Cell Shape and Cell Lineage Conversion

Yukiko Nakaya and Guojun Sheng
Laboratory for Early Embryogenesis, RIKEN Center for Developmental Biology,
Kobe, Hyogo 650-0047, Japan

The organization of animal cells in vivo can be categorized as being either epithelial or mesenchymal. Interconversions between these two states, epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET), often represent key events in animal development and pathogenesis. The molecular and cellular mechanisms by which cell-cell and cell-substrate interactions are governed during EMT/MET have been examined extensively. Recent studies have also shown that EMT/MET is implicated in the acquisition of stemness in cancer cells and is accompanied by changes in epigenetic modifications. Ongoing progresses in regenerative medicine suggest that morphological and physical changes can facilitate somatic cell reprogramming and help achieve stemness. In this review, we will describe the principles of EMT/MET, their roles in cancer and normal animal development, and their relationship to stemness. We will conclude by emphasizing that studying cell shape changes in development is important for mechanistic understanding of how EMT/MET contributes to cell lineage conversion in cancer research and therapeutic medicine.

Key words: cell shape, cell biology, chick, epithelial mesenchymal transition, pluripotency, cancer stem cell

J. Poult. Sci., 52: 1-6, 2015

Introduction

Epithelial cells display an apico-basal polarity and maintain relatively stable interactions with their neighbors and with the extracellular basement membrane (BM) (Fig. 1). Epithelial cells collectively form a sheet with individual cells abutting each other in a uniform array. Mesenchymal cells, on the other hand, form structures with irregular shape and non-uniform composition and density. Mesenchymal cells also adhere to each other, but this adhesion is more transitory and less strong than in the epithelium, allowing for their increased migratory capacity (Fig. 1). EMT and MET are phenomena in which epithelial and mesenchymal cells interconvert their cellular morphology. They occur frequently during normal and abnormal physiological processes in development, wound healing, fibrosis and cancer progression (Polyak and Weinberg, 2009; Lim and Thiery, 2012; Nakaya and Sheng, 2013; Nieto, 2013).

EMT is marked by changes in several cell-biological features which include the loss of epithelial specific intercellular junctions, apico-basal polarity and the BM. Molecurally, cells in epithelial or mesenchymal state can be distinguished from each other by the expression level of several specific markers (Lee et al., 2006; Thiery et al., 2009; Lamouille et al., 2014). Epithelial cells are tightly interconnected through two adhesion complexes, the adherens junctions (AJs) and tight junctions (TJs). They contribute to the formation and maintenance of the apical domain (Martin-Belmonte and Perez-Moreno, 2012; Rodriguez-Boulan and Macara, 2014). The key component of the AJ is E-cadherin, a transmembrane protein molecule (Takeichi, 2014). Its proper regulation is crucial for controlling the epithelial morphology, and its protein re-localization or degradation, or transcriptional downregulation promotes the phenotypic transition from the epithelial to the mesenchymal state. Coincident with the downregulation of this epithelial marker gene, several mesenchymal marker genes encoding transcriptional repressors of E-cadherin (e.g., Snail, Twist, Zeb genes), and N-cadherin (mediating mesenchymal AJ interaction), matrix metalloproteinases (MMPs) (involved in matrix degradation/remodeling) and vimentin (a mesenchymal intermediate filament molecule) are upregulated during the EMT process. However, reversibility of EMT means that cells after undergoing EMT can halt their migratory behavior and downregulate N-cadherin, vimentin and EMT initiating-transcription factors through the process MET, culminating in the re-appearance of epithelial specific marker genes and epithelial cell morphology (Nieto, 2013). Many signaling pathways and molecules that underlie EMT have been identified, but mechanisms controlling MET remain elusive, suggesting that MET may not be the simple
reverse process of EMT (Polyak and Weinberg, 2009; Chen et al., 2013).

**Main Text**

At the cellular level, pathological EMTs are similar to developmental EMTs in that they are triggered in response to similar molecular signals and transcriptional factors. Developmental EMTs are highly reproducible events, and often take place concomitant with cell fate changes with precise spatial and temporal control. Pathological EMTs on the other hand are highly variable in their cellular compositions and molecular cues, and as a consequence are less amenable for in-depth analysis. Many pathological EMTs, however, are considered to have their underlying causes in the failure to suppress normal developmental EMT programs (Thiery et al., 2009; Lim and Thiery, 2012). Moreover, acquisition of malignancy and stem cell traits during cancer EMTs are intrinsically connected to cell fate regulation programs during development. Therefore, developmental EMTs represent not only models for molecular and cellular studies of EMT, but also have direct links to many cancer EMT events (Nakaya and Sheng, 2013).

