E-cadherin, catenin, cytoskeletal interactions and induced pluripotency

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July 2013

Abstract
The glycoprotein, E-cadherin, mediates tight intercellular contacts and promotes an epithelial cellular phenotype. An early requirement for induced pluripotency from somatic fibroblasts is a mesenchymal-to-epithelial transition which includes an upregulation of E-cadherin expression at the cell surface. This review summarizes the current knowledge on the induction and maintenance of pluripotent cells mediated by E-cadherin’s specific cellular signaling and cytoskeletal interactions.

Keywords: pluripotency, E-cadherin, human embryonic stem cells (hESC), induced pluripotent stem cells (iPSC), mesenchymal-to-epithelial transition (MET)

E-cadherin-mediated cell contacts are essential for induced pluripotency
Pluripotency is defined as the capability of cells to differentiate into cell types derived from each of the three embryonic germ layers (Kelly et al., 2011). Induced pluripotency defines a process of epigenetic reprogramming in which epigenetic changes implemented during differentiation are reversed to generate cells with a stem-like phenotype. Fibroblasts (Takahashi and Yamanaka, 2006; Sommer et al., 2009), human keratinocytes (Aasen et al., 2008) and nasal mucosal cells (Ono et al., 2012) can be reprogrammed to stem-like cells with introduction of four transcription factors OCT4, SOX2, KLF4 and c-MYC (OSKM) through the introduction of the four transcription factors OCT4, SOX2, KLF4 and c-MYC (OSKM) was demonstrated by Takahashi and Yamanaka (2006). Using the same four OSKM transcription factors the reprogramming of human skin fibroblasts into iPSC was performed successfully (Park et al., 2008; Takahashi et al., 2007).

Similar to ESC from other species, human ESC and human iPSC derived from somatic cells form tightly adherent cell colonies, an assembly that is essential for maintaining pluripotency (Park et al., 2008; Takahashi et al., 2007; Takahashi and Yamanaka, 2006; Thomson et al., 1998; Yu et al., 2007). Human ESC and iPSC display high levels of E-cadherin (Ohgushi and Sasai, 2011). This E-cadherin cell surface expression was shown to play a role for self-renewal and the maintenance of the undifferentiated state in mouse and human ESC (Li et al., 2010c; Redmer et al., 2011; Soncin et al., 2009; Soncin et al., 2011) and was down-regulated during differentiation (Redmer et al., 2011). Cadherins are calcium-dependent type I transmembrane cell adhesion proteins (Cavallaro and Christofori, 2004;
Takeichi, 1995; Yoshida and Takeichi, 1982). Strikingly, dissociation of human ESC or iPSC colonies during culture resulted in massive apoptosis (Ohgushi et al., 2010; Ohgushi and Sasai, 2011). Trypsin, a serine hydrolase most commonly used to disaggregate multicellular ESC colonies, was shown to cleave E-cadherin from the cell surface (Xu et al., 2010) suggesting that the intercellular adhesion mediated by E-cadherin provides essential stimuli for the survival of human ESCs and iPSCs. Similarly, blocking or siRNA-mediated down-regulation of E-cadherin impairs ESC survival (Li et al., 2010c; Xu et al., 2010).

**Upregulation of cell adhesion molecules promotes Mesenchymal-to-epithelial Transition (MET)**

Cell adhesion constitutes an essential component for pluripotency. When pluripotency is induced in MEFs a transition from a mesenchymal to an epithelial phenotype was observed. Mesenchymal-to-epithelial transition (MET) is considered the hallmark for the initial phase during reprogramming of somatic fibroblasts (Li et al., 2010c; Qin et al., 2007) and includes the up-regulation of epithelial cell surface proteins such as E-cadherin, several claudins and epithelial cell adhesion molecule (EpCam) (Huang et al., 2011; Li et al., 2010c; Samavarchi-Tehrani et al., 2010). Following induction of reprogramming in MEFs, E-cadherin expression was detected in SSEA1-positive and NANOG-positive cells (Redmer et al., 2011). Expression of E-cadherin and cytokeratin even preceded the presence of the pluripotency marker SSEA1 (Li et al., 2010c). Cre-mediated deletion of floxed E-cadherin in MEFs prevented reprogramming emphasizing the importance of tight-junctional cell contacts during the initial phase of reprogramming (Redmer et al., 2011).

Krüppel-like factor 4 (Klf4), one of the four reprogramming transcription factors, upregulated E-cadherin by binding to its promoter, and omission of Klf4 prevented induced pluripotency (Li et al., 2010c). Klf4 was shown to regulate the expression of V-E-cadherin in tight junctions of endothelial cells (Cowan et al., 2010) and to bind to the E-cadherin promoter in mammary epithelial cells thus promoting an epithelial phenotype through formation of E-cadherin-mediated cell contacts (Yori et al., 2010). Consistent with the epithelial transition being an important requirement for reprogramming, Klf4 was dispensable for reprogramming of epithelial cells suggesting the pre-existing expression of epithelial cell E-cadherin-based tight junctions to bypass the need for KLF4 during reprogramming. Indeed, higher reprogramming efficiency was observed using human epithelial cells such as keratinocytes (Aasen et al., 2008) and mammary gland epithelial cells (Li et al., 2010c).

