Epithelial-mesenchymal Transition and Cell Invasion

Hwajin Son and Aree Moon
College of Pharmacy, DukSung Women’s University, Seoul 132-714, Korea

(Received October 20, 2010; Revised November 2, 2010; Accepted November 14, 2010)

Epithelial-mesenchymal transition (EMT) is a complex process in which epithelial cells acquire the characteristics of invasive mesenchymal cells. EMT has been implicated in cancer progression and metastasis as well as the formation of many tissues and organs during development. Epithelial cells undergoing EMT lose cell-cell adhesion structures and polarity, and rearrange their cytoskeletons. Several oncogenic pathways such as transforming growth factor (TGF)-β, Wnt, and Notch signaling pathways, have been shown to induce EMT. These pathways have activated transcription factors including Snail, Slug, and the ZEB family which work as transcriptional repressors of E-cadherin, thereby making epithelial cells motile and resistant to apoptosis. Mounting evidence shows that EMT is associated with cell invasion and tumor progression. In this review, we summarize the characteristic features of EMT, pathways leading to EMT, and the role of EMT in cell invasion. Three topics are addressed in this review: (1) Definition of EMT, (2) Signaling pathways leading to EMT, (3) Role of EMT in cell invasion. Understanding the role of EMT in cell invasion will provide valuable information for establishing strategies to develop anti-metastatic therapeutics which modulate malignant cellular processes mediated by EMT.

Key words: EMT, cell invasion, TGF-β, Wnt, Notch

INTRODUCTION

Epithelial-mesenchymal transition (EMT) is a central process required for normal embryonic development. Recent evidence suggests roles of EMT for pathological conditions such as cancer progression, fibrosis, and chronic inflammation (Thiery, 2002, 2003; Grunert et al., 2003; Kalluri and Neilson, 2003; Balkwill, 2004). Multi-step processes and various oncogenic signaling pathways are involved in EMT (Derynck and Zhang, 2003; Larue and Bellacosa, 2005; Yee et al., 2010). Conditions where epithelial cells are transformed to a mesenchymal phenotype usually have poor clinical outcomes. The acquisition of EMT features correlates with carcinogenesis, invasion, metastasis, poor survival and an increased risk of cancer recurrence among patients with various solid tumor types, including breast (Trimboli et al., 2008), bladder (Adam et al., 2009), gastric (Kim et al., 2009), and colon cancer (Brabletz et al., 2005).

These findings suggest that EMT is required for cell invasion involved in cancer progression.

The present review highlights the current understanding of the role of EMT in cell invasion. The following topics are addressed in this review: (1) Definition of EMT - EMT consists of multiple steps in which epithelial cells lose epithelial-specific features and acquire fibroblastoid and mesenchymal features. (2) Pathways leading to EMT - Diverse signaling pathways are responsible for regulation of EMT. Mainly, TGF-β, Wnt, and Notch signaling pathways lead to EMT. (3) Role of EMT in cell invasion - Tumor cell invasion is necessary for the malignant progression of cancer and EMT is considered as a crucial event in developing invasive potential (Comijn et al., 2001; Christiansen and Rajasekaran, 2006; Lewis-Tuffin et al., 2010; Vuoriluoto et al., 2010). Recent studies on the correlation between EMT and tumor cell invasion are discussed in this review.

Definition of EMT. EMT-associated changes of cell phenotype in embryonic and adult epithelia were first proposed by Trelstad’s group and Hay’s group (Trelstad et al., 1967; Hay, 1968). In the 1980s, the mesenchymal transformation of early epithelial cells during development was confirmed in chick embryonic lens epithelium (Greenburg and Hey, 1982, 1986). The process of EMT is composed of multiple steps (Voulgaris and Pintzas, 2009; Zavadil and...
Bottinger, 2005). During EMT, epithelial cells undergo dramatic changes. Epithelial cells lose epithelial cell-specific phenotypes and develop features of mesenchymal cells. Tight- and adherens-junction proteins such as E-cadherin and α-catenin are downregulated and mesenchymal cell-specific marker proteins including N-cadherin, vimentin, and fibronectin are upregulated (Grunert et al., 2003; Christofori, 2006). Subsequently, epithelial cells lose cell-cell adhesion structures and polarity and then obtain cell motility and invasiveness. Finally, epithelial cells undergoing EMT digest and migrate through the extracellular matrix (ECM) and acquire motile and invasive characteristics (Cordon-Cardo and Prives, 1999; Thiery, 2002; Hay, 2005).

