E-cadherin, catenin, cytoskeletal interactions and induced pluripotency

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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 stem cell transcription factors Oct4, KLF4, Sox2 and c-Myc (OKSM). Previous studies have provided evidence that the adhesion and cellular signaling provided by a glycoprotein, known as E-cadherin, has essential functions in pluripotency. Induced pluripotency has potential medical application where the induced pluripotent stem cells (iPSCs) apply as clinical tools for modeling diseases, drug development, and to deliver cell-replacement therapy to support regenerative medicine (Goldthwaite, 2011).

The present review summarizes our current knowledge on the role of E-cadherin, its cellular signaling and cytoskeletal interactions in the induction and maintenance of pluripotent cells.

Induced pluripotency describes the result of reprogramming somatic cells into pluripotent stem cells (iPSC) which are capable of self-renewal and of developing into all three germ layers (Zhao and Daley, 2008), similar to embryonic stem cells (ESC). Reprogramming of murine somatic cells, mouse embryonic fibroblasts (MEFs), 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 somatic 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- 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 VE-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).

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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

TGFβ: transforming growth factor-beta

ROCK: Rho-associated kinase

References

1. Aasen, T., Raya, A., Barrero, M. J., Garreta, E., Consiglio, A., Gonzalez, F., Vassena, R., Bilic, J., Pekarik, V., Tiscornia, G., *et al.* (2008). Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nature Biotechnology 26*(*11)*, 1276-1284.
2. Arce, L., Yokoyama, N.N., and Waterman, M.L. (2006). Diversity of LEF/TCF action in development and disease. *Oncogene 25(57)*, 7492–7504
3. Cavallaro, U., and Christofori, G. (2004). Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nature Review Cancer 4*, 118-132.
4. Chen, T., Yuan, D., Wei, B., Jiang, J., Kang, J., Ling, K., Gu, Y., Li, J., Xiao, L., and Pei, G. (2010). E-cadherin-mediated cell-cell contact is critical for induced pluripotent stem cell generation. *Stem Cells 28(8)*, 1315-1325.
5. Cowan, C. E., Kohler, E. E., Dugan, T. A., Mirza, M. K., Malik, A. B., and Wary, K. K. (2010). Kruppel-like factor-4 transcriptionally regulates VE-cadherin expression and endothelial barrier function. *Circulation Research 107(8)*, 959-966.
6. Cowin, P., and Burke, B. (1996). Cytoskeleton-membrane interactions. *Current Opinion in Cell Biology 8(1)*, 56-65.
7. Davis, M. A., Ireton, R. C., and Reynolds, A. B. (2003). A core function for p120-catenin in cadherin turnover. *Journal of Cell Biology 163(3)*, 525-534.
8. Drees, F., Pokutta, S., Yamada, S., Nelson, W. J., and Weis, W. I. (2005). Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell 123(5)*, 903-915.
