Review Article

Molecular signatures of epithelial-mesenchymal transition and their role in cancer progression

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Abstract

The differentiation of epithelial cells to mesenchymal cells is known as epithelial-mesenchymal transition (EMT) which plays a significant role in embryo and organ development, wound healing and tissue regeneration. Off late, EMT has emerged as a major factor contributing towards cancer metastasis. Cancer metastasis is a key challenge faced during the treatment of cancer. Apart from promoting cancer, EMT is also considered as a factor helping in the generation of cancer stem cells which can eventually lead to drug resistance making EMT a potential target in anticancer therapies. In this review, we highlight the different types of EMT, biomarkers of EMT which could be targeted to halt the EMT process which may provide new hope in cancer treatment.

***Keywords*: Epithelial-mesenchymal transition, cancer, biomarkers, metastasis**

Introduction

Epithelial-mesenchymal transition (EMT) is a phenomenon in which a polarized epithelial cell, undergoes biochemical changes transforming it into a mesenchymal cell phenotype, resulting in invasiveness, increased migration, hoisted protection from apoptosis, improved generation of extracellular matrix (ECM) components (Kalluri and Nelson, 2003). EMT is initiated through some molecular processes which include activation of transcription factors, expression of specific cell-surface proteins, cytoskeletal proteins, production of ECM-degrading enzymes, and changes in the expression of specific microRNAs. At the end of an EMT process, the basement membrane undergoes degradation, and mesenchymal cells are formed which can migrate from their origin (Kalluri and Weinberg, 2009). The inverse of EMT is mesenchymal-epithelial transition (MET) in which the migratory ability of the cells is lost, with cells depicting hallmarks of epithelial tissues namely undergoing apicobasal polarization and expressing the junctional complexes (Thiery et al., 2009). The process of EMT was first described by Elizabeth Hay who observed EMT transition in the primitive streak of chick embryos (Hay, 1995). EMT is an important developmental process, usually activated in wound healing, fibrosis, and cancer metastasis (Thiery et al., 2009; Kalluri and Weinberg, 2009; Chapman, 2011). Differentiation of embryonic stem cells, behavior of cancer stem cells and induced pluripotency are also regulated by EMT and MET (Lamouille et al., 2014).

Epithelial and mesenchymal cells differ phenotypically as well as in function, although both types of cells have inherent plasticity in common (Lamouille et al., 2014). Epithelial cells are linked together by gap junctions, tight junctions, adherens and also have an apicobasal polarity, the polarization of the actin cytoskeleton and are joined by a basal lamina at their basal surface. Mesenchymal cells, on the other hand, do not have the polarity, morphologically are spindle-shaped and interact with each other through focal points (Thiery and Sleeman, 2006). Epithelial cells express high levels of E cadherin, while mesenchymal cells express N cadherin, vimentin, and fibronectin. Thus, EMT involves significant morphological as well as phenotypical changes to a cell.

Biologically, EMTs are of three types: Type I or developmental, Type II or fibrosis (Phua et al., 2013) and wound healing, and Type III or cancer metastasis (Li and Li, 2015).

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EMT classification

EMT is now being studied vastly to understand its role in different signaling and transcription pathways (Kalluri and Zeisberg, 2006). Thus, a proposal to classify EMTs into three different biological subtypes based on their occurrence was considered in a meeting on EMT in Poland (2007) followed by a meeting in March 2008 at Cold Spring Harbor Laboratories (Kalluri and Weinberg, 2009).

Type 1 EMT: During implantation, embryo and organ development

Type 1 EMT involves primitive epithelial cells transitioning to motile mesenchymal cells as part of gastrulation and primitive neuroepithelial cells generating migrating neural crest cells. In both situations, some of the cells generated by EMT are re-induced as secondary epithelial cells in mesodermal and endodermal organs by MET (Zeisberg and Neilson, 2009). Further, following the earliest stages of embryogenesis, embryo implantation and the initiation of the formation of the placenta are both associated with an EMT that involves the parietal endoderm (Vicovac and Aplin, 1996). In particular, the trophectoderm cells, which are precursors of the cytotrophoblast, undergo an EMT to facilitate the invasion of the endometrium and the subsequent proper anchoring of the placenta, enabling its function in nutrient and gas exchange (Bischof et al., 2006). At the biochemical level, the EMT associated with gastrulation is dependent on and orchestrated by canonical Wnt signaling, and embryos deficient in Wnt3 cannot undergo the EMT associated with gastrulation (Skromne and Stern, 2001). Subsequently, the formation of a primitive streak is related to the expression of Wnt8c, and ectopic expression of Wnt8c in embryos that leads to multiple primitive streaks (Thomas et al., 1997).

During embryonic development, an EMT involving the epithelial cells of the neuroectoderm gives rise to migratory neural crest cells (Duband and Thiery, 1982). Initially, the premigratory neural crest cells express genes such as *Sox, Snail, Slug, and forkhead box D3* (*FoxD3*), and these cells subsequently undergo an EMT (Liem et al., 2000). As a consequence, they then dissociate from the neural folds, become motile, and disperse to the different parts of the embryo, where they undergo further differentiation into other cell types (Kalluri and Weinberg, 2009).

Type 2 EMT: Related with regeneration of tissues and fibrosis of organ

EMT-Type 2 is associated with wound recovery, tissue regeneration, and organ fibrosis. In various epithelial tissues, organ fibrosis arises by the inflammatory cells and by fibroblast that induces the numerous inflammatory signals of ECM complex. This type of EMT is usually seen in kidney, lung, liver and intestine fibrosis (Potenta et al., 2008; Zeisberg et al., 2007; Zeisberg et al., 2007; Kim et al., 2006). Furthermore essential tumor nodules of epithelial origin have cancer-related fibroblasts that acquire certain types of genetic alteration /mutation with a tumor cell, thus leading to tumorigenesis (Yu et al., 2014).

Type 3 EMT: Related with the progression of cancer and metastasis

During cancer progression, cancer cells invade adjacent tissues and migrate from their primary site to distant sites causing cancer metastasis (Hanahan and Weinberg, 2000). In many cases, EMT has been observed to be a part of one of the many causes of cancer metastasis (Thiery 2002). Many mesenchymal markers such as S100A4 (also termed as FSP1 or fibroblast-specific protein-1) or vimentin (Thompson et al., 1994), nuclear overexpression of β-catenin (Brabletz et al., 1998) and E-cadherin (loss of epithelial adhesion molecule) are the biomarkers for cancer metastasis (Mareel et al., 1989). Type 3 EMT is essential for the transition and preparation of epithelial nodular tumor cells for metastasis, movement, invasion but not for the formation of fibroblasts (Xue et al., 2003).

Complete knowledge of signaling events contributing towards EMT is still unclear. One of the proposed ideas is that the cancer cells undergoing genetic and epigenetic changes during the formation of primary tumor make them responsive to EMT-inducing heterotypic changes which originate in the tumor-associated stroma (Smit and Peeper, 2008). EMT-inducing signals arising from PDGF (Platelet-derived growth factor), EGF (Epidermal growth factor), HGF (Hepatocyte growth factor), and TGF-β (Transforming growth factor beta), play an essential role in activating EMT inducing transcription factors like Snail, Slug, Twist, Goosecoid, zinc finger E-box binding homeobox 1 (ZEB1) and FOXC2 (Forkhead box protein C2) in cancer cells and thus helping in cancer metastasis (Thiery, 2002; Jechlinger et al., 2002; Niessen et al., 2008; Medici et al., 2008). These transcription factors then help in activating the EMT program in cancer cells. A successful execution of EMT in cancer cells also depends on different signaling networks and signal transducing proteins such as PI3K, Akt, MAPK, ERK, Smads, β-catenin, lymphoid enhancer binding factor (LEF), RhoB, Ras, and c-Fos β4 integrins, α5β1 integrin, and αVβ6 integrin (Tse and Kalluri, 2007). Any disruption of cell-cell adherens junctions also promotes EMT process and the cell-ECM adhesions mediated by integrins facilitated by the disruption of cell-cell adheren junctions and the cell-ECM adhesions mediated by integrins (Gupta et al., 2005; Mani et al., 2007; Taki et al., 2006). Therefore, EMT plays an essential role in cancer metastasis and thus helping in the formation of secondary tumors.

Role of Tumor Microenvironment in EMT

The tumor microenvironment plays a fundamental role in tumor progression, induction of EMT, and cancer metastasis. There are several cells in the tumor microenvironment, such as inflammatory and immune cells, cancer-associated fibroblasts (CAFs), extracellular matrix components (ECM), endothelial and epithelial cells, mesenchymal stem cells, etc. (Tse and Kalluri, 2007). Immune cells induce EMT by infiltrating the primary tumor and activating TFG-β, EGF, and HGF from several signaling pathways. Two signaling pathways namely TFG-β/Smad and NF-kB cause changes in mesenchymal phenotype and initiates the process of metastasis in vivo. Thus, targeting the NF-kB signaling pathway in cancer may protect against cancer cell metastasis (Labelle et al., 2011). Positive regulation of EMT markers such as N-cadherin, Vimentin, Twist and Snail and negative regulation of E-cadherin expression was noticed in co-culture of breast tumor cells with bone marrow-derived mesenchymal stem cells (MSCs). Therefore, these cells can promote breast cancer metastasis by facilitating the EMT process (Martin et al., 2010). Tumor-associated macrophages (TAM) can also induce EMT in the intra-tumor environment by the activation of the β-catenin pathway and signaling of TFG-β. TAM can also induce EMT in the intra-tumor environment by the activation of the β-catenin pathway and signaling of TFG-β. A study conducted by Bonde et al. showed a positive correlation between TAM density and expression of mesenchymal markers, activation of β-catenin pathway, increase in mesenchymal markers expression, decrease in E-cadherin expression, and an invasive phenotype (Bonde et al., 2012). Thus, discerning the markers that help in the induction of EMT may help in thoroughly understanding the complicated process of EMT and its relation with tumor-microenvironment.

Biomarkers for EMT

EMT biomarkers are widely used to characterize the cancer cells. Some of the most common biomarkers used in EMT studies are TFG-β, Wnts, SNAIL and TWIST, cadherins and vimentin (Thompson et al., 2005). TGF-β is one of the most important inducers of EMT and a vital suppressor of epithelial cells (Pickup et al., 2013). TGF-β triggers cells to lose epithelial markers, like E-cadherin and helps them in gaining mesenchymal markers, such as vimentin. Wild-type TGF-β helps in cell proliferation but mutated TGF-β helps in the uncontrolled proliferation of cancer cells (Bellam and Pasche, 2010). Another transcription factor which is induced by TGF-β is Snail. It controls the protein expression of mesenchymal phenotype cells and also suppresses epithelial proteins like E-cadherin (Lamouille et al., 2014). Snail also helps in promoting epithelial cell migration and differentiation while in the embryonic stage it promotes the formation of mesoderm (Fidler and Poste, 2008). Another transcription factor namely Twist helps in the migration and differentiation of epithelial to mesenchymal cells. Twist also promotes the conversion of E-cadherin to N- cadherin. N-cadherin is one of the important biomarkers of mesenchymal cells (Vaittinen et al., 2001). The expression of E-cadherins and N-cadherins are usually checked to screen the presence of EMT and to determine the presence of mesenchymal stage of cancer cells (Strutuz et al., 2002). Studies related to breast cancer have shown that the level of E-cadherin is inversely proportional to metastasis and poor prognosis. Thus, E-cadherin level is monitored to determine tumor progression (Singhai et al., 2011). Expression of E-cadherin is also affected by Wnt signaling pathway whose function is to control the transcription of genes that helps in cell proliferation, differentiation, and migration (Wang and Zhou, 2011). In EMT process, epithelial cells acquire mesenchymal phenotype and start expressing mesenchymal markers like vimentin, tyrosine kinase 2 receptor discoidin domain (DDR2), fibroblast-specific protein 1 (S100A4) and collagen-specific tyrosine kinase receptor (Ren et al., 2014). Vimentin is an intermediate filament, and upregulation in its expression has been observed in several cancer types such as breast, colon, prostate. Vimentin expression in these cancers is associated with metastasis and poor prognosis (Kalluri and Weinberg, 2009; Lehtinen et al., 2013). Thus, these results show that the expression of vimentin can be used as a biomarker to predict invasiveness and survival in breast cancer (Patel et al., 2015).

