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Article

A Mesenchymal-to-Epithelial Transition Initiates and Is Required for the Nuclear Reprogramming of Mouse Fibroblasts

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SUMMARY

Epithelial-to-mesenchymal transition (EMT) is a developmental process important for cell fate determination. Fibroblasts, a product of EMT, can be reset into induced pluripotent stem cells (iPSCs) via exogenous transcription factors but the underlying mechanism is unclear. Here we show that the generation of iPSCs from mouse fibroblasts requires a mesenchymal-to-epithelial transition (MET) orchestrated by suppressing pro-EMT signals from the culture medium and activating an epithelial program inside the cells. At the transcriptional level, Sox2/Oct4 suppress the EMT mediator Snail, c-Myc downregulates TGF-β1 and TGF-β receptor 2, and Klf4 induces epithelial genes including E-cadherin. Blocking MET impairs the reprogramming of fibroblasts whereas preventing EMT in epithelial cells cultured with serum can produce iPSCs without Klf4 and c-Myc. Our work not only establishes MET as a key cellular mechanism toward induced pluripotency, but also demonstrates iPSC generation as a cooperative process between the defined factors and the extracellular milieu.

INTRODUCTION

The direct reprogramming of somatic cells to an embryonic stem cell (ESC)-like state by defined factors is an approach commonly known as iPS (Takahashi and Yamanaka, 2006). This technology is devoid of ethical concerns and can produce pluripotent stem cells from an individual that are compatible with his/her own immune system. Compared to somatic cell nuclear transfer (SCNT) (Wilmut et al., 1997), iPS not only has solved a technical hurdle but also offers a unique experimental system to investigate key questions regarding cell fate determination and epigenetic regulation. iPSC generation is viewed as a multiple-step course of action mediated by transcription factors that progressively induce the expression of ESC-like genes and suppress the somatic cell genetic program (Brambrink et al., 2008). Because the appearance of iPSCs takes at least 12–24 days and is very inefficient, nuclear reprogramming is considered a stochastic process in which successive barriers must be overcome to reach a state toward pluripotency (Hanna et al., 2009b; Yamanaka, 2009), but the nature of these barriers is poorly understood. Remarkably, a series of compounds including TGF-β (transforming growth factor β) inhibitors have been identified that accelerate or improve the reprogramming (Esteban et al., 2010; Huangfu et al., 2008; Ichida et al., 2009; Maherali and Hochedlinger, 2009; Mikkelsen et al., 2008), and this may shed light into the putative reprogramming roadblocks.

One of the first noticeable changes during the reprogramming of fibroblasts is their transformation into tightly packed clusters of rounded cells in a process that resembles a MET (Qin et al., 2007; Takahashi and Yamanaka, 2006), the opposite of EMT. During development, EMT events occur as early as gastrulation and are frequent afterwards, for example in the delamination of the neural crest (Thiery et al., 2009). EMT consists of coordinated changes in cell-cell and cell-matrix interactions that lead to loss of epithelial features and acquisition of mesenchymal characteristics. A previous analysis has estimated that EMT changes the expression of about 4000 genes (~10% of the human genome) (Zavadil et al., 2001), with two genes reproducibly changed in all forms of EMT: E-cadherin (downregulated) and Snail (upregulated). Snail is a basic helix-loop-helix transcription factor that binds to specific cognate sequences termed E-boxes and represses the transcription of E-cadherin and other key epithelial regulators (Batlle et al., 2000; Cano et al., 2000; Nieto, 2002). E-cadherin is a transmembrane constituent of intercellular adherens junctions responsible for maintaining epithelial cohesion (Cavallaro and Christofori, 2004) and has also been linked to the control of ESC pluripotency (Chou et al., 2008; Soncin et al., 2009). Here we demonstrate that the exogenous reprogramming factors activate an epithelial program and shut down key mesenchymal genes to overcome the EMT epigenetic barrier of fibroblasts and allow their successful reprogramming into pluripotent stem cells.

