MINI-REVIEW

Roles of Signaling Pathways in the Epithelial-Mesenchymal Transition in Cancer

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Abstract

The epithelial-mesenchymal transition (EMT) is a cellular process though which an epithelial phenotype can be converted into a phenotype of mesenchymal cells. Under physiological conditions EMT is important for embryogenesis, organ development, wound repair and tissue remodeling. However, EMT may also be activated under pathologic conditions, especially in carcinogenesis and metastatic progression. Major signaling pathways involved in EMT include transforming growth factor β(TGF-β), Wnt, Notch, Hedgehog and other signaling pathways. These pathways are related to several transcription factors, including Twist, Smads and zinc finger proteins snail and slug. These interact with each other to provide crosstalk between the relevant signaling pathways. This review lays emphasis on studying the relationship between EMT and signaling pathways in carcinogenesis and metastatic progression.

Keywords: EMT - signaling pathway - cancer

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Introduction

Epithelial-mesenchymal transition (EMT) is a process during which cells lose epithelial characteristics, and acquire mesenchymal features. This includes the changes that apical-basolateral polarity is lost, cell-cell junctions dissolve, the actin cytoskeleton is remodeled and those changes can increased migration and metastasis properties of cancer cells (Kalluri and Neilson, 2003; Kalluri and Weinberg, 2009). EMT was first described in corneal epithelial cells in vitro in 1982 (Hay, 1982). Normal epithelia is comprised of cells with aligned apical-basal polarity that are interconnected laterally by several types of junctions, including adherers junction, which play important roles in establishing and regulating cell-cell adhesion (Nelson, 2008). EMT occurs in the early embryonic epithelium that is internalized to lead to the mesodermal tissue formation structures and peripheral nervous system. Its molecular hallmark is the downregulation of epithelial markers, E-cadherin, α-catenin, β-catenin, γ-catenin, CK, ZO-1, and upregulation of a number of mesenchymal markers, including N-cadherin, Vimentin, α-SMA ,fibronectin and so on (Luo et al., 2012; Zhu et al., 2013).

EMT is important for migration and metastasis of cancer cells (Xia et al., 2015). E-cadherin is the most important mediator in EMT. Increased expression of E-cadherin was associated with good survival and can be used in survival prediction in serous ovarian cancer patients (Taskin et al., 2012). E-cadherin and the β-catenin were significant factors in predicted the histological grade in oral squamous cell carcinoma (OSCC) patients (Zaid, 2014). E-cadherin expression and positive Snail expression were correlated with a poor outcome for gastric adenocarcinoma patients (Liu et al., 2015). EMT allows cancer cells to leave the primary cancer environment and migrate to distant sites. Cell scratch experiments showed that the cells which occurred EMT can migrate more quickly and make scratch healing in less time, suggested that EMT can promote cancer invasion and metastasis. Cancer cells can be induced to undergo EMT by several signaling pathways, such as TGF-β signaling, Wnt, Notch, Hedgehog pathways and so on (Moustakas and Heldin, 2007). In addition, certain transcription factors (TF), including twist, snail, slug, ZEB1 and FOXC2 can induce EMT (Yang and Weinberg, 2008). They all can bind to the E-box site in the promoter of E-cadherin and repress its transcription, inducing EMT (Cano et al., 2000). Data showed that overexpression of Snail appeared to promote the prostate cancer progression. Except snail to and twist, ZEB1 has an important role in inducing EMT in non-small sell cancer (NSCLC). Mitsuo found that ZEB1 expression significantly correlates with increased Vimentin and decreased E-cadherin expression in lung cancer, while knockdown of ZEB1 resulted in dramatic growth inhibition in lung cancer cell lines (Takeyama et al., 2010) .

