Commentary

Stem Cell INVESTIGATION

E-cadherin adhesion-mediated Wnt activation for mesoderm specification in human embryonic stem cells needs a soft mattress

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Received: 15 October 2016; Accepted: 28 October 2016; Published: 14 November 2016.
doi: 10.21037/sci.2016.10.12
View this article at: http://dx.doi.org/10.21037/sci.2016.10.12

While the roles of transcription factors and morphogens in stem cell differentiation have been well studied, little is known about how extracellular forces are translated into intracellular signals to direct tissue specific differentiation and development.

A report published in *Cell Stem Cell* by Przybyla *et al.* reveal that E-cadherin-mediated cell-cell adhesion and integrin β1-medicated cell-extracellular matrix (ECM) interaction regulate Wnt/β-catenin signaling and mesoderm specification in human embryonic stem cells (hESCs) (1). By accumulating β-catenin at cell-cell adhesion, hESCs on soft gel show enhanced Wnt-dependent mesoderm differentiation. Mechanistically, E-cadherin, β-catenin, and P120-catenin at cell-cell junctions are stabilized when hESCs are cultured on soft hydrogel (400 kPa). Knockdown of E-cadherin, β-catenin or P120-catenin significantly impairs cell-cell adhesion and also diminishes mesoderm specification (1).

In contrast, hESCs on stiff hydrogel express high levels of integrin β1, which activates SFK (Src-family kinases) to promote cell-ECM adhesion. Junctional stabilization of E-cadherin, P120-cadherin and β-cadherin inhibits integrin β1 activity in hESCs on soft hydrogel. Conversely, integrin β1 activation destabilizes the cell-cell adhesion, inhibits β-catenin and E-cadherin expression and ultimately compromises mesoderm specification (1).

E-cadherin is a transmembrane glycoprotein that mediates calcium-dependent, homophilic cell-cell adhesion in all epithelial tissues including hESCs. Cytoplasmic tails of E-cadherin bind to its cytoplasmic partners P120-catenin and β-catenin proteins. β-catenin plays an important role in structural organization and E-cadherin function by linking E-cadherin to the actin cytoskeleton through α-catenin (2,3). β-catenin is also a pivotal effector of the canonical Wnt signaling pathway via association with the transcription factor—T cell factor (TCF) in the nucleus (3). It is well known that Wnt/β-catenin signaling is one of potent drivers for mesoderm differentiation. The stabilization and accumulation of β-catenin in the cytoplasm is crucial for Wnt signaling.

One of interesting observations made by Przybyla *et al.* (1) is the stabilization and rapid release of β-catenin in cell-cell adhesion in hESCs on soft but not stiff substrates. Upon morphogen stimulation, abundant β-catenin in cell-cell adhesion is quickly released to cytoplasm and subsequently enters nucleus to initiate the mesoderm gene transcription essential for epithelial-to-mesenchymal transition (EMT) and tissue-specific differentiation (1).

It appears that Cbl-Like 1 (CBLL1) and SFKs are involved in the above regulation. CBLL1 is an E3 ubiquitin ligase capable of promoting the internalization of E-cadherin. CBLL1 is activated by SFKs, and SFKs is stimulated by integrin β1-medicated cell-ECM adhesion. The majority of the SFKs in hESCs on the soft gels are inactivated and localized at cell-cell junctions due to the formation of cell-cell adhesion. Morphogen stimulation of hESCs on soft gels leads to activation of SFKs in cell-cell junctions within 2 h, which induces a rapid release

of abundant β-catenin from cell-cell adhesion through CBLL1-mediated E-cadherin endocytosis. Concurrently, P120-catenin is also released from cell-cell adhesion into cytoplasm, which further enhances transcriptional activity of β-catenin by antagonizing Kaiso-mediated transcriptional suppression of β-catenin.

On a stiff substrate, however, CBLL1 activity is elevated, which promotes E-cadherin internalization to destabilize cell-cell adhesion and decreases β-catenin pool in cell membrane. The slowly released β-catenin in the cytoplasm is phosphorylated by β-catenin destruction complex and marked for proteasomal degradation. This makes β-catenin insufficient or unavailable for mesoderm induction upon morphogen stimulation.