MEFs with E-cadherin knock-down failed to undergo reprogramming (Redmer et al., 2011) further emphasizing the essential role for cell-cell contacts in induced pluripotency. Surprisingly, exogenous expression of E-cadherin was able to replace OCT4 as one of the reprogramming factors in the induction of pluripotent cells (Redmer et al., 2011). It is currently not known whether E-cadherin signalling induces expression of endogenous OCT4 and which E-cadherin downstream factors mediate the induction/support of pluripotency transcription factors. Intracellular mediators of E-cadherin functions are β-catenin and p120-catenin which connect to the cytoplasmic tail of E-cadherin (Cowin and Burke, 1996; Davis et al., 2003). P120-catenin stabilized the E-cadherin-catenin complex by inhibiting its endocytosis (McCrea and Park, 2007). The actin-binding protein α-catenin functions in linking the E-cadherin to the actin cytoskeleton, thus promoting an epithelial phenotype (Drees et al., 2005; Ozono et al., 2011; Yamada et al., 2005).

**Regulation of E-cadherin in stem cells**

In the presence of Wnt-signaling, β-catenin accumulation was shown to induce endogenous OCT4 and to enhance OCT4-mediated transcriptional activity in a TCF-independent manner (Kelly et al., 2011). T-cell factor proteins (TCFs) are nuclear execution factors for the Wnt/β-catenin signaling pathway recruiting co-activators to Wnt response elements of respective target genes (Arce et al., 2006). However rescue experiments with mutant E-cadherin lacking the cytoplasmic β-catenin binding domain demonstrated that E-cadherin induced reprogramming was independent of its β-catenin-binding domain (Chen et al., 2010). Thus, the importance for E-cadherin in the reprogramming process is not given by its signalling through the canonical β-catenin pathway but the tight-junctional cell contacts.
Furthermore, E-cadherin-negative mouse ESC were capable of Wnt-induced β-catenin/TCF signalling (Soncin et al., 2011) indicating the structural requirements for E-cadherin to be independent of its intracellular mediator β-catenin. The role for the canonical Wnt-β-catenin signalling in ESC renewal and pluripotency was recently reviewed (Miki et al., 2011; Sanges and Cosma, 2011; Watanabe and Dai, 2011).

The activity of myosin II in epithelial cells determines the dynamics of the actin filaments, thus influencing cell shape, motility and cell polarity (Vicente-Manzanares et al., 2009). Myosin II controls E-cadherin mediated cell-cell contacts in hESC (Li et al., 2010a) via p120-catenin which binds to the transmembrane domain of E-cadherin (Thoreson et al., 2000). The non-muscle myosin II and p120 catenin pathways are upstream of E-cadherin in the reprogramming of human primary fibroblasts and keratinocytes (Li et al., 2010a).

Another protein regulating E-cadherin function is the small G-protein Rap1. Loss of function mutation for Rap1 showed a similar phenotype than those for E-cadherin with early embryonic lethality (Kan et al., 2007; Larue et al., 1994; Ohba et al., 2001). Rap1 was shown to regulate the rapid turnover of membrane-bound E-cadherin by endocytosis and by promoting E-cadherin re-assembly into adherens junctions (Li et al., 2010b). Consequently, inhibition or knock-down of Rap1 in hESC caused a decrease in E-cadherin expression and clonogenic capacity and increased apoptosis (Li et al., 2010b). At the same time, Rap1 was rapidly degraded in lysosomes following disruption of cell adhesion contacts in hESCs causing reduced recycling of E-cadherin to the cell membrane (Li et al., 2010b), a mechanisms which may contribute to decreased survival of hESC following dissociation in culture. Thus, the mutual positive regulation of the E-cadherin-Rap1 interaction contributes to maintaining pluripotency in hESC.

The broad clinical potential in the application for iPSC launched the search for conditions to improve the reprogramming efficiency of somatic cells, in part by promoting cell adhesion. The miRNA cluster 302-367 was shown to accelerate reprogramming of MEFs with just three factors SOX2, Klf4, and OCT4 by increasing E-cadherin and down-regulating TGFβ receptor 2, thus promoting MET (Liao et al., 2011). The small molecule Thiazovivin (2,4-disubstituted thiazole) was able to enhance hESC survival by stabilizing surface E-cadherin protein through inhibition of its endocytosis (Xu et al., 2010). Rho-associated kinase (ROCK) is one of the effectors of Rho which signals from the extracellular matrix to regulate actin cytoskeleton dynamics and cell contractility (Hammar et al., 2009). Thiazovovin prevented ROCK-Rho-mediated cytoskeletal remodelling and cell motility and favoured cell adhesion to extracellular matrices (Xu et al., 2010). The efficiency of induced pluripotency from MEFs was also enhanced by the two chemicals Apigenin and Luteolin, both of which up-regulated E-cadherin expression during the early phase of reprogramming (Chen et al., 2010).

Outlook

In summary, the structural functions of intercellular adhesions are predominantly involved in MET and are crucial requirements for reprogramming and pluripotency. Knowing the molecular mechanism that regulate the reprogramming processes will help with developing new and safer procedures to obtain iPSCs for clinical use (Sanges and Cosma, 2010). Induced pluripotent stem cells (iPSCs) present promising clinical tools in the near future for modeling disease, drug development, and to deliver cell-replacement therapy to support regenerative medicine (Goldthwaite, 2011).

Acknowledgments

I am very grateful to Dr. Sabine Hombach-Klonisch and Dr. Marek Los for engaging me in such an interesting field of research. A special thank you to Dr. Hombach-Klonisch for her guidance and support, as well as her critical comments and insight during the writing of this article.

Abbreviations:

MEF: murine embryonic fibroblasts
MET: mesenchymal-to epithelial transition
hESC: human embryonic stem cells
mESC: mouse embryonic stem cells
iPSC: induced pluripotent stem cells
miRNA: microRNA
Klf4: Krüppel-like factor 4
Oct-4: Octamer-binding transcription factor 4
TCF1: T-cell factor-1
Rap-1: Ras-related protein
TGFB: transforming growth factor-beta
ROCK: Rho-associated kinase

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