TGF-β, Wnt, Notch, Hedgehog signaling pathways have been shown to promote EMT by involving in the EMT process that occurs during cancer progression. Phenotype of epithelial cells is converted into phenotype of mesenchymal cells during the EMT, which affects the

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epithelial integrity and target downstream transcriptional regulators to regulate epithelial to mesenchymal genes expression. Mostly, these signaling pathways share common endpoints, the central target being the regulation of expression of the adherens junction protein, E-cadherin(Hofman and Vouret-Craviari, 2012).

Tgf-B Signaling Pathway

TGF-β is the most important factor in EMT and cancer cell dissemination. TGF-β pathway promotes invasiveness and metastasis by induced the expression of transcription factors snail, slug, twist, ZEB1 and TCF3 (Katsuno et al., 2013). These factors inhibit E-cadherin expression and upregulate mesenchymal markers such as N-cadherin, vimentin and promote the secretion of matrix metalloproteases. TGF-β has a dual effect in cancers, on the one hand it suppresses cancer development of early stages by preventing proliferation and inducing cell death, on the other hand it contribute to the malignant progression later by promoting invasion and metastasis (Massague, 2008; Heldin et al., 2009). TGF-β and its receptors regulate transcription by Smad-dependent and Smad-independent TGF-β receptor signaling pathway. TGF-β binds to type I and type II serine-threonine kinase receptors, termed TβRI and TβRII, respectively (Attisano and Wrana, 2002). After ligand binding the TβRII receptors activate TβRI receptors through direct phosphorylation, which phosphorylate the receptor-regulated Smad2 and Smad3 protein at two C-terminal serines. Activated Smad2 and Smad3 then combine with Smad4 to from trimeric Smad compiexes, a DNA binding partner common to all receptor-regulated Smads, and translocates into the nucleus. The Smad complexes interact with various transcription factors and transcriptional co-activators and regulate the transcription of target genes. TGF-β restrained the proliferation of epithelial cells in both developing organs and adult organs (Romero-Gallo et al., 2005). When the TGF-β suppressive effects were lost, TGF-β over expression was commonly observed in many solid cancers. NADPH oxidase (Nox) 4-generated reactive oxygen species(ROS) mediated TGF-β-induced morphological transformation from epithelial to fibroblast-like cells, restrained the expression of E-cadherin, and promoted the expression of snail. Furthermore p38MAPK transmitted activation signals from the TGF-β- Nox4 axis, which in turn alters the expression of snail and E-cadherin. Nox4-derived ROS mediate TGF-β-induced EMT by inhibiting PTP1B as a negative regulator of EMT signaling. Thus, PTP1B as a redox-sensor for Nox4-derived ROS participated in TGF-β-induced EMT. In summary, Nox4 contributed to TGF-β signaling pathway induced EMT through p38MAPK and PTP1B in pancreatic cancer cells (Hiraga et al., 2013). To investigate the role of TGF-β/Smad2 signaling pathway in long multi-walled carbon nanotubes (LMWCNT, length=5-15um)-induced EMT in human pulmonary epithelial cell line A549. SB431542, a TGF-β1 type I receptor inhibitor, could inhibit downregulation of E-cadherin and upregulation of α-SMA induced by L-MWCNT. L-MWCNT increased collagen deposition and pulmonary, and approximately 20% of pro-surfactant protein C positive epithelial cells translated into fibroblasts, suggested that occurrence of EMT in C57BI/6J male mice treated with 60ug L-MWCNT. All in all, these data indicated that TGF-β/Smad2 signaling pathway played a critical role in the EMT induced by L-MWCNT. (Chen et al., 2014a). In human esophageal squamous cell carcinoma (ESCC) cell lines in vitro TGF-β1 induced EMT to under go a transition from the epithelial to the spindle-like mesenchymal. This changes were accompanied by the loss of E-cadherin and the gain of vimentin, and the cells obtained increased ability of invasion and migration. In addition, PTEN/PI3K signaling pathway was activated in ESCC and it was negatively correlated with E-cadhrein, but was positively correlated with vimentin and contributed to tumor differentiation, invasion depth and lymph node. The levels of PTEN was increased after inhibited by pc-DNA3.1-PTEN after TGF-β1 treated EC-1 cells, upregulated TGF-β1-inhibited E-cadhrein expression, but downregulated TGF-β1-promoted vimentin expression. In conclusion, TGF-β1 induced EMT was driven by the PTEN/PI3K signaling pathway in ESCC. They first hypothesized that the PTEN/PI3K signaling pathway was a downsream target of TGF-β1 signaling (Zhang et al., 2014). The mechanism for TGF-β1-induced EMT in HK-2 cells was that TGF-β1 through activation of PI3K lead to activation of Akt2. Activated Akt2 caused the phosphrylation of GSK3β resulted in upregulation of snail, which repressed the E-cadherin expression, ultimately lead to EMT. Taken together, Akt2 mediated TGF-β1-induced EMT through GSK3β/snail signaling (Lan et al., 2014). In human lens epithelial cells (LECs) TGFβ2-induced activation of extracellular signal-regulated kinase(ERK)1/2 was independent of TGFβ/Smad signaling. TGFβ2-induced EMT was completely inhibited after repressed ERK1/2 signaling. In addition, the blockaded of ERK1/2 signaling inhibited the canonical TGFβ/Smad signaling pathway, as well as the Jagged/Notch pathway. However, the non-canonical TGFβ/ERK1/2 signaling can be mediated by the Notch pathway. So ERK1/2 signaling cross-interact with the canonical TGF-β/Smad and the Jagged/Notch pathway mediated EMT (Chen et al., 2014b). For the purpose of research the role of TGF-β's effect on EMT in benign hyperplasia cells (BPHs), monoclonal antibody against TGF-β1 treated the BPHs under supernatant-conditioned medium, found that the morphology of BPHs changed to a spindle-like shape, the expression of E-cadherin and cytokeratin5/8 were significantly lower than in ordinary medium. As well as these BPHs expressed the mesenchymal markers vimentin, α-SMA and snail. The stromal cell supernatant was able to induce EMT in BPHs through secreting TGF-β1 to activate Smad3 signaling (Hu et al., 2014).