9. Goldthwaite C. (2011). The Promise of Induced Pluripotent Stem Cells (iPSCs). In *Stem Cell Information*. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2011 *<http://stemcells.nih.gov/info/Regenerative_Medicine/pages/2006chapter10.aspx>*
10. Hammar, E., Tomas, A., Bosco, D., Halban, P. (2009). Role of the Rho-ROCK (Rho-associated kinase) signaling pathway in the regulation of pancreatic beta-cell function. *Endocrinology 150(5*), 2072-9.
11. Huang, H. P., Chen, P. H., Yu, C. Y., Chuang, C. Y., Stone, L., Hsiao, W. C., Li, C. L., Tsai, S. C., Chen, K. Y., Chen, H. F., *et al.* (2011). Epithelial cell adhesion molecule (EpCAM) complex proteins promote transcription factor-mediated pluripotency reprogramming. *Journal of Biological Chemistry 286(38)*, 33520-33532.
12. Kan, N. G., Stemmler, M. P., Junghans, D., Kanzler, B., de Vries, W. N., Dominis, M., and Kemler, R. (2007). Gene replacement reveals a specific role for E-cadherin in the formation of a functional trophectoderm. *Development 134(1)*, 31-41.
13. Kelly, K. F., Ng, D. Y., Jayakumaran, G., Wood, G. A., Koide, H., and Doble, B. W. (2011). beta-catenin enhances Oct-4 activity and reinforces pluripotency through a TCF-independent mechanism. *Cell Stem Cell 8(2)*, 214-227.
14. Larue, L., Ohsugi, M., Hirchenhain, J., and Kemler, R. (1994). E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proceedings of National Academy of Sciences of the United States of America 91(17)*, 8263-8267.
15. Li, D., Zhou, J., Wang, L., Shin, M. E., Su, P., Lei, X., Kuang, H., Guo, W., Yang, H., Cheng, L., *et al.* (2010a). Integrated biochemical and mechanical signals regulate multifaceted human embryonic stem cell functions. *Journal of Cell Biology 191(3)*, 631-644.
16. Li, L., Wang, S., Jezierski, A., Moalim-Nour, L., Mohib, K., Parks, R. J., Retta, S. F., and Wang, L. (2010b). A unique interplay between Rap1 and E-cadherin in the endocytic pathway regulates self-renewal of human embryonic stem cells. *Stem Cells 28(2)*, 247-257.
17. Li, R., Liang, J., Ni, S., Zhou, T., Qing, X., Li, H., He, W., Chen, J., Li, F., Zhuang, Q., *et al.* (2010c). A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. *Cell Stem Cell 7(1)*, 51-63.
18. Liao, B., Bao, X., Liu, L., Feng, S., Zovoilis, A., Liu, W., Xue, Y., Cai, J., Guo, X., Qin, B., *et al.* (2011). MicroRNA cluster 302-367 enhances somatic cell reprogramming by accelerating a mesenchymal-to-epithelial transition. *Journal of Biological Chemistry 286(19)*, 17359-17364.
19. McCrea, P. D., and Park, J. I. (2007). Developmental functions of the P120-catenin sub-family. *Biochimica et Biophysica Acta 1773(1)*, 17-33.
20. Miki, T., Yasuda, S. Y., and Kahn, M. (2011). Wnt/beta-catenin Signaling in Embryonic Stem Cell Self-renewal and Somatic Cell Reprogramming. *Stem Cell Reviews 7(4)*, 836-846.
21. Ohba, Y., Ikuta, K., Ogura, A., Matsuda, J., Mochizuki, N., Nagashima, K., Kurokawa, K., Mayer, B. J., Maki, K., Miyazaki, J., and Matsuda, M. (2001). Requirement for C3G-dependent Rap1 activation for cell adhesion and embryogenesis. *EMBO Journal 20(13)*, 3333-3341.
22. Ohgushi, M., Matsumura, M., Eiraku, M., Murakami, K., Aramaki, T., Nishiyama, A., Muguruma, K., Nakano, T., Suga, H., Ueno, M., *et al.* (2010). Molecular pathway

