**Perspective**

**EMT and MET as paradigms for cell fate switching**

Jiekai Chen†, Qingkai Han†, and Duanqing Pei*

Key Laboratory of Regenerative Biology, South China Institute for Stem Cell Biology and Regenerative Medicine at Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China
† These authors contributed equally to this work.
* Correspondence to: Duanqing Pei, E-mail: pei_duanqing@gibh.ac.cn

Cell fate determination is a major unsolved problem in cell and developmental biology. The discovery of reprogramming by pluripotent factors offers a rational system to investigate the molecular mechanisms associated with cell fate decisions. The idea that reprogramming of fibroblasts starts with a mesenchymal-epithelial transition (MET) suggests that the process is perhaps a reversal of epithelial to mesenchymal transition (EMT) found frequently during early embryogenesis. As such, we believe that investigations into MET-EMT may yield detailed molecular insights into cell fate decisions, not only for the switching between epithelial and mesenchymal cells, but also other cell types.

In any given animal tissue, one may find two very common cell types: the epithelial cells that line the surface of a tissue or organ and mesenchymal cells that are embedded in the three-dimensional matrix. Microscopically, the epithelial cells are attached to the basement membrane, establish an apical–basal axis of polarity, and communicate with each other through the gap junction. Across and underneath the basement membrane, there is the stroma made of the three-dimensional extracellular matrix synthesized by the resident mesenchymal or stromal cells. Throughout development, much of cell fate decisions revolve around the formations of these two cell types. Two critical processes have been recognized dealing with inter-conversion between epithelial and mesenchymal cells, namely EMT for epithelial to mesenchymal transition and MET for mesenchymal to epithelial transition. In this review, we focus on these two processes and describe the molecular machineries that control these transitions. Given the newly recognized role of MET in cellular reprogramming, we discuss the implication of this process in cell fate transition in greater detail.

In metazoans, the formation of mesoderm represents a typical EMT during gastrulation. Even though the critical structural changes where cells involute or ingress vary among species, such as the primitive streak in mouse, the gastrulation process involving active EMTs is conserved throughout evolution (Thiery et al., 2009). In essence, EMT gives rise to all the mesenchymal cells in early embryogenesis. Taking mouse embryogenesis for example, gastrulation occurs around E6.0, which gives rise to ectoderm, mesoderm, and definitive endoderm (DE) (Arnold and Robertson, 2009) (Figure 1A). A large body of evidence suggests that extracellular factors transmit instructive signals to the nuclei and the cells are programmed to express specific genes for a particular cell type within those three germ layers. Specifically, nodal plays a critical role in the formation of primitive streak from epiblast cells (Arnold and Robertson, 2009). Inside the nuclei, Snail genes are considered as the most important downstream targets of the nodal-SMAD2/3 pathway during gastrulation (Thiery et al., 2009). The Snails may in fact be the guardians of the mesenchymal phenotype by activating mesenchymal genes and suppressing epithelial genes. Indeed, Snails have been shown to down-regulate E-cadherin effectively, which is one of hallmarks for epithelial cells. Snail-deficient embryos could not proceed through gastrulation and form mesodermal cells as they could not down-regulate the expression of E-cadherin in the primitive streak (Thiery et al., 2009). It is generally recognized that the embryonic EMT process is orchestrated and maintained through the collaboration of extracellular signals and intracellular transcription factors.

In 2006, Takahashi and Yamanaka (2006) first discovered that mouse embryonic fibroblasts (MEFs) can be converted into ES-like cells by forced expression of several defined factors, including Oct4, Sox2, Klf4, and c-Myc. This remarkable process resets the differentiation clock of MEFs back to the pluripotent state equivalent to a blastocyst. The recapture of pluripotency by defined factors has tremendous implication both in basic biology and in regenerative medicine.