In a recent report, E-cadherin has been shown to coordinate with Wnt signaling in hESC self-renewal and mesodermal differentiation (4). Self-renewal *vs.* mesoderm differentiation of hESCs in response to Wnt signaling seems to be determined by a two-layer regulatory circuit involving E-cadherin, β-catenin, PI3K/AKT and Slug in a time-dependent manner (4). Short-term upregulation of β-catenin promotes E-cadherin expression, thereby enhancing hESC self-renewal through E-cadherin-associated PI3K/AKT signaling. Conversely, long-term Wnt activation or loss of E-cadherin intracellular β-catenin binding domain promotes mesoderm differentiation of hESCs through β-catenin-induced up-regulation of *Slug*. The enhanced Slug expression leads to further reduction of E-cadherin that serves as β-catenin "sink" sequestering free cytoplasmic β-catenin (4). Beyond the above findings, Przybyla *et al.* provide new insights into the communications between ECM stiffness, cell membrane E-cadherin, intracellular Wnt/β-catenin signaling, SFKs and CBLL1 activities in mesoderm specification.

One interesting question yet to be addressed is whether the crosstalk revealed by Przybyla *et al.* affects hESCs to express different levels of Oct4, Nanog and Sox2. While importance of Oct4, Nanog and Sox2 in hESC self-renewal has been clearly demonstrated, several studies also reveal that each factor differentially affects cell fate determination (5,6). High levels of Oct4 enable self-renewal in the absence of BMP4 but specify mesendoderm (i.e., precursor for both mesoderm and endoderm) in the presence of BMP4. Low levels of Oct4 induce embryonic ectoderm differentiation in the absence of BMP4 but specify extraembryonic lineages in the presence of BMP4. Nanog represses embryonic ectoderm differentiation whereas Sox2 repress mesendoderm differentiation (5). It seems that E-cadherin enhances Oct4 and Nanog expression in hESCs (4). It is also possible that E-cadherin mediated cell-cell adhesion in hESCs on soft gels upregulates Oct4 and Nanog to contribute to subsequent mesoderm specification.

ECM stiffness, E-cadherin mediated cell-cell adhesion and cell spreading also significantly regulate YAP/TAZ signaling. YAP/TAZ (key transducers along Hippo pathway) has been shown to incorporate into β-catenin destruction complex to orchestrate Wnt response (7) and to drive cell cycle entry in an E-cadherin and β-catenin-dependent manner (8). It is unknown whether YAP/TAZ signaling contributes to ECM stiffness-induced E-cadherin adhesion and Wnt activation, warranting further studies.

Another interesting question associated with this report is whether and to what extent Wnt signaling in cancer cells is influenced by ECM stiffness. Wnt signaling has been implicated in cancer development and malignance, its activation has been shown either to stimulate or inhibit cancer growth depending on yet to be determined molecular mechanisms (9,10). Investigation of communications between ECM stiffness, E-cadherin and Wnt signaling in hESCs may shed light on the study of cancer development as the tumorigenesis and normal embryogenesis may share many of the same basic processes and molecular pathways. This may lead to the development of a new approach for more effective treatment of cancers.

Acknowledgements

Funding: This work is supported by operating grants from the CIHR MOP-111224, HSFO NA7186 and Canadian Breast Cancer Foundation-Ontario Region to L Wang.

Footnote

Provenance: This is an invited Commentary commissioned by Editor-in-Chief Zhizhuang Joe Zhao (Pathology Graduate Program, University of Oklahoma Health Sciences Center, Oklahoma City, USA).
Conflicts of Interest: The authors have no conflicts of interest to declare.

Comment on: Przybyla L, Lakins JN, Weaver VM. Tissue Mechanics Orchestrate Wnt-Dependent Human Embryonic Stem Cell Differentiation. Cell Stem Cell 2016;19:462-75.

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doi: 10.21037/sci.2016.10.12
Cite this article as: Sulaiman A, Li L, Wang L. E-cadherin adhesion-mediated Wnt activation for mesoderm specification in human embryonic stem cells needs a soft mattress. Stem Cell Investig 2016;3:77.