Epithelial cells function as an important barrier (Peinado et al., 2007). E-cadherin is a hallmark of epithelial cells, and is encoded by the CDH1 gene (Ghoul et al., 2009; Humar et al., 2009). This protein is localized at the basolateral membrane in adherens-junctions (Perez-Moreno et al., 2003), leading to the cell-cell adhesion (Peinado et al., 2007; Roger et al., 2010). Transcription factors involved in EMT such as Snail, Slug, TWIST, and the ZEB family mainly repress expression of E-cadherin during EMT (Batlle et al., 2000; Cano et al., 2000; Takkunen et al., 2006; Yee et al., 2010).

EMT is associated with intracellular events including embryonic development, wound healing, organ development, and cancer progression (Thiery, 2002, 2003; Grunert et al., 2003; Peinado et al., 2007). Many processes which occur in embryonic development and tissue repair in adult organisms might cause epithelial cells to lose their polarized phenotype. Redistribution of tight-, adherens-junction proteins and migration/scattering of cells occurs when epithelial cells lose of their polarity during many developmental processes. These phenomena are observed in several normal processes including tubulogenesis and branching in the mammary gland, or mesoderm formation; gastrulation, kidney and palate development (reviewed in Grunert et al., 2003).

EMT is controlled by several transcriptional repressors including Snail, Slug, TWIST, and the ZEB family that recruit histone deacetylases to E-box elements that are located in the E-cadherine promoter and a variety of other genes (Peinado et al., 2007). In general, EMT is very complex and strictly controlled in temporal and spatial fashions to ensure proper homing and reversion of cells to non-invasive phenotypes after migration to a destined location and to prevent abnormal development (Hay, 1995; Oft et al., 1998; Brabletz et al., 2001). Cancer cells undergoing EMT lose proper target recognition and activate self-sufficient autocrine loops of growth signals to avoid apoptosis (reviewed in Gotzmann et al., 2004).

**Signaling pathways leading to EMT.** The key signaling pathways which induce EMT include the TGF-β, Wnt, and Notch signaling pathways. Many of the EMT-inducing pathways control transcription factors of the Snail family (Snail, SLUG) and the ZEB family (ZEB1/2) as well as TWIST which repress expression of E-cadherin (Batlle et al., 2000; Cano et al., 2000; Takkunen et al., 2006; Yee et al., 2010). Here, we summarize the signaling pathways that contribute to the regulation of EMT.

**TGF-β signaling pathways:** TGF-β is a known multifunctional cytokine, which triggers diverse cellular processes including growth arrest, tissue fibrosis, and EMT (Pieè et al., 1999; Moustakas and Heldin, 2007; Schilling et al., 2008). To activate TGF-β signaling pathways, TGF-β binds to TGF-β type II (TβRII) and type I (TβRI) serine/threonine kinase receptors, respectively. TβRII binding to TGF-β transphosphorylates TβRI and the activated TβRI in turn activates R-Smads (Smad2 and Smad3) via phosphorylation at their C-terminal serine residues. As a result, activated R-Smads form a heterocomplex with Smad4 and translocate into the nucleus to regulate gene expression (Akiyoshi et al., 1999; Heldin et al., 1997; Nishihara et al., 1998; Massague and Chen, 2000; Feng and Derynck, 2005). These pathways are termed Smad-dependent pathways. In addition to the Smad signaling pathways, TGF-β also elicits diverse types of non-Smad signaling pathways. Among them, activation of Ras, mitogen-activated protein kinases (MAPks) such as ERK and p38 MAPks, and PI3K/Akt signaling has been linked to TGF-β-induced EMT (Bakin et al., 2000, 2002; Xie et al., 2004; Tavares et al., 2006). These pathways regulate distinct processes including cytoskeleton organization, cell survival, migration, and invasion. Recent studies have shown a crucial role for TGF-β signaling pathways that induce EMT through the Smad-dependent and Smad-independent pathways (Derynck and Zhang, 2003; Davies et al., 2005; Moustakas and Heldin, 2005; Valcourt et al., 2005).