Notch Signaling Pathway

Notch signaling is a core signaling system that regulates many cellular processes, including apoptosis, migration, invasion and angiogenesis (Miele et al., 2006a; Miele et al., 2006b), it can be either oncogenic or anti-proliferative (Hassan et al., 2014). Mammals

express four Notch receptors isotypes ,namely Notch-1, Notch-2, Notch-3, Notch-4 and five ligands Delta-like1,3,4, Jagged-1, and Jagged-2.The five ligands belong to Serrate family and Delta family, respectively. The pathway is activated through the interaction of a Notch receptor with a Jagged or Delta-like ligand, leading to proteolytic cleavages of the Notch receptor at two distinct sites. Importantly, the second cleavage releases a short extracellular peptide and generates a short-lived intermediate by the γ-secretase complex. The third cleavage releases the Notch intracellular domain (NICD), allowing it to enter the nucleus and binds to the transcriptional repressor RBP-JK (also known as CSL/CBF-1), then recruiting coactivators such as Mastermind-like (MAML) protein and function as a transcriptional activator, activating transcription of downstream target genes. Notch expression has been reported to be up-regulated in many human malignances (Penton et al., 2012). Inhibition expression of Notch-1 could suppress the occurrence of EMT in esophagel carcinoma EC-9706 cells and was accompanied by declined metastasis and invasion properties. The expression of E-cadherin was elevated and the vimentin was decreased in the EC-9706 cells transfected with Notch-1 siRNA. Their data showed Notch-1 was a major regulator of EC-9706 cells invasion and metastasis. They elevated the snail expression by transfecting the eukaryotic expression vector of snail in the EC-9706 cells, which transfected with Notch-1 siRNA, found that snail could deplete epithelial characteristics and rescue mesenchymal features of EC-9706 cells and also increased the invasion, migratory and proliferative ability. The data showed that the Notch-1 induced the EMT through the activation of snail in the EC-9706 cells (Wang et al., 2014a). The expression of the Notch1 target gene Hes1 was dramatically decreased in gastric cancer (GS) cell lines after treated by DAPT. In addition, Snail expression was downregulated and EMT was impaired. In conclusion, Notch1 signaling pathway induced EMT in GC cell lines Vial the regulation of Snail(Li et al., 2014). Snail was the first discovered and most important transcriptional repressor of E-cadherin. Hypoxia decreased the expression of E-cadherin and increased cell motility and invasion in three oral squamous cell carcinoma (OSCC) cell lines compared with that cultured under normoxia. As well as, there OSCC cell lines showed upregulation of Notch receptors, ligands and target genes, indicated that hypoxia induced EMT. GSI inhibited this upregulation of cell motility and invasion, and decreased the expression of E-cadherin under hypoxia. Thus hypoxia induced EMT through upregulation of the Notch signaling activation in OSCC cell lines (Ishida et al., 2013). Notch1 and ICN (the intracellular domain of Notch1) expression were high in intrahepatic cholangiocarcinoma (ICC) cell lines, the same time, enhanced expression ofα-SMA and lost of E-cadherin. Notch1 cDNA was transfected in ICC-9810 cells, inhibited the migration of ICC-9810 cells and decreased Rac1 activity. These results revealed that Notch1 could induce EMT and promote ICC migration and invasion through Rac1 activation (Zhou et al., 2013). In MCF7 and MCF10A cells overexpression Notch1 and N-caddherin. The vimentin was upregulated, butα-SMA and occluding were downregulated, meanwhile the expression of p65 and IL-1β were increased in these cell lines. Found that expression of p65 and IL-1β were downregulated upon inhibition of STAT3 and inhibition of STAT3 restrained EMT. Finally, results suggest that STAT3 phosphorylation was a key downstream target of Notch signaling and induced EMT in breast cancer cell lines(Zhang et al., 2015). NICD and its target gen Hes1 were increased in adenoid cystic carcinoma(AdCC), otherwise TGF-β1 was closely related to Slug and was negatively correlated with E-cadherin. So TGF-β1/Slug induced EMT and played an important role in AdCC development. Inhibition of Notch could notably decreased the TGF-β1, Slug and E-cadherin in the process of EMT, which was the possible mechanism for Notch signaling induced EMT in AdCC (Zhao et al., 2015). In non-small cell lung cancer (NSCLC) bone metastasis, Notch3 upregulation increased ZEB-1 expression. ZEB-1 was a novel target gene of Notch3 and increased its transcriptional activity, which contribute to the TGF-induced EMT phenotype. Further data suggested that ZEB-1 contribute to Notch3-induced EMT in NSCLC bone metastasis (Liu et al., 2014).

Wnt/B-Catenin Signaling Pathway

The Wnt signaling has an significant role in many adult developmental processes, such as gastrulation, axis formation, cell polarity, organ development and maintenance of cancer stem cell pluripotency (Logan and Nusse, 2004; Nusse et al., 2008). The protein β-catenin is the central regulator of Wnt/β-catenin signaling. The structure of β-catenin is consisted of three domains: the N-terminal domain, the armadillo domain and the C-terminal domain. When Wnt signaling pathway is activated by various intercellular factors, Wnt ligands bind to its cell surface receptors, such as Frizzled (FZD) and the low-density lipoprotein receptor-related protein (LRP), then activate the targeting protein APC and Axin, causing dephosphorylation of GSK-3β and recruitment of the cyosolic proteins Dishevelled(Dvl), which lead to the accumulation of β-catenin in the cytoplasm. Elevated levels of β-catenin lead to its translocation into the nucleus to form a complex with lymphoid enhancer factor (LEF) and T-cell factor (TCF) then regulates the expression of Wnt target gene (Clevers and Nusse, 2012). The characterized Wnt signaling pathway is the canonical Wnt/β-catenin signaling pathway. Numerous studies have demonstrated that Wnt ligands via their cell membrane bound receptors and coreceptors exert many fundamental physiological and pathophysiological functions in multiple organs and cell lineages, including organogenesis, carcinogenesis, fibrosis (Schinner et al., 2009). Importantly, it has been reported that the Wnt pathway can cross talk with transforming growth factor-β (TGF-β)/Smad, Notch pathways and connective tissue growth factor (CTGF). Wei showed that binding of β-catenin to Tcf4 is need for the activation of Wnt pathway (Wei et al., 2010). The expression of Tcf4 and the β-catenin/Tcf4 transcriptional activity were dose-dependently inhibited by destruxin B (DB) in Sk-Hep1 cells, and the β-catenin was decreased. Furthermore,

Wnt/β-catenin target genes, including cyclin D1, c-myc and survivn were downregulated after DB treatment. Both the migratory and the invasive abilities of Sk-Hep1 cells were downregulated. This observation supported that DB-mediated downregulation of EMT. Consistently, the levels of mesenchymal markers N-cadherin, slug and vimentin, were downregulated, whereas the expression of E-cadherin was enhanced. This study provided the evidence that the role of DB as a novel compound for the treatment of hepatocellular carcinoma through the suppression of the Wnt/β-catenin signaling pathway. (Huynh et al., 2014). Viviane found that in human sarcomatoid carcinomas, Mucin-1 (MUC1) and snail were overexpressed. MUC1 overexpression was associated with morphologic and phenotypic changes consistent with EMT. MUC1 C-terminal domain (MUC1-C) and β-catenin increased snail transcriptional activity by interaction with its promoter and blocking MUC1-C nuclear localization decreased Wnt/β-catenin signaling pathway activation and snail expression. MUC1 was an actor in EMT and Snail through Wnt pathway activation (Gnemmi et al., 2014). VGLL4 overexpression could suppress migration and invasion in gastric cancer cells, also increased the levels of E-cadherin and decreased N-cadherin and vimentin, whereas the reverse results were obtained following knockdown of VGLL4. From study the role of Wnt/β-catenin signaling pathway in VGLL4 induced EMT, results showed that VGLL4 overexpression suppressed the levels of β-catenin, however, reverse results were obtained following knockdown of VGLL4. Confirmed that β-catenin was a significant downstream factor of VGLL4, which suppressed EMT via regulation of Wnt/β-catenin signaling pathway (Li et al., 2015). ZNF488 is a zinc finger protein of ZNF family. ZNF488 expression was over-expressed in nasopharyngeal carcinoma (NPC) cell lines, which stimulated migration and invasion, however, inhibition of ZNF488 reversed these observations. E-cadherin andα-catenin were down-regulated, whereas the vimentin and N-cadherin were up-regulated in ZNF488-over-expression cell lines. Wnt pathway triggered the translocation of oncoprotein β-catenin to nucleus, where acted as coactivator of Tcf/Lef factor in transcriptional activation of target genes. They found that over-expression of ZNF488 activated the Wnt/β-catenin pathway to enhance Tcf/Lef transcriptional activity, thereby contributed to the EMT(Zong et al., 2015). Paired-related homeobox 1(PRRX1) expression was higher in gastric cancer tissues than in adjacent normal gastric mucosa and was significantly correlated with EMT markers and the metastasis of gastric cancer. Another PRRX1 could promote invasion, migration and cell proliferation in gastric cancer cells. Upon PRRX1 over-expression, β-catenin and c-Myc were upregulated and the translocation of β-catenin into the nucleus was increased. When the Wnt/β-catenin pathway was activated, the binding of Wnt molecules to frizzled receptors inhibits the activity of the destruction complex and allowed β-catenin to accumulate and translocate to the nucleus, which result in the loss of E-cadhrein. Findings demonstrated that PRRX1 promote EMT in gastric cancer cells through the activation of Wnt/β-catenin signaling. Inhibited Wnt/β-catenin signaling, counteracted the effect of EMT induced by PRRX1 overexpression, and suppressed the invasion and migration of gastric cancer cells. In short, PRRX1 promoted EMT via Wnt/β-catenin signaling in gastric cancer cells (Guo et al., 2015). Two major noncanonical Wnt pathways are the Calcium-dependent pathway and the planar cell polarity pathway. Both the canonical Wnt pathway and noncanonical Wnt pathway are involved in the EMT induction process. There is interaction between different Wnt signaling pathway , which makes the molecular mechanisms become very complicated.

