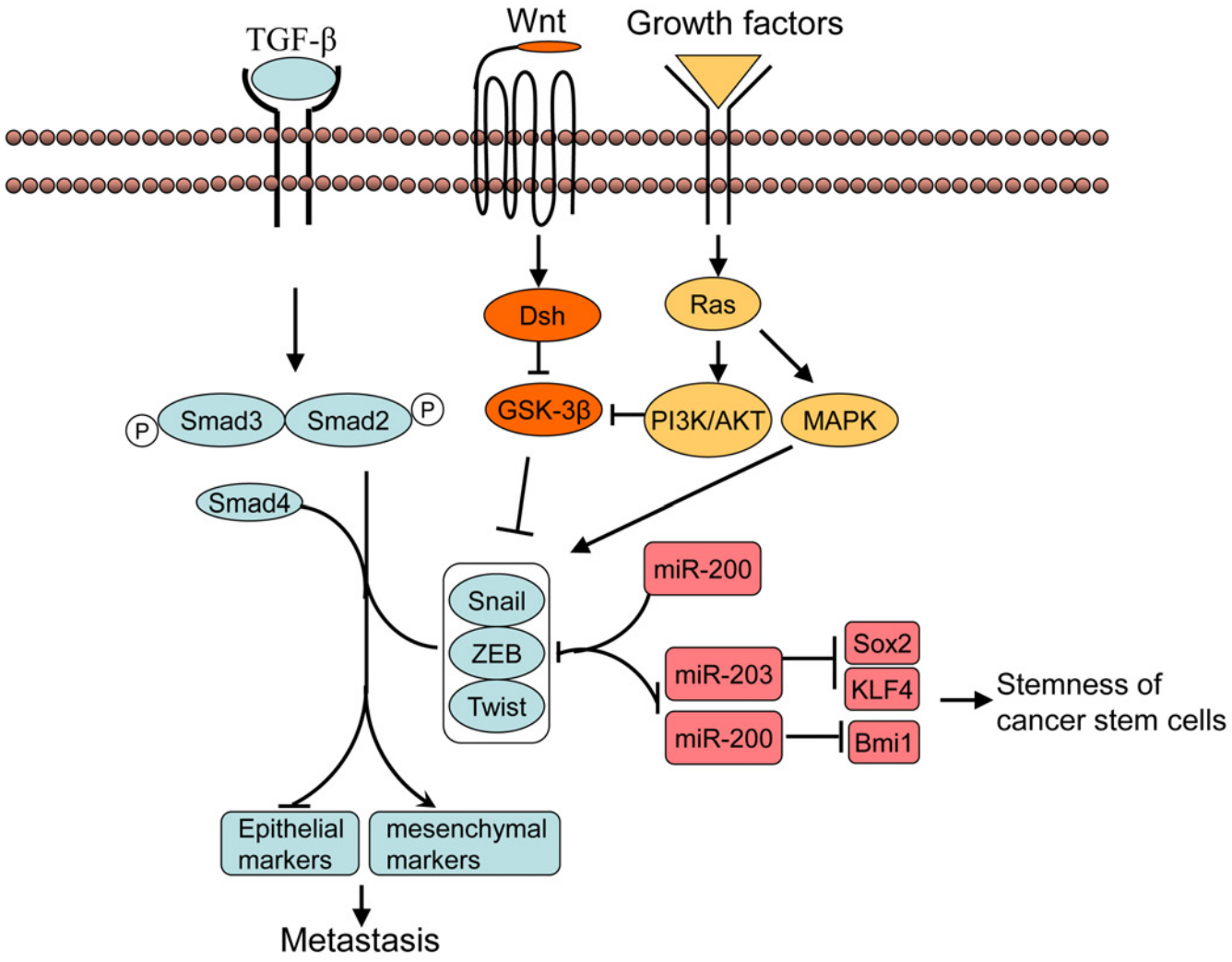

Fig 1. Schematic of the signal transduction pathways associated with EMT. TGF-β is a major inducer of EMT. It binds to the receptors leading to the phosphorylation of Smad2 and Smad3. Activated Smad2 and Smad3 form trimers with Smad4, Smads complex are then translocated into nucleus where they associate and cooperate with DNA binding transcriptional factors such as Snail, ZEB and Twist to regulate the expression of TGF-β target genes, resulting in the downregualtion of epithelail markers and the upregualtion of mesenchymal markers. TGF-β also cooperates with other signal factors such as Wnt and growth factors that act through receptor tyrosine kinase to regulate EMT. Several microRNAs have been indentified to regulate EMT. miR-200 suppresses EMT mainly through targeting ZEB factors and ZEB factors also regulate the expression of miR-200 and miR-203, linking the EMT and stem maintenance of cancer stem cells.

epithelial cells to motile, invasive and migratory mesenchymal cells. In the process, epithelial cells lose cell-cell adhesion and cell polarity, decrease the expression of epithelial cells marker such as E-cadherin, increase the expression of mesenchymal cell markers such as Vimentin, fibronectin, N-cadherin, alpha-smooth muscle actin (α-SMA), as well as increase the activity of matrix metalloproteinases (MMPs) like MMP-2, MMP-3 and MMP-9, associated with invasive phenotype (7). Thus, cancer cells acquire the capacity to migrate and invade the surrounding stroma and subsequently spread through the blood and lymphatic vessels to distant site. The conversion of epithelial cells to mesenchymal cells is coordinately regulated by a number of signaling pathways, transcriptional factors, eventually resulting in the loss of epithelial markers and acquistion of mesenchymal features (Fig 1).

EMT and signaling pathways

EMT can be induced by various signal factors, including TGF- β, growth factors that act through receptor tyrosine kinases such as fibroblast growth factor, hepatic growth factor, and Wnt, Notch and hedgehog proteins. Among these signaling factors, TGF-β is the most extensively studied inducer of EMT. TGF-β binding

to its receptors leads to the activation of Smad2 and Smad3 through direct C-terminal phosphorylation by TGF-β receptor I, phosphorylated Smad2 and Smad3 then form trimers with Smad4, Smads complex are then translocated into nucleus where they associate and cooperate with DNA binding transcriptional factors to regulate the expression of TGF-β target genes (8). However, Smad transcription factors have low affinity to DNA and need to interact with transcriptional cofactors such as Snail and ZEB factors (see below) to achieve high affinity and selectivity for target genes. TGF-β receptors, Smad3 and Smad4 are all essential for TGF-β-induced EMT, as suppressing the expression of those genes by dominant negative forms blocks TGF-β-induced EMT (9).

In addition, TGF-β also cooperates with other signaling pathways such as Wnt (10, 11), Hedgehog (12), Notch (13), Ras-MAPK (14) to induce EMT.

Transcriptional regulation of EMT

The loss of epithelial markers and acquisition of mesenchymal markers are typical feature of EMT. Among these, loss of E-cadherin is considered as a hallmarker of EMT. E-cadherin is a calcium dependent glycoprotein constituting the major transmembrane component of adherens junctions, and is responsible for epithelial cell-cell adhesion and maintenance of cytoskeleton organization. Loss of function of E-cadherin is thought to contribute to progression of cancer by increasing proliferation, invasion and metastasis. A number of transcriptional factors have been identified as transcriptional repressor of E-cadherin during EMT such as Snail, ZEB, and bHLH family factors like Twist, KLF8 and FoxC2. They suppress the transcription of E-cadherin through binding the E-box sequence containing a core 5'-CACCTG-3' motif within its promoter. Based on their effects on E-cadherin promoter, Thiery JP et al. (3) classified these transcriptional repressors into two groups : Snail1, Snail2, ZEB1/δEF1, SIP1/ZEB2, E47 and KLF8 directly bind and repress the activity of E-cadherin, whereas transcriptional factors such as Twist, Goosecoid, E2.2, and FoxC2 repress E-cadherin transcription indirectly. Among these transcriptional repressors, Snail1 is the first repressor identified to regulate the transcription of E-cadherin and promote EMT. In response to signal from EMT inducer like TGF- β, Snail factors are induced with the cooperation of smads and HMGA2 (15, 16). In addition, the TGF- β pathway can also cooperate with Ras, Notch and Wnt signaling to induce Snail expression. Furthermore, Snail is also induced by other grow factor such as EGF, HGF and FGF via Ras-MAPK or PI3K-AKT pathway. Upon activation, Snail1 and other transcriptional factors such as Smads complex can bind to the E-box consensus sequences in E-cadherin promoter, recruit the transcriptional cofactor such as mSIN3A, HDAC1 and HDAC2 (17, 18) to modify the chromatin structure, leading to the transcriptional repression of E-cadherin. Additionally, Snail factors not only regulate expression of E-cadherin, but also modify the epithelial and mesenchymal phenotype. For example, Snail1 was shown to repress the expression of claudin-3,-4 and -7 (19, 20), which are major components of tight junctions. Snail proteins also activate the mesenchymal proteins such as fibronectin (17, 21) and N-cadherin (22).