[Smad pathway] Many studies have investigated the roles of the Smad-dependent pathway in TGF-β-induced EMT. When expression of Smad2 or Smad3 was downregulated by a dominant negative mutant, induction of EMT in response to the TGF-β-mediated Smad pathway was blocked in NMuMG cell system (Valcourt et al., 2005). Smad3 is a more important factor than Smad2 in the induction of EMT. Smad3-deficient renal tubular epithelial cells and Smad3−/− lens epithelial cells fail to undergo TGF-β-induced EMT (Sato et al., 2003; Saika et al., 2004). In contrast, effects of Smad2 on induction of EMT are controversial. In some cases, Smad2 has negative effects on EMT and cancer progression, e.g., Smad2 deficiency promotes skin tumor formation and EMT in human skin cancer patients and Smad2−/− hepatocytes develop mesenchymal characteristics and migratory capability (Ju et al., 2006).

[Non-Smad pathways] Numerous studies have demonstrated that TGF-β also induces EMT via non-Smad pathways, including the Ras-Raf-MEK-ERK, p38 MAPK, and
PI3K/Akt signaling pathways.

< Ras-Raf-MEK-ERK pathway>

When TβRI receptor is activated by TGF-β, it phosphorylates Shc, which offers a docking site for the recruitment of Grb2/Sos complex. Phosphorylated Shc forms a complex with Grb2/Sos. Shc/Grb2/Sos complex initiates activation of the Ras signaling cascade (Lee et al., 2007). Many observations support a collaboration of TGF-β with the Ras-Raf-MEK1/2-ERK1/2 pathway. Hyper-activated Ras-ERK1/2 pathway due to expression of mutant Ras enhances TGF-β-induced EMT through the loss of epithelial cell-specific features such as the downregulation of E-cadherin expression (Lehmann et al., 2000; Grande et al., 2002; Uttamsingh et al., 2008). Consistent with this observation, MEK1/2, an upstream kinase of ERK1/2, induces activation of ERK1/2 and enhances TGF-β-induced EMT were blocked using a chemical inhibitor of MEK1/2 (Xie et al., 2004).

< p38 MAPK pathway>

TGF-β also triggers phosphorylation of p38 MAPK as well as MKK3/6 and TAK1, which is an activator of MKK3/6 (Santibañez, 2006). Several studies have demonstrated that an inhibitor of p38 MAPK blocks TGF-β-induced EMT in mammary epithelial cells (Bakin et al., 2002; Yu et al., 2002). The Ras-Rac signaling pathway induces several transcription factors such as Elk-1 and ATF2 through the MKK3/6-p38 MAPK pathways. Eventually, this signaling pathway upregulates expression of Snail-activator for the MKK3/6-p38 MAPK pathways. Eventually, this signal- tumor invasion. It is well established that EMT is a key step in metastasis and cancer progression (Bakin et al., 2000). However, the roles of the PI3K/Akt pathway in EMT are not fully understood at present.