Termed induced pluripotent stem cells (iPSCs), these cells may be utilized to model a specific disease or differentiate into functional cells for transplantation. On the other hand, the reprogramming process can be a useful system to investigate mechanisms involving cell fate decision; indeed, compared with somatic cell nuclear transfer, the generation of iPSCs is relatively easy and suitable for detailed biochemical and genome-wide analysis (Yamanaka and Blau, 2010). Furthermore, a better understanding of reprogramming mechanisms may provide a rationale for improving this technology such that safer iPSCs can be produced reliably and routinely. However, much effort was devoted to global and high throughput strategies that generated large data sets associated with the reprogramming process, providing significant insights in terms of gene expression associated with the reprogramming process.
Empirically, reprogramming appears to be a multi-step metamorphosis transforming fibroblasts into pluripotent stem cells. Microscopically, this dramatic change in morphology is well programmed by the reprogramming factors, resulting in uniform and tightly packed ES-like cells from individually disperse fibroblasts. To put this process into a cellular context, it was proposed and then demonstrated that reprogramming starts with MET, mirroring the embryonic EMT observed frequently during gastrulation and throughout development and carcinogenesis; experimentally, upon the introduction of ectopic reprogramming factors including Oct4/Sox2/Klf4/c-Myc, fibroblasts undergo an obligatory transformation in morphology to become epithelial-like, expressing the corresponding markers such as E-cadherin, accompanied by becoming smaller and establishing more cell–cell contacts (Li et al., 2010). Such a transformation indicated that the fibroblasts were losing their mesenchymal identity. By functional genomics, the reprogramming process can be segregated into initiation, maturation and stabilization phases. During the initiation phase, a MET signature was also found through microarray analysis of genes (Samavarchi-Tehrani et al., 2010). Detailed analysis of gene expression and surface marker expression showed that the MET comprised two concrete changes, i.e. the down-regulation of mesenchymal markers and key transcriptional factors such as N-cadherin, Snail, and Zeb1 as well as up-regulation of epithelial markers such as E-cadherin and Occludin (Ocn) (Li et al., 2010; Samavarchi-Tehrani et al., 2010). The changes occur at the early phase of reprogramming in which pluripotent-associated genes were still not to be reactivated. Thus MET can be defined as an initiated response of factorial-mediated somatic reprogramming (Figure 1B).

One may wonder why it takes four functionally unrelated transcription factors to turn a fibroblast into iPSCs. This question appears to be quite difficult to address. The discovery of MET by accident offered a reasonable system to address this question. Given the concrete gene expression and marker profiles associated with MET, it became feasible to dissect the role of each reprogramming factor towards MET. As MET starts upon the transduction of reprogramming factors, the relationship between ectopic expression of these factors and the MET-associated variation were examined. It became apparent that the transforming growth factor beta (TGFβ) signaling pathway is the primary target of the reprogramming factors: Tgfb1, Tgfb3, two members of TGFβ superfamily, were repressed by c-Myc and Oct4/Klf4, respectively, TgfbR2 was suppressed by c-Myc, TgfbR3 was suppressed by Oct4/Sox2 (Li et al., 2010). Given the established role of the TGFβ pathway in activating EMT during embryonic development and carcinogenesis (Thiery et al., 2009), the primary role of the reprogramming factors at the early phase seems to attempt to reverse the EMT process. Furthermore, Oct4 and Sox2 also target the nuclear transcription factors that specify the mesenchymal cell fate in development such as Snail. Therefore, it appears that the defined factors initiate reprogramming by extinguishing the mesenchymal cell fate of fibroblasts through the silencing of TGFβ signaling and the expression of Snail, both critical for EMT (Li et al., 2010).
Which fate will those cells that lost their mesenchymal identity have? It has been demonstrated that many of those cells assume an epithelial-like morphology a few days after the transduction of reprogramming factors (Takahashi and Yamanaka, 2006; Li et al., 2010). Then, which of the reprogramming factors initiate the epithelial program? Among the four original ‘Yamanaka’ factors, only Klf4 has the capability to activate the expression of E-cadherin, the hallmark of epithelial identity, and several other markers such as EpCAM and Ocn (Li et al., 2010). So, it becomes apparent that there is a clear division of labors among the defined factors during reprogramming: SKOM work to suppress the TGFβ pathway and erase the identity of fibroblasts, Klf4 activates E-cadherin and other epithelial program to start MET (Figure 1C).

Accumulating evidence now supports the role of MET in reprogramming. For instance, knocking down the expression of E-cadherin reduces the number of fully reprogrammed iPSC colonies (Li et al., 2010). During a genome-wide siRNA screening, siRNAs targeting E-cadherin, Par3 and Crb3 all reduced the number of AP positive colonies significantly (Samavarchi-Tehrani et al., 2010). Conversely, TGFβ, a potent inducer of EMT, as well as overexpression of Snail, blocked reprogramming potently (Maherali and Hochedlinger, 2009; Li et al., 2010). Since TGFβ is abundant in serum, which was widely used in the culture system for iPSCs generation, a better practice would be the elimination of serum in the reprogramming system. Therefore, knowledge about MET should provide a rationale to further improve reprogramming.

As MET or other fibroblasts are constantly synthesizing TGFβ, inhibitors for the TGFβ signaling pathway should be able to enhance the reprogramming of fibroblasts. Indeed, selective inhibitors of the activin receptor-like kinase 4, 5, and 7 (ALK4/5/7), including A83-01, SB431542, and E-616452 (RepSox), have been demonstrated to improve reprogramming, presumably by antagonizing EMT and facilitating MET (Ichida et al., 2009; Lin et al., 2009; Maherali and Hochedlinger, 2009). Like the chemical compounds, Smad7, one of the I-Smad proteins, was able to block TGFβ pathway signaling selectively and replace Sox2 to enhance reprogramming (Li et al., 2010).

On the other hand, any means to boost MET should be a positive force in enhancing reprogramming. Indeed, overexpression of E-cadherin can both enhance the generation of iPSCs and even replace Oct4 (Chen et al., 2010; Redmer et al., 2011). Interestingly, two small molecule compounds, apigenin and luteolin, have been reported to promote the expression of E-cadherin and consequently improve the efficiency of iPSCs generation (Chen et al., 2010). Further studies showed recently that the bone morphogenetic proteins (BMPs), another class of morphogens in the TGFβ superfamily, can enhance the reprogramming by activating MET and the blockage of BMPs pathway would impair the reprogramming process (Samavarchi-Tehrani et al., 2010). It has been shown that reprogramming requires only a short-time expression of Klf4 at early phase to induce epithelial markers, suggesting that the primary function of Klf4 is to activate MET as originally envisioned; since BMPs are considered as a potential inducer of MET, they are used to replace Klf4 in an optimized culture system. Indeed, BMPs induce MET in the absence of Klf4 and can replace Klf4 efficiently (Chen et al., 2011). In sum, either blocking mesenchymal signaling or inducing epithelial program can not only enhance reprogramming but also replace some of the reprogramming factors. These results demonstrated that MET plays a critical role in reprogramming and may serve as a guide for rational improvement of reprogramming methodologies (Figure 1C).

MicroRNAs are RNA molecules with important roles in post-transcriptional regulation of gene expression. The miR-200 family is a well-known microRNA family that regulates MET and has been well studied during oncogenesis and for cell migration (Brabetz and Brabetz, 2010). The miR-200a/200b/200c has been shown to improve the expression of epithelial genes such as E-cadherin and EpCAM, and increase the ratio of SSEA-1 positive cells during the reprogramming process (Samavarchi-Tehrani et al., 2010).

Recent studies showed that the miR-302 family (including miR-302a/b/c/d and miR-367) can improve the generation of iPSCs, and more remarkably, can induce pluripotency without any transcription factors. Recently, three independent reports showed that the miR-302 cluster is able to reprogram somatic cells alone (Anokye-Danso et al., 2011; Lin et al., 2011; Miyoshi et al., 2011). Subsequently, two additional reports demonstrated that the miR-302 cluster can enhance reprogramming by targeting TgfbR2 and facilitating MET (Liao et al., 2011; Subramanyam et al., 2011) (Figure 1C).

Gls1 is a maternal transcription factor and has been shown to improve reprogramming mediated by defined factors. Mechanistically, it has been shown that Gls1 enhances reprogramming by activating several genes including Foxa2, which in fact antagonizes EMT (Maekawa et al., 2011). Taken together, MET emerges as a critical step in the reprogramming process in which multiple factors and microRNAs can exert their functions (Figure 1C). One can envision that additional factors may impact MET in a similar fashion, which awaits discovery in the near future.

In summary, cell fate determination is a major unsolved problem in cell and developmental biology. The discovery of reprogramming by pluripotent factors offers a rational system to investigate the molecular mechanisms associated with some important aspect of cell fate decision. The idea that reprogramming of fibroblasts starts with MET suggests that the process is perhaps a reversal EMT found frequently during early embryogenesis. As such, we believe that investigations into MET–EMT may yield detailed molecular insights into cell fate decisions, not only for the switching between epithelial and mesenchymal cells, but also other cell types.

[We are grateful to Guanjing Pan and Jieying Zhu for help and discussion. This work as funded in part by the grants from Ministry of Science and Technology 973 Program (2009CB941102, 2011CB965200, and 2011CBA01106), ‘Strategic Priority Research Program’ of the Chinese Academy of Sciences (Grant No. XDA01020401, XDA01020106, XDA01020202, XDA01020404).]
reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 8, 376–388.