Role of E-cadherin and other cell adhesion molecules in survival and differentiation of human pluripotent stem cells

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Introduction

Our understanding of the molecular and cellular mechanisms that control self-renewal and pluripotency of human pluripotent stem cells and our progress toward harnessing the regenerative potential of these cells to treat human diseases are advancing at a rapid rate. Human pluripotent stem cells include human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). The first hESCs were generated in 1998 from the inner cell mass of blastocyst-stage embryos.1 Their unique capacity for indefinite self-renewal (unlimited proliferation) in vitro coupled with their ability to differentiate into almost any cell type present in the adult body (pluripotency) provide a potentially unlimited source of cells for cell replacement therapies. The demonstration, in 2007, that hiPSCs could be generated from human fibroblasts has further expanded this therapeutic potential.2,3 Like hESCs, hiPSCs are capable of differentiating into almost any tissue type in the body.2,4 Production of hiPSCs, therefore, have been hailed as a transformative breakthrough in regenerative medicine with potential to lead to disease-based modeling and patient-tailored therapies.5 Clearly, understanding the mechanisms that control self-renewal, pluripotency and differentiation of hESCs and hiPSCs is key to realizing this potential and validating new therapies for many human degenerative diseases that currently have no cure.

Our understanding of the regulatory networks underlying the initiation and maintenance of hESC and hiPSC self-renewal and pluripotent states has expanded considerably during the past 14 years. Key transcription factors, including Oct4, Sox2 and Nanog, have been found to act in autoregulatory modules to specify the pluripotent state of both mouse and human pluripotent stem cells.6 Other regulatory cues, such as supporting cells,7,8 extracellular matrix,9,10 low oxygen culture,11,12 growth factors,13,14 small molecules15–20 and various signaling pathways21–26 have also been extensively studied and their relevance in self-renewal and differentiation of human pluripotent stem cells is now well recognized. However, the long-standing question as to why hESCs are so sensitive to the disruption of cell-cell contact, undergoing massive cell death when dissociated into single cell suspensions has only begun to be understood in recent years, which is key to propagating sufficient numbers of hESCs and hiPSCs for regenerative therapy.

A growing number of reports now reveal that, in addition to the support cells, extracellular matrix and growth factor cues, cell adhesion molecule (CAM)-mediated cohesive interaction among hESCs (and hiPSCs) and between the cells and their neighboring instructive/support cells and extracellular matrix contribute significantly to the self-renewal and the pluripotent state of human pluripotent stem cells. CAMs are the proteins on the surface of...
mammalian cells that contribute to juxtacrine cell-cell binding or cell-matrix binding. Two decades of structural biology studies have characterized domain topologies and binding mechanisms at the molecular/atomic level for key members of all the main classes of CAMs: the cadherins, integrins, selectins and immunoglobulin superfamily (IgSF). Cell biology approaches have shown that CAMs perform a wide range of functions at cell-cell and cell-matrix interfaces, such as mechanical support, target recognition, cell differentiation, initiation and regulation of signaling platforms.10,36 Today, numerous CAM family members have recently been identified on the surface of human pluripotent stem cells and found to regulate their self-renewal and pluripotency.12,31-35

This review will summarize recent progress in the study of CAM function in human pluripotent stem cells. We will focus on the roles of E-cadherin and several other molecules from following CAM superfamilies: the cadherins (N-cadherin and VE-cadherin), the integrins and the IgSF CAMs. In addition, we will also briefly discuss the significant role of heparin sulfate proteoglycans (HSPGs), a group of cell-matrix adhesion molecules critical in mediating the interaction between human pluripotent stem cells and extracellular matrix, in the regulation of hESCs and hiPSC fate.