The chick is one of the main model organisms in vertebrate developmental biology. It is also the leading model for developmental EMT/MET investigation. Through studies ranging from the formation of pluripotent epiblast, to the gastrulation event which generates the three principal germ layers, to neural crest cell formation, somitogenesis and beyond, the chick embryo has been indispensible in shaping our basic molecular and cellular understanding of developmental EMT/MET. Amenable to cell labeling, imaging, molecular perturbation, cell biology and systems-level analy-

---

**Fig. 1.** Morphology of epithelial and mesenchymal cells and EMT/MET. Epithelial cells have an apico-basal polarity and show tight cell-cell connections mediated through tight junction (TJ), adherence junction (AJ), gap junction (GAP) and desmosome. They also interact with the basement membrane (BM), which is a specialized form of extracellular matrix (ECM). These cellular and extracellular features, or at least a subset of them, are present in all epithelial structures. In contrast, mesenchymal cells tend to maintain loose and dynamic interactions with their neighbors and the surrounding ECM. EMT and MET are phenomena in which epithelial and mesenchymal cells interconvert their cellular morphology.
Gastrulation: Gastrulation is a developmental process in which the three germ layers, the ectoderm, mesoderm, and endoderm, are generated from an initial single-layered epiblast. The epiblast cells have an epithelial organization and have to undergo EMT to form mesoderm and endoderm cells. Gastrulation EMT takes place at a special region of the embryo called the primitive streak, and is the best-studied example of in vivo EMTs. Mesoderm precursor cells located in the epiblast first move within the epiblast epithelium to get close to the primitive streak (Bortier et al., 2001; Lawson and Schoenwolf, 2001; Hardy et al., 2011). While these cells approach the primitive streak, they have a columnar epithelial morphology, with an apico-basal polarity and tight junctions and epithelial adherens junctions, and move on the BM underneath the epiblast sheet. When these cells reach the primitive streak, with molecular mechanisms still not fully understood, the BM components are degraded underneath the mesoderm precursor cells poised to initiate the ingression process, whereas at the same time both TJs and AJs are still intact (Wakely and England, 1977; Solursh and Revel, 1978; Shook and Keller, 2003; Nakaya et al., 2008, 2013). Ingression, i.e., the completion of morphological transition from epithelial to mesenchymal, is marked by the loss of TJs and epithelial AJs and of the apico-basal polarity. This transition, the gastrulation EMT, allows epiblast cells to delaminate basally and invade inside of the embryo, and to become migratory mesoderm cells ready to initiate differentiation and further cellular reorganization (Fig. 2). During this temporal sequence of cellular steps, the epiblast cells, originally expressing E-cadherin, gradually shift to N-cadherin expression. Moreover, the pluripotency marker Nanog is expressed in most epiblast cells, but not in mesoderm cells (Shin et al., 2011), suggesting that gastrulation EMT coincides not only with changes in cell morphology, but also with cell fate specification and loss of cellular pluripotency features.

Somite formation: After mesoderm cells ingress from the epiblast and migrate away from the primitive streak, they undergo secondary, lineage-specific morphological changes according to their dorso-ventral fate and finally distribute themselves along the entire medio-lateral axis of the embryo. A prominent feature of the paraxial mesoderm lineage (cells destined to form the dermis, muscles and axial bones) is to initiate an MET event, giving rise to transitory epithelialized
structures called somites (Fig. 2). Somites form periodically through the MET of presomitic mesoderm (mesenchymal shaped paraxial mesoderm). Presomitic mesenchymal cells at the front of the prospective boundary for the next segmentation begin to reorganize their actin cytoskeleton, polarize, re-construct epithelial cell-cell adhesion and synthesize BM proteins, eventually resulting in the re-epithelialization of these mesoderm cells. Such MET event also occurs, although involving more complicated cell biological changes, in the axial (generating notochord), intermediate (nephrogenic) and lateral plate (splitting into somatic and splanchnopleuric layers) mesoderm. This mesoderm re-epithelialization behavior of mesenchymal cells shares some resemblance to that when disseminated cancer cells colonize by re-epithelialization a distant organ to complete metastasis (Fig. 2). It is also important to note that developmental EMT/METs are extremely dynamic. The re-epithelialized somitic cells quickly initiate further cellular reorganization to generate sclerotome, myotome and dermatome cells through a complex series of tertiary morphological change events.

In cancer biology, EMT has recently been shown to be one of the mechanisms underlying the generation of cells with stem-like characteristics (Mani et al., 2008; Ocana et al., 2012; Scheel and Weinberg, 2012; Lamouille et al., 2014). For example, in high grade breast tumors (estrogen receptor, progesterone receptor and Her2/neu triple-negative subtype), small populations of cells with features of tumor-initiating cancer stem cells (CSCs) also express molecular signatures associated with EMT (Aktas et al., 2009; Dunning et al., 2011). Because CSCs are more resistant to conventional radiation- or chemo-therapy, molecular intervention of associated EMT events in CSC generation or maintenance is viewed as a new and promising therapeutic means in treating malignancies. Furthermore, MET has also been suggested to be required for transforming somatic cells into pluripotent stem cells, execution of which is accompanied to and enabled by DNA demethylation and miRNA activation (Li et al., 2010; Samavarchi-Tehrani et al., 2010; Esteban et al., 2012). Thus, in addition to morphological changes in cell biological features, epigenetic reprogramming is clearly implicated in mechanistic regulation of EMT/MET (Stadler and Allis, 2012; Tam and Weinberg, 2013).

Epigenetic events such as DNA methylation, histone modification and chromatin remodeling are known to play a role in regulating gene expression in development and during tumor progression. E-cadherin, an important hallmark for epithelial cell-cell interaction, has been reported to be under the control of epigenetic modifications in cultured cells derived from cancer and normal tissues (Tam and Weinberg, 2013). Epigenetic silencing of E-cadherin during EMT is orchestrated by polycomb group (PcG) proteins. For instance, Ezh2, a component of the multi-subunit polycomb repressive complex (PRC) 2, catalyzes trimethylation of histone 3 lysine 27 (H3K27me3) in the nucleosome surrounding Cdh1 (encoding E-cadherin) promoter and represses Cdh1 transcription in TGF-beta induced EMT (Tam and Weinberg, 2013; Tiwari et al., 2013). Histone acetylation by histone acetyltransferases (HATs) or deacetylation by histone deacetylases (HDACs) is also associated with transcriptional regulation. Snail, an EMT inducing transcription factor, can recruit HDAC1 to the Cdh1 promoter (Peinado et al., 2004) and is also involved in the recruitment of LSD1, lysine-specific demethylase, to silence epithelial genes, including those encoding E-cadherin, claudin and cytokeratin in human mammary epithelial cells (Lin et al., 2010). In addition, EMT genes come under both positive and negative regulations at the post-transcriptional level by microRNAs (miRNAs). miR-200 family and miR-205 maintain the epithelial state through the interaction with both ZEB1 and ZEB2 (E-cadherin repressors) to repress their activity (Tam and Weinberg, 2013). However, in breast CSCs, loss of miR-200 expression results in the upregulation of SUZ12 protein and in polycomb-mediated repression of the Cdh1 gene (Iliopoulos et al., 2010). MET, implicated in the somatic cell reprogramming (Esteban et al., 2012), can also be controlled by miRNAs. Induction of the miR-200 family by BMP promotes the reprogramming of mouse embryonic fibroblast (MEF) and MET, and blocking of MET impairs MEF reprogramming, suggesting that MET is a key cellular mechanism toward induced pluripotency (Li et al., 2010). Taken together, epigenetic modifications clearly enable and drive phenotypic plasticity in both normal and cancer cells, and their roles in EMT/MET events during normal development, and in stem cell differentiation and somatic cell reprogramming are increasingly being appreciated.

Conclusions and Future Prospect

The relationship between cell morphology and cell fate had been underappreciated in the past. Recent studies highlighted the role of EMT in both cell morphological and fate conversions as exemplified by metastatic cancer cells which can de-differentiate and acquire a stem-cell like phenotype through EMT. In development and disease, EMT programs are activated by similar signaling molecules and transcriptional regulators. These EMT inducers are tightly regulated in developmental processes, but are aberrantly expressed in various types of carcinomas. Enablers for abnormal EMTs may lie in the complex changes in epigenetic regulation in CSCs and the yet poorly-understood extracellular chemical and physical properties of cancer tissues. MET has also been associated with cellular reprogramming, with recent data showing that somatic cell reprogramming can be induced by either extracellular stimulation or stress (Ohta et al., 2012). Altered cell morphology such as in MET due to exogenous stimuli may represent a more direct mechanism underpinning reprogramming in somatic cells. In animal development, cells undergo constant morphological changes and lineage differentiation, depicted by the “epigenetic landscape” model proposed half a century ago. In contrast, aside from homeostatic maintenance, adult cells are relatively quiescent with little or no change in either morphology or lineage identity. However, the existence of tissue specific adult stem cells and the
possibility for differentiated adult cells to acquire multipotent or pluripotent stem cell-like properties both in vitro and in vivo underscore the importance of EMT/MET studies not only in cancer research but also in therapeutic and regenerative medicine. In regeneration, the aim is to harness the power of normal EMT/MET during animal development, and in cancer research, a crucial challenge is to prevent unwanted EMT/MET in adult tissues. Finally, epigenetic regulation of EMT/MET during lineage conversion both in cancer and during reprogramming deserves more investigations in the future. Research in these areas has been carried out mainly using cultured cells. Yet in vivo tissue complexity can rarely be recapitulated in vitro, and studies using animal/developmental models remain irreplaceable for therapeutic applications and mechanistic understanding of normal and abnormal EMT/METs. In this respect the poultry research community is presented with a rare opportunity. With a long history of making significant contributions to the developmental biology field (Stern, 2005), the birds are increasingly becoming a tractable model system for genetics (Nishijima and Iijima, 2013), cancer (Kain et al., 2014) and pluripotency (Alev et al., 2013; Jean et al., 2013; Sheng, 2014) studies. Poultry science researchers have the advantage of combining recent advances in these fields with avian resources often uniquely available to them to help establish the avian system as an attractive alternative research model for cancer, cancer EMT, ES cell maintenance and differentiation and its therapeutic applications.

References

Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R and Kasimir-Bauer S. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Research, 11: R46. 2009.

Alev C, Nakano M, Wu Y, Horiuchi H and Sheng G. Manipulating the avian epiblast and epiblast-derived stem cells. Methods in Molecular Biology, 1074: 151–173. 2013.

Bortier H, Callebaut M, van Nueten E and Vakaet L. Auto- radiographic evidence for the sliding of the upper layer over the basement membrane in chicken blastoderms during gastrulation. European Journal of Morphology, 39: 91–98. 2001.

Chen Y, Wang K, Qian CN and Leach R. DNA methylation is associated with transcription of Snail and Slug genes. Biochemical and Biophysical Research Communications, 430: 1083–1090. 2013.

Dunning NL, Laversin SA, Miles AK and Rees RC. Immunotherapy of prostate cancer: should we be targeting stem cells and EMT? Cancer Immunol Immunother, 60: 1181–1193. 2011.

Esteban MA, Bao X, Zhuang Q, Zhou T, Qin B and Pei D. The mesenchymal-to-epithelial transition in somatic cell reprogramming. Current Opinion in Genetics and Development, 22: 423–428. 2012.

Hardy KM, Yatskievych TA, Konieczka J, Bobbs AS and Antin PB. FGF signalling through RAS/MAPK and PI3K pathways regulates cell movement and gene expression in the chicken primitive streak without affecting E-cadherin expression. BMC Developmental Biology, 11: 20. 2011.

Iliopoulos D, Lindahl-Allen M, Polytarchou C, Hirsch HA, Tsichlis PN and Struhl K. Loss of miR-200 inhibition of Suz12 leads to polycomb-mediated repression required for the formation and maintenance of cancer stem cells. Molecular Cell, 39: 761–772. 2010.

Jean C, Aubel P, Soleihavoup C, Bouhallier F, Voisin S, Laviol F and Pain B. Pluripotent genes in avian stem cells. Development Growth and Differentiation, 55: 41–51. 2013.

Kain KH, Miller JW, Jones-Paris CR, Thomason RT, Lewis JD, Bader DM, Barnett JV and Zijlstra A. The chick embryo as an expanding experimental model for cancer and cardiovascular research. Developmental Dynamics, 243: 216–228. 2014.

Lamouille S, Xu J and Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nature Reviews. Molecular Cell Biology, 15: 178–196. 2014.

Lawson A and Schoenwolf GC. Cell populations and morphogenetic movements underlying formation of the avian primitive streak and organizer. Genesis, 29: 188–195. 2001.

Lee JM, Dedhar S, Kalluri R and Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. Journal of Cell Biology, 172: 973–981. 2006.