Similarly, ZEB factors are also upregulated in response to TGF-β or other growth factors (23). ZEB proteins then interact with Smad3 and repress the expression of epithelial marker genes during EMT (24).

In addition, epigenetic alteration is also involved in the regulation of E-cadherin, for example, hypermethylation in the promoter region results in the loss of E-cadherin expression, associated with EMT phenotype in breast cancer (25).

miRNA and EMT

miRNAs are single-stranded, 18-24nt non-coding RNA molecules that regulate gene expression at the post-transcriptional level through binding to 3'UTRs of target mRNAs, usually resulting in gene silencing. Recently several miRNAs have been identified to regulate EMT in development and cancer. miR-200 family members (miRNA-141,miRNA-200a, b and c, miR-429) suppress EMT mainly through targeting the transcriptional activator of EMT, ZEB1 and ZEB2 (26, 27). Interestingly, ZEB1 and ZEB2 have also been shown to suppress the expression of miR-200 family members through binding to E-box in the promoter of miR-200 family member, suggesting that miR-200 members and ZEB factors reciprocally control each other in a double negative feedback loop (28).

EMT and cancer progression

EMT has been shown to be associated with progression of several type of cancer. Upregulation of genes involved in EMT is associated with poorly differentiated tumors relative to low-grade tumors in breast cancer (29). A switch from E-cadherin to N-cadherin showed strong and significant associations with prostate cancer progression (30). However, the correlation of EMT and the progression or prognostic significance of lung cancer is still controversial. Several studies have found the association between loss of E-cadherin expression and poor prognosis in lung cancer. And co-expression of hypoxia-inducible factor 1α (HIF-1α), EMT inducer twist and snail was also associated with inverse outcome. However Pruklin et al. (31)showed that although the high expression of EMT associated markers were found in most lung cancer specimens, especially in squamous cell carcinoma, neither reduced E-cadherin or N-cadherin overexpression is associated with poor outcome in patients with NSCLC. Interestingly, brain metastases of NSCLC had decreased EMT phenotype

expression compared to the primary tumors, showing the characteristics of mesenchymal-epithelial transition, which is consistent to the observation that metastatic foci commonly appear more differentiated than the primary tumor. Importantly, in some studies, survival data related to the EMT profile is lacking. Clearly, further investigation is needed to identify and characterize the role of EMT in progression of lung cancer.

EMT and drug resistance

Although the mechanisms responsible for drug resistance have been investigated intensively over past decades, the clinical causes of drug resistance are still incompletely understood. Recent studies have shown that EMT is important in conferring drug resistance to cancer cells against conventional therapeutics. Several chemotherapeutic drugs resistant cell lines established *in vitro* such as genmcitabine-resistant pancreatic (32) cancer cells, paclitaxel-resistant ovarian carcinoma cells (33) showed phenotypic changes consistent with EMT. In NSCLC, Rapid advance in our understanding of molecular events in cancer biology leads to discovering molecular targeted drugs. EGFR inhibitors have been proven to be highly effective in the treatment of NSCLC harboring EGFR mutations (34, 35). However, the majority of patients will eventually develop treatment resistance. Recent studies have identified EGFR T790M mutation and c-Met overexpression are responsible for the acquired resistance to EGFR inhibitor, which account for only approximately 50% of cases of EGFR inhibitor-acquired resistance (36). Of note, human NSCLC lines harboring wild-type EGFR also displayed a range of sensitivity to EGFR inhibition dependant on the degree to which they have undergone EMT (37). NSCLC lines expressing E-cadherin showed greater sensitivity to EGFR inhibition. In contrast, NSCLC lines expressing vimintin and/or fibronectin were insensitive to EGFR inhibition both in vitro and xenograft. Accordingly, lung cancer cells resistant to EGFR inhibitors such as gefitinib and erlotinib, regardless of their EGFR status, also display mesenchymal phenotype with a decrease in the expression of E-cadherin and an increase in the expression of vimentin (38, 39), suggesting EMT might be a determinant of insensitivity to EGFR inhibition in lung cancer (37, 40). Studies on the underlying molecular mechanisms revealed that upregulated snail1 and Snail2 (Slug) are associated with chemoresistance in ovarian cancer cells (41), and silencing the expression of Snail or Twist restore the sensitivity of A549 cells to cisplatin (42, 43). Yao et al. also provided data to show that activation of TGF-β signaling pathway is required and sufficient to EMT and drug resistance to EGFR inhibitor. However, activation of IL-6 axis, not Zeb2 nor Snai knockdown can modify the drug sensitivity, suggesting that although a correlation of EMT and drug resistance is observed, the programs that lead to acquisition of mesenchymal phenotype and drug resistance appear to be mediated by distinct signaling transduction pathways (39).