Cell-Cell Adhesion Molecules in Human Pluripotent Stem Cells: E-, N- and VE-Cadherins

The cadherin superfamily is comprised of over 100 members in vertebrates and can be further divided into classical and non-classical subfamilies. Cadherins were originally identified as cell surface glycoproteins responsible for Ca2+-dependent homophilic cell-cell adhesion during morula compaction in the preimplantation mouse embryo and during chick development in the early 1980s.36-39 In the three decades since their discovery, it has become clear that the role of cadherins is not limited to calcium-dependent, homophilic cell-cell adhesion in all epithelial tissues including embryonic stem cells.40 As shown in Figure 1, E-cadherin has long extracellular and cytoplasmic domains. The extracellular domain of E-cadherin establishes homophilic interactions between neighboring cells, while its cytoplasmic tail associates with an array of multifunctional adapter proteins. These intracellular adapter proteins link cell-cell adhesion to the actin-myosin network and other intracellular signaling pathways.

The cytoplasmic part of E-cadherins contains binding sites for two catenins, p120-catenin and β-catenin. While p120-catenin is involved in the delivery and stabilization of adhesion complexes at the plasma membrane,41 β-catenin provides the connection to the actin-myosin complex. The β-catenin complex in turn interacts with forms to nucleate actin filaments at the adherens junctions and uses an unknown mechanism to link cadherin-catenin complexes at the membrane with the actin cytoskeleton.42 Strengthening of E-Cadherin-mediated cell-cell adhesion is dependent on intact actin cytoskeleton, which includes motor protein non-muscle myosin (NM) II. The NM II motor protein is a hexamer of three subunits (two heavy chains with a globular N-terminal motor domain for each, two regulatory light chains and two essential light chains) that converts the energy from ATP hydrolysis into mechanical work and is post-translationally regulated by the phosphorylation of its light and heavy chains.43 In addition to linking cadherin and β-catenin with the actin-myosin cytoskeleton, β-catenin is also a central part of the canonical Wnt signaling pathway.44 The molecular structure and the binding sites of E-cadherins, and its connection with the actin-myosin network, are illustrated in Figure 1A.

Among the classic cadherin subfamily, the roles of E-cadherin, N-cadherin and VE-cadherin have been mostly studied in human pluripotent stem cells. These three classical cadherins were originally named for the tissue in which they were prominently expressed: epithelial cadherin (E-cadherin) in skin epithelia, neural cadherin (N-cadherin) in the central nervous system and vascular endothelial cadherin (VE-cadherin) in blood vessel endothelia. Our current understanding of how these three classical cadherins modulate hESC phenotype is described below.

The Molecular Structure and the Binding Sites of E-Cadherin

E-cadherin is a transmembrane glycoprotein that mediates calcium-dependent, homophilic cell-cell adhesion in all epithelial tissues including embryonic stem cells.40 As shown in Figure 1, E-cadherin has long extracellular and cytoplasmic domains. The extracellular domain of E-cadherin establishes homophilic interactions between neighboring cells, while its cytoplasmic tail associates with an array of multifunctional adapter proteins. These intracellular adapter proteins link cell-cell adhesion to the actin-myosin network and other intracellular signaling pathways.

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The role of E-cadherin in hESCs. As one of the archetypal cadherin families, E-cadherin is essential for epithelialization of the early mouse embryo, cell rearrangement, tissue morphogenesis, establishment of cell polarity and maintenance of tissue architecture. Given the inherent origin of undifferentiated hESCs (derived from the inner cell mass of the blastocyst wherein E-cadherin was found on human oocytes and early embryos), one may speculate that E-cadherin expression reflects the tissue of origin from which the hESCs are initially derived and, as such, that E-cadherin mediated cell-cell cohesion plays an important role in the survival and self-renewal of hESCs. Indeed, E-cadherin has been used as an undifferentiated marker to identify hESCs. E-cadherin is co-expressed with other typical undifferentiated markers, such as SSEA4, Tra-1-60, Tra-1-81, alkaline phosphatase, as well as pluripotency factors Oct4 (also known as Pou5f1), Nanog and Sox2 in undifferentiated hESCs. The use of E-cadherin to demarcate differentiated and undifferentiated hESCs is based on definitive expression kinetics. Expression in undifferentiated hESCs is decreased immediately after induction of differentiation. The positive expression of E-cadherin in
characteristic colonies is usually considered as one of the undifferentiated properties during the hESC maintenance.\textsuperscript{75,76} Accordingly, in the recent derivation of iPSCs from somatic cells, development of compact colonies with tight cellular association and E-cadherin expression has been used and is now widely accepted as a simple and reliable readout for conversion of non-hESCs to an ESC-like state.\textsuperscript{73-76}