Wnt signaling pathway: The Wnt pathway has been implicated in EMT during embryonic development and cancer progression as summarized in several recent reviews (Huber et al., 2005; Larue and Bellacosa, 2005; Xu et al., 2009). In the absence of a Wnt signal, β-catenin is phosphorylated by GSK3 and then undergoes ubiquitin-dependent degradation. This system helps to maintain cytoplasmic β-catenin at a low level. When the Wnt signaling pathway is activated, cytoplasmic β-catenin is accumulated in the nucleus, where it associates with a transcription factor (TCF/LEF) and promotes the expression of target genes involved in EMT. β-catenin/TCF targets Slug (Conacci-Sorrell et al., 2003) and stabilizes Snail through blocking of its phosphorylation and degradation (Yook et al., 2005).

Mounting studies have documented that the Wnt signaling pathway cooperates with the TGF-β and PI3K/Akt signaling pathways in the context of EMT. For example, Smad2 and Smad4 form a complex with LEF, resulting in repression of E-cadherin gene expression in palate medial-edge epithelial cells. In addition, Smad4 and LEF induce mesenchymal features such as upregulation of fibronectin and vimentin, and the acquisition of migratory capacity (Nawshad et al., 2007). At the same time, the PI3K/Akt signaling pathway stabilizes β-catenin through blocking activation of GSK3 (Zhou et al., 2004).

Notch signaling pathway: Notch is critical for regulation of cell-cell communication during embryonic development and cancer progression. However, the function of the Notch signaling pathway in EMT is not currently understood. The Notch signaling pathway is activated via ligand-receptor interactions between neighboring cells. In turn, Notch is cleaved by proteases, and then the intracellular cleaved form, Notch intracellular domain (NIC), releases from its membrane anchoring. Upon activation, the NIC translocates into the nucleus, where it binds to the transcription factor CSL which upregulates Hey1, a well-known target gene of Notch signaling, and Snail (Timmerman et al., 2004; Zavadil et al., 2004). The Notch signaling pathway crosstalks with the TGF-β pathway. Hey1 is required for TGF-β-induced EMT and migration in epithelial cells (Zavadil et al., 2004).

Role of EMT in cell invasion. Metastasis, the major cause of death in cancer patients, is the spread of a tumor from its primary site to another location in the body. Cell invasion has been recognized as a key step in metastasis and results from various factors and oncogenic signaling pathways which overlap with EMT-inducing pathways (Boyer et al., 2000). Cancer cells undergoing EMT generally lose proper target recognition and activate self-sufficient autocrine loops of growth signals - mechanisms to avoid apoptosis (reviewed in Gotzmann et al., 2004). It has been
documented that cell invasion during cancer progression may be critically dependent on the acquisition of EMT features (Kang and Massague, 2004; Thiery and Morgan, 2004). These findings suggest that EMT is associated with cell invasion involved in cancer malignancy.

The association between EMT and cell invasion has been demonstrated in cancer progression. Matrix metalloproteinases (MMPs), especially MMP-2 and -9, are deeply involved in invasion and metastasis. Increased enzymatic degradation of extracellular matrix components by MMPs induces cell invasion and tumor spread (Platten et al., 2001; Wick et al., 2001). Studies suggest that MMPs stimulate EMT and that EMT produces MMPs (reviewed in Radisky and Radisky, 2010). In addition, signaling pathways involved in EMT, such as TGF-β, Wnt, and Notch signaling pathways are associated with cancer progression.

MMPs are an important mediator of various cellular events, including ECM degradation and remodeling, cell proliferation, apoptosis, cell invasion/migration and morphological change (Sternlicht and Werb, 2001). MMPs act in malignant cancer progression through degradation of ECM, stimulation of EMT and the induction of cell invasion (Kim et al., 2003; Duong and Erickson, 2004; Illman et al., 2006; Song et al., 2006). Each of the MMPs has a different substrate, for example, MMP-1, -8, and -13 are collagen-cleaving MMPs, while MMP-2 and -9 are gelatin-cleaving MMPs (reviewed in Snoek-van Beurden and Von den Hoff, 2005). Increased expression of MMPs has been suggested as a marker for tumor aggressiveness in breast, lung, pancreatic, and prostate cancers (Jones et al., 2004; Morgia et al., 2005; Somiari et al., 2006; Liu et al., 2007).