Although relationship between drug resistance and EMT is well established, the mechanisms underlying drug resistance and EMT remain unclear. Chemotherapeutic drugs can enrich mesenchymal-like cells by eradicating non-mesenchymal cells. Another possibility is chemotherapeutic drugs can promote EMT. For example, adriamycin induces EMT in breast cancer cells, and NF-κB and miR-448 are shown to be involved in this process (44).

However, all these drug resistant cell lines in above studies are artificially generated in vitro from parental cell lines. Recently we isolated and established a non small lung cancer cell line, Am1010, directly from the muscle metastases of a patient diagnosed with lung adenocarcinoma after conventional therapy (45). This cell line is resistant to several drugs *in vitro* like cisplatin, taxol, gefitinib and radiotherapy. Of importance, it also displays mesenchymal-like morphology (unpublished data). Compared with other drug resistant cell models established in vitro, Am1010, derived directly from a patient with lung cancer, could better mimic the cancer cells resistant to treatment in patients and might provide a valuable tool to study the mechanisms of therapeutics resistance.

EMT and cancer stem cells (CSCs)

CSCs is a rare subpopulation of tumor cells that possess the ability to self-renew and differentiate into heterogeneous lineages of cancer cells that comprise the cancer mass (46). Emerging evidence suggests cells derived from EMT exhibit cancer stem cell-like features, thus linking the EMT and cancer stem cells. Mani and colleagues found mammary epithelial cells induced to undergo EMT by overexpression of snail,twist or TGF-β treatment acquired a $CD44^{high}/CD24^{low}$ signature (47), similar to the breast stem cells isolated and identified previously (48). The resulting population displayed mesenchymal phenotype, could form mammosphere and differentiate into cells of different lineages. Further study also from Mani group showed that the mesenchymal-like cells derived from EMT have the potential to differentiate into mature osteoblasts, adipocytes and chondrocytes similar to mesenchymal stem cells derived from human bone marrow (49). More recently, the molecular linkage between EMT and cancer stem cells was also defined. Transcriptional repressor ZEB1 was shown to repress the expression of miR-200 and stemness-inhibiting miR-203. And the candidate targets of miR-200 are also involved in stem cell, such as Sox2 and Klf4. Moreover, miR-200c, miR-203 and miR-183 cooperate to suppress expression of stem cell factors in cancer cells and mouse embryonic stem (ES) cells like bmi1. Thus ZEB1 links EMT and stemness-maintenance by regulating the expression of miR-200 and miR-203, providing the direct

link between EMT and cancer stem cells (50). And the linkage between EMT-like signature and cancer stem-like cells is also demonstrated in prostate cancer via modulating miR-200.

Recently, bronchioalveolar Stem Cells which express the AT2 cell-specific marker prosurfactant apoprotein-C (SP-C) and Clara cell-specific marker CCA have been identified from normal lung and lung cancer (51). However, the study on the role of EMT in lung cancer stem cells is rarely reported.

Perspective

EMT is a multistep process and many signal factors, signaling pathways and transcriptional factor are involved in this event. Importantly, EMT is also associated with metastasis, drug resistance and cancer stem cell. Emerging evidence demonstrated the promoting effects of therapeutic drugs on EMT and cancer stem-like cells (52), and molecular targeted therapy was also shown to promote metastasis (53), suggesting the side effects of anticancer drugs. Considering the role of EMT in cancer progression, targeting the proteins involved in EMT might provide a therapeutic strategy to preventing metastasis, drug resistance and recurrence (54).

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