RESULTS

The Reprogramming of Fibroblasts Starts with a MET

Morphological differences between elongated fibroblasts and mouse ESCs are profound (Figure 1A). During reprogramming,

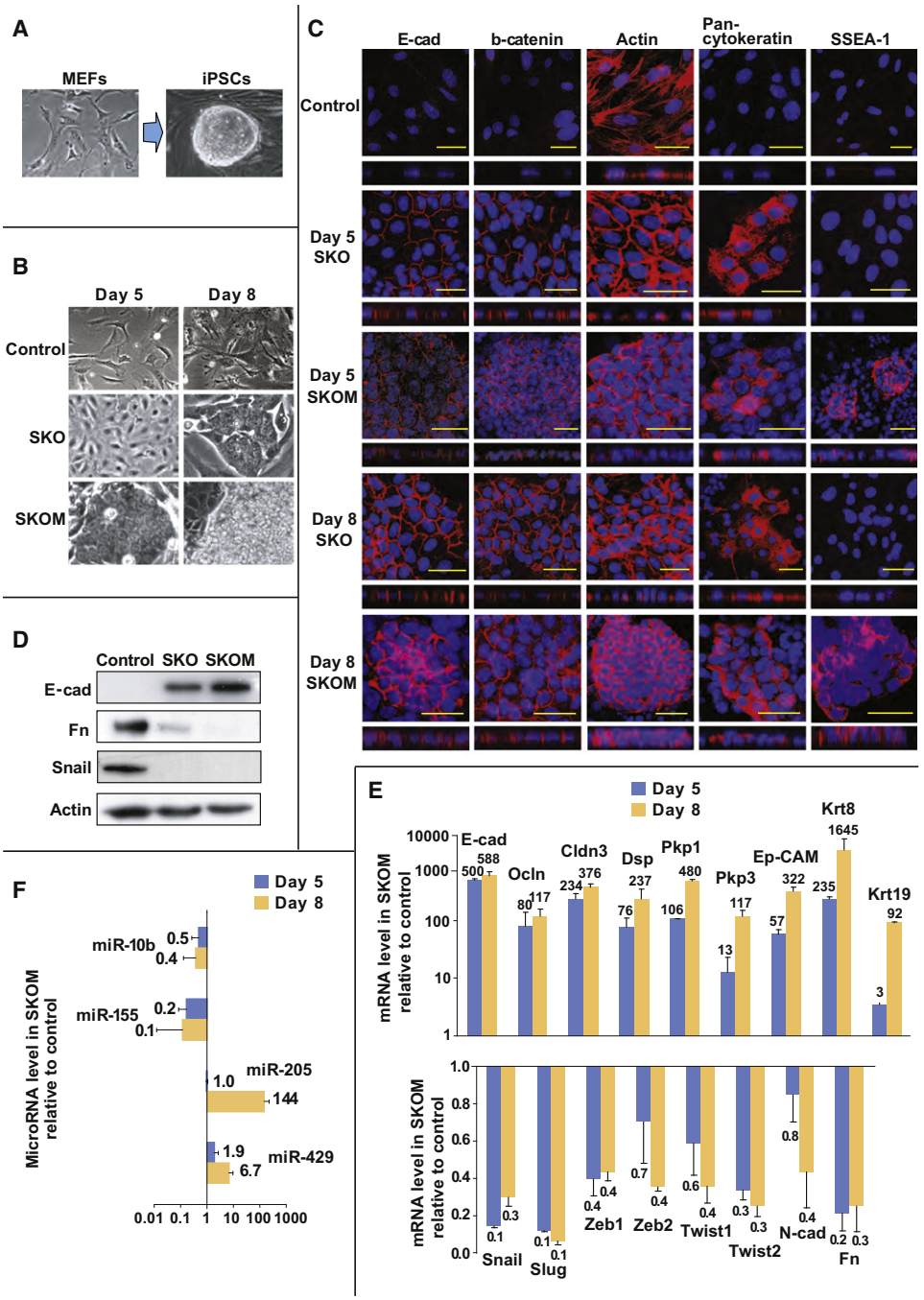

Figure 1. MET Is an Early Event during the Reprogramming of Mouse Fibroblasts

(A) Phase-contrast photographs of nontransduced MEFs and an iPSC line generated from the same MEFs.

(B) Phase-contrast photographs of MEFs transduced with empty vector, and SKOM or SKO combinations at days 5 and 8.

(C) Immunofluorescence microscopy for the indicated markers in a similar experiment as in (B); the nuclei are stained in blue with DAPI. Vertical computer reconstructions are shown below each item. A representative experiment (this applies hereafter unless otherwise indicated) is shown. E-cad stands for E-cadherin (also hereafter); scale bars indicate 50 μm (also hereafter).

(D) Western blotting for the indicated proteins of lysates corresponding to a similar experiment; actin is the loading control.

(E) qPCR for the indicated genes of three independent SKOM time-course experiments. Samples were measured in triplicate and the mean values + standard deviation (SD) are shown (this applies hereafter to any graph containing error bars).

(F) qPCR for the indicated microRNAs of a time course experiment with SKOM-transduced MEFs; values are referred to MEFs.

Related to Figure S1.