Li R, Liang J, Ni S, Zhou T, Qing X, Li H, He W, Chen J, Li F, Zhuang Q, Qin B, Xu J, Li W, Yang J, Gan Y, Qin D, Feng S, Song H, Yang D, Zhang B, Zeng L, Lai L, Esteban MA and Pei D. A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. Cell Stem Cell, 7: 51–63. 2010.

Lim J and Thiery JP. Epithelial-mesenchymal transitions: insights from development. Development, 139: 3471–3486. 2012.

Lin T, Ponn A, Hu X, Law BK and Lu J. Requirement of the histone demethylase LSD1 in Snail-mediated transcriptional repression during epithelial-mesenchymal transition. Oncogene, 29: 4896–4904. 2010.

Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polypak K, Brisken C, Yang J and Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell, 133: 704–715. 2008.

Martin-Belmonte F and Perez-Moreno M. Epithelial cell polarity, stem cells and cancer. Nature Reviews. Cancer, 12: 23–38. 2012.

Nakaya Y, Sukowati EW, Wu Y and Sheng G. RhoA and micro- tubule dynamics control cell-basement membrane interaction in EMT during gastrulation. Nature Cell Biology, 10: 765–775. 2008.

Nakaya Y and Sheng G. EMT in developmental morphogenesis. Cancer Letters, 341: 9–15. 2013.

Nakaya Y, Sukowati EW and Sheng G. Epiblast integrity requires CLASP and Dystroglycan-mediated microtubule anchoring to the basal cortex. Journal of Cell Biology, 202: 637–651. 2013.

Nieto MA. Epithelial plasticity: a common theme in embryonic and cancer cells. Science, 342: 1234850. 2013.

Nishijima K and Iijima S. Transgenic chickens. Development, 139: 3486. 2010.

Ocana OH, Corcoles R, Fabra A, Moreno-Bueno G, Acloque H, Vega S, Barrallo-Gimeno A, Cano A and Nieto MA. Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. Cancer Cell, 22: 709–724. 2012.

Ohta K, Kawano R and Ito N. Lactic acid bacteria convert human fibroblasts to multipotent cells. PLoS ONE, 7: e51866. 2012.

Peinado H, Ballestar E, Esteller M and Cano A. Snail mediates E-
cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. Molecular and Cellular Biology, 24: 306–319. 2004.
Polyak K and Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nature Reviews. Cancer, 9: 265–273. 2009.
Rodriguez-Boulan E and Macara IG. Organization and execution of the epithelial polarity programme. Nature Reviews. Molecular Cell Biology, 15: 225–242. 2014.
Samavarchi-Tehrani P, Golipour A, David L, Sung HK, Beyer TA, Datti A, Woltjen K, Nagy A and Wrana JL. Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. Cell Stem Cell, 7: 64–77. 2010.
Scheel C and Weinberg RA. Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. Seminars in Cancer Biology, 22: 396–403. 2012.
Sheng G. Day-1 chick development. Developmental Dynamics, 243: 357–367. 2014.
Shin M, Alev C, Wu Y, Nagai H and Sheng G. Activin/TGF-beta signaling regulates Nanog expression in the epiblast during gastrulation. Mechanisms of Development, 128: 268–278. 2011.
Shook D and Keller R. Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. Mechanisms of Development, 120: 1351–1383. 2003.
Solursh M and Revel JP. A scanning electron microscope study of cell shape and cell appendages in the primitive streak region of the rat and chick embryo. Differentiation, 11: 185–190. 1978.
Stadler SC and Allis CD. Linking epithelial-to-mesenchymal transition and epigenetic modifications. Seminars in Cancer Biology, 22: 404–410. 2012.
Stern CD. The chick; a great model system becomes even greater. Developmental Cell, 8: 9–17. 2005.
Takeichi M. Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling. Nature Reviews. Molecular Cell Biology, 15: 397–410. 2014.
Tam WL and Weinberg RA. The epigenetics of epithelial-mesenchymal plasticity in cancer. Nature Medicine, 19: 1438–1449. 2013.
Thiery JP, Acloque H, Huang RY and Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell, 139: 871–890. 2009.
Tiwari N, Tiwari VK, Waldmeier L, Balwierz PJ, Arnold P, Pachkov M, Meyer-Schaller N, Schubeler D, van Nimwegen E and Christofori G. Sox4 is a master regulator of epithelial-mesenchymal transition by controlling Ezh2 expression and epigenetic reprogramming. Cancer Cell, 23: 768–783. 2013.
Wakely J and England MA. Scanning electron microscopy (SEM) of the chick embryo primitive streak. Differentiation, 7: 181–186. 1977.