Overcoming Drug Resistance by Targeting EMT

EMT plays a vital role in drug resistance. In a study conducted by Gupta et al., EMT cells were generated by E-cadherin shRNA, and these cells were further used to identify the CSC-selective small molecule inhibitors. After screening, it was observed that salinomycin, an antibiotic can selectively kill breast CSCs (Gupta et al., 2009). It has also been observed that salinomycin can also inhibit EMT induced by doxorubicin treatment and further sensitize HCC cells towards doxorubicin (Zhou et al., 2015). Apart from salinomycin, other small molecule inhibitors of EMT have also been tested in-vitro and in-vivo. Curcumin was able to sensitize colorectal cells that were initially resistant to 5-fluorouracil via miRNA-mediated suppression of EMT (Toden et al., 2015). Mocetinostat, a histone deacetylase (HDAC) inhibitor, inhibited the expression of EMT transcription factor namely ZEB1 by restoring miR-203, changed the EMT phenotype in pancreatic cancer and sensitized cells towards docetaxel (Meidhof et al., 2015). It has also been reported that Akt/GSK3β/Snail1 pathway-driven EMT causes gemcitabine resistance in pancreatic cancer. Further, the sensitivity of gemcitabine towards pancreatic cancer cells was restored by the administration of zidovudine, an antiviral drug, which led to inhibition of the Akt/GSK3β/Snail1 signaling pathway. Tumor formation in mice having gemcitabine-resistant pancreatic tumor xenograft was inhibited when zidovudine with gemcitabine was co-administered and hence, halted the pancreatic cancer cells from acquiring EMT phenotype (Namba et al., 2015). Metformin an anti-diabetic drug has also been shown to inhibit cancer and is now being investigated as a potential anti-cancer agent (Evans et al., 2005). Hirsch et al. (2009) showed that metformin could selectively target breast cancer stem cells (BCSCs). Vazquez-Martin et al. (2010) reported that metformin downregulated the expression of important EMT transcription factors such as ZEB1, Twist1, and SNAI2 and induced transcriptional reprogramming of BCSCs (Vazquez-Martin et al., 2010). Metformin also blocked the IL-6/STAT3 pathway and further inhibited the lung cancer cells from acquiring mesenchymal phenotype (Zhao et al., 2014). The exact mechanism of action of metformin by which it inhibits EMT is still unclear, but it is speculated that metformin may work through AMPK activation and inhibits EMT formation (Chou et al., 2014). Thus metformin can become a suitable candidate as an anti-cancer compound possessing EMT inhibiting potential (Lv and Shim, 2015).

Conclusion

It is now strongly suggested that EMT plays a significant role in cancer progression by targeting multiple transcription factors which in turn can activate different pathways. EMT is becoming a potential target to treat cancer, and many studies suggest that targeting EMT can help in halting cancer metastasis and this provides a new ray of hope in cancer treatment.

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Conflict of interest

The authors declare no conflict of interest

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