and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells. *Cell Stem Cell 7(2)*, 225-239.

23. Ohgushi, M., and Sasai, Y. (2011). Lonely death dance of human pluripotent stem cells: ROCKing between metastable cell states. *Trends in Cell Biology 21*, 274-282.
24. Ono M, Hamada Y, Horiuchi Y, Matsuo-Takasaki M, Imoto Y, Satomi K, et al. (2012). Generation of induced pluripotent stem cells from human nasal epithelial cells using a sendai virus vector. *Public Library of Science 7(8)*:e42855.
25. Ozono, K., Komiya, S., Shimamura, K., Ito, T., and Nagafuchi, A. (2011). Defining the roles of alpha-catenin in cell adhesion and cytoskeleton organization: isolation of F9 cells completely lacking cadherin-catenin complex. *Cell Structure and Function 36(1)*, 131-143.
26. Park, I. H., Zhao, R., West, J. A., Yabuuchi, A., Huo, H., Ince, T. A., Lerou, P. H., Lensch, M. W., and Daley, G. Q. (2008). Reprogramming of human somatic cells to pluripotency with defined factors. *Nature 451(7175)*, 141-146.
27. Qin, D., Li, W., Zhang, J., and Pei, D. (2007). Direct generation of ES-like cells from unmodified mouse embryonic fibroblasts by Oct4/Sox2/Myc/Klf4. *Cell Research 17(11)*, 959-962.
28. Redmer, T., Diecke, S., Grigoryan, T., Quiroga-Negreira, A., Birchmeier, W., and Besser, D. (2011). E-cadherin is crucial for embryonic stem cell pluripotency and can replace OCT4 during somatic cell reprogramming. *EMBO Reports 12(7)*, 720-726.
29. Samavarchi-Tehrani, P., Golipour, A., David, L., Sung, H. K., Beyer, T. A., Datti, A., Woltjen, K., Nagy, A., and Wrana, J. L. (2010). Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell 7(1)*, 64-77.
30. Sanges, D., and Cosma, M. P. (2011). Reprogramming cell fate to pluripotency: the decision-making signalling pathways. *International Journal of Developmental Biology 54(11)*, 1575-1587.
31. Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, Mostoslavsky G. (2008). Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells 27(3)*:543-9.
32. Soncin, F., Mohamet, L., Eckardt, D., Ritson, S., Eastham, A. M., Bobola, N., Russell, A., Davies, S., Kemler, R., Merry, C. L., and Ward, C. M. (2009). Abrogation of E-cadherin-mediated cell-cell contact in mouse embryonic stem cells results in reversible LIF-independent self-renewal. *Stem Cells 27(9)*, 2069-2080.
33. Soncin, F., Mohamet, L., Ritson, S., Hawkins, K., Bobola, N., Zeef, L., Merry, C. L., and Ward, C. M. (2011). E-cadherin acts as a regulator of transcripts associated with a wide range of cellular processes in mouse embryonic stem cells. *Public Libratry of Science One 6(7)*, e21463.
34. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell 131(5)*, 861-872.
35. Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell 126(4)*, 663-676.
36. Takeichi, M. (1995). Morphogenetic roles of classic cadherins. *Current Opinion in Cell Biology 7(5)*, 619-627.
37. Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., and Jones, J. M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science 282(5391)*, 1145-1147.
38. Thoreson, M. A., Anastasiadis, P. Z., Daniel, J. M., Ireton, R. C., Wheelock, M. J., Johnson, K. R., Hummingbird, D. K., and Reynolds, A. B. (2000). Selective uncoupling of p120(ctn) from E-cadherin disrupts strong adhesion. *Journal of Cell Biology 148(1)*, 189-202.
39. Vicente-Manzanares, M., Ma, X., Adelstein, R. S., and Horwitz, A. R. (2009). Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nature Reviews Molecular Cell Biology 10(11)*, 778-790.
40. Watanabe, K., and Dai, X. (2011). A WNTer revisit: new faces of beta-catenin and TCFs in pluripotency. *Science Signaling 4(193)*, pe41.
41. Xu, Y., Zhu, X., Hahm, H. S., Wei, W., Hao, E., Hayek, A., and Ding, S. (2010). Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. *Proceedings of National Academy of Sciences of the United States of America 107(18)*, 8129-8134.
42. Yamada, S., Pokutta, S., Drees, F., Weis, W. I., and Nelson, W. J. (2005). Deconstructing the cadherin-catenin-actin complex. *Cell 123(5)*, 889-901.
43. Yori, J. L., Johnson, E., Zhou, G., Jain, M. K., and Keri, R. A. (2010). Kruppel-like factor 4 inhibits epithelial-to-mesenchymal transition through regulation of E-cadherin gene expression. *Journal of Biological Chemistry 285(22)*, 16854-16863.
44. Yoshida, C., and Takeichi, M. (1982). Teratocarcinoma cell adhesion: identification of a cell-surface protein involved in calcium-dependent cell aggregation. *Cell 28(2)*, 217-224.
45. Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R., *et al.* (2007). Induced

pluripotent stem cell lines derived from human somatic cells. *Science 318(5858)*, 1917-1920.

46. Zhao, R., and Daley, G. Q. (2008). From fibroblasts to iPS cells: induced pluripotency by defined factors. *Journal of Cellular Biochemistry 105(4)*, 949-955.