Recent studies have shown that MMPs stimulate EMT in various epithelial cell types, including kidney, ovary, lung, prostate, lens and breast epithelial cells (reviewed in Radisky and Radisky, 2010). According to several findings, MMPs are implicated in EMT during cancer progression via three mechanisms: (a) increased MMPs in the tumor microenvironment can directly induce EMT in epithelial cells, (b) cancer cells undergoing EMT can produce more MMPs and then facilitate cell invasion, and (c) epithelial cells can undergo EMT through further MMP production. For example, overexpression of MMP-7, 28 induces stable EMT in A549 lung adenocarcinoma cells (McGuire et al., 2003; Illman et al., 2006), and MMP-3 in MCF-7 human breast cancer cells and SCp2 mouse mammary epithelial cells (Noel et al., 2000; Radisky et al., 2005). MMP-2 becomes activated in neural cells undergoing EMT (Duong and Erickson, 2004) and MMP inhibitors block mesenchyme formation (Song et al., 2006).

Recent studies suggest that microRNA (miRNA, miR) is associated with EMT (reviewed in Zavadil et al., 2007; McConkey et al., 2009). miRNAs have been known as non-coding RNAs which act as post-transcriptional repressors of gene functions through translation inhibition and degradation. miRNAs are often deregulated in diseases such as cancer. Several findings have shown that the miR-200 family plays a critical role in EMT through regulation of ZEB family levels. Interestingly, the ZEB family can also repress the miR-200 family. When the miR-200 family is downregulated by the ZEB family, E-cadherin expression is suppressed and cell motility and invasiveness are increased. These feedback loops are in place to fine-tune EMT-related signaling. Recently, Zavadil et al. (2007) revealed a novel EMT-specific microRNA, miR-21, which is a candidate oncogenic microRNA associated with carcinogenesis.

Further functional studies on the interactions of MMP, miRNA and EMT may provide promising therapeutic approaches for the treatment of cancer. The above mentioned pathways leading to EMT, and crosstalk among these pathways can promote cell invasion and the progression of malignant cancer (Reiss and Barcellos-Hoff, 1997; Taki et al., 2003; Brabletz et al., 2005; Kim et al., 2005; Larue and Bellacosa, 2005; Wang et al., 2006).

CONCLUSIONS

Cancer cell invasion is a major factor and the first step in cancer metastasis which is the feature of malignant tumors resulting in dissemination of primary tumor cells to distant organs. To develop effective treatments for invasion and substantially improve patient prognosis, we need to understand the mechanism of cancer cell invasion and identify potential therapeutic targets related to metastasis.

Mounting evidence shows that EMT has been linked to an invasive phenotype as well as solid tumor progress (reviewed in Miclalizzi et al., 2010). EMT promotes malignancy in various cancer types including prostate, gastric, bladder, lung and breast cancer. Epithelial cells change their phenotype and acquire features of mesenchymal cells in a cancer microenvironment via EMT. Since EMT plays a key role in tumor cell invasion and cancer metastasis, establishing strategies to control EMT-inducing signaling should be a high priority for the development of anti-metastatic therapeutics.

In this review, we summarized a portion of recent findings on EMT-inducing signaling and discussed how EMT facilitates cell invasion and cancer progression. EMT is induced through diverse signaling pathways, including the TGF-β, PI3K/Akt, Wnt, and Notch signaling pathways. These signaling pathways crosstalk with each other and then facilitate EMT and cell invasion.

Although much is known about the functions of EMT in cell invasion, more must be understood to provide detailed information on the molecular mechanisms leading to an invasive phenotype. Understanding the relationship between EMT and cell invasion might provide useful information that can lead to the future development of novel targets for prognosis and/or therapy of cancer progression.
ACKNOWLEDGMENTS

This research was supported by a 2009 Duksgn Women's University Research Grant.

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