Hedgehog Signaling Pathway

The Hedgehog (Hh) signaling pathway is essential for numerous processes during embryonic development including cell growth, cell differentiation, patterning and organogenesis. In normal adult tissues, this pathway is involved in stem cell population maintenance, tissue repair and regeneration (Beachy et al., 2004; Karhadkar et al., 2004; Teglund and Toftgard, 2010). According to the most models, canonical pathway activation is initiated by peptide ligands, called hedgehogs. In humans, three homologous Hh ligands exist: Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert Hedgehog (Dhh). Hh signaling is orchestrated by two trans-membrane receptors, Patched (Ptch) and Smoothened (SMO). It is initiated by the Hh ligand binds to a twelve-span transmembrane protein, PTCH. After binding to Hh ligands, the inhibitory effect of SMO is released, leading to active the three Gli zinc finger to transport into the nucleus and increase transcription of Hh target genes. Without binding to Hh ligands, PTCH catalytically inhibits the activity of SMO, a seven-span transmembrane receptor protein, and prohibits signaling to downstream genes. Expression of SMO and Gli are the markers of the Hh pathway activation. The expression of key Shh signaling components including Shh, Smo, Ptch1 and Gli1 were detected in lung squamous cell carcinomas (SCC), indicated the activation of Shh pathway. Data revealed reverse correlation between Gli1 and E-cadherin, as well as Gli1 and β-catenin. Taken together, the role of Gli1, a downstream effecter of Shh pathway, in enhancing EM, which in turn promotes recurrence and metastasis in lung SCC(Yue et al., 2014). RNAi-mediated Gli1 interference inhibited the hypoxia-induced EMT and decreased invasion of pancreatic cancer cells. Gli1 siRNA could not interrupt the hypoxia-mediated increase in SMO; conversely, blocking SMO decreased the expression of Gli1. These results indicated that hypoxia activated Hh signaling via up-regulation of SMO expression. Hypoxia induced EMT process as well as invasion, was largely driven by activation of Hh signaling pathway in pancreatic cancer cells. (Lei et al., 2013). Immunohistochemistry was performed to detect the expression of the Hh-induced transcriptional factor Gli-1 and the EMT markers Snail and E-cadherin in 121 patients with progressive gastric cancer. Gli-1 expression increased markly, and was positively correlated with Snail and was negatively correlated with E-cadherin. These findings suggested that activation of the Hh signaling pathway was closely related to the presence of EMT (Wang et

al., 2014b). HH pathway transcription factor Gli1 is in charge of epithelial differentiation in pancreatic ductal adenocarcinoma (PDAC). Knockdown of Gli1 abolished characteristics of epithelial differentiation, increased cell motlity, and induce EMT. The mechanism of EMT conversion in PDAC cells was Gli1 directly regulated the transcription of E-cadherin, rather than induction of Snail or Slug (Joost et al., 2012).

Discussion

In summary, increasing evidence shows that signaling pathways play key roles in the regulation of EMT. EMT is considered to play fundamental roles in the invasion and progression of cancer. The occurs of EMT involved in many mechanisms, the investigation of EMT is not thorough enough. Therefore deeply study of the mechanisms of Signaling pathways in EMT, will provide a better understanding of the roles of signaling pathways in the progression of cancer and lead to the development of new therapeutic or prognostic strategies for the treatment of cancers.

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