Beyond use as a supplementary marker for undifferentiated hESCs,\textsuperscript{77,78} the active role of E-cadherin in hESC self-renewal was further pursued. One possible explanation for this might be a clear demonstration in 1996 that E-cadherin was dispensable for mouse ESC maintenance.\textsuperscript{79} More recent studies, however, report that hESCs may resemble mouse epiblast stem cells (EpiSCs)\textsuperscript{80,81} to a greater extent rather than mouse ESCs in their molecular profile and growth factors requirements.\textsuperscript{82-84} Intriguingly, a current report described a mouse EpiSC-like cell type, termed FAB-SCs (i.e., fibroblast growth factor, Activin and BIO-derived stem cells),\textsuperscript{85} that share expression markers with EpiSCs but are unable to differentiate. Remarkably, these differentiation-resistant FAB-SCs can be steered to convert into their naïve pluripotent state (like ESCs) by ectopic expression of E-cadherin, suggesting an unexpected role for E-cadherin-mediated cell-cell adhesions in the maintenance of pluripotent state.\textsuperscript{86} Taken together, the molecular and biological similarities between hESCs and mouse EpiSCs indicate that E-cadherin corresponds, at least partially, to the survival, self-renewal and pluripotency state of hESCs.

Recent reports from several groups, including our own, have indeed demonstrated that E-cadherin directly contributes to hESC survival and self-renewal.\textsuperscript{82,83,85,86} Unlike mouse ESCs, dissociated hESCs are highly susceptible to apoptosis and undergo massive cell death upon seeding as single cells at low cell density.\textsuperscript{87,88} or in suspension culture.\textsuperscript{86} Several lines of evidence recently suggest that the primary cause of hESC death following cell dissociation comes from an irreparable disruption of E-cadherin signaling.\textsuperscript{89-91} Even attenuation of E-cadherin expression by siRNA or by blocking antibodies lead to a rapid loss in the expression of pluripotency markers Oct4, Nanog and Sox2 in hESCs.\textsuperscript{92,93} Oct4, Nanog and Sox2 constitute a core set of transcription factors crucial for the maintenance of pluripotent state of ESCs.\textsuperscript{94,95} It has yet to be established how (or indeed if) signaling events downstream of E-cadherin connect with these core set of transcriptional factors.

In addition to modulating survival and proliferation, E-cadherin also contributes to the self-renewal and pluripotency of hESCs. Upregulation of E-cadherin expression markedly enhances the cloning efficiency and self-renewal capacity of hESCs.\textsuperscript{96} Further, small molecules capable of upregulating E-cadherin expression facilitate hESC self-renewal.\textsuperscript{97} Conversely, inhibition of E-cadherin expression with specific blocking antibodies suppresses hESC self-renewal\textsuperscript{98,99} and results in not only an altered response to growth factor stimulation but also an overall decrease in hESC proliferation.\textsuperscript{100} Moreover, suppression of E-cadherin expression by siRNA or by blocking antibodies lead to a rapid loss in the expression of pluripotency markers Oct4, Nanog and Sox2 in hESCs.\textsuperscript{92,93} Oct4, Nanog and Sox2 constitute a core set of transcription factors crucial for the maintenance of pluripotent state of ESCs.\textsuperscript{94,95}

Molecular mechanisms underlying the role of E-cadherin in hESCs. Recent studies have identified multiple regulatory mechanisms involved in the interplay between E-cadherin and cell signaling in the regulation of cell fate decisions of hESCs. As illustrated in Figure 1B, several key mechanistic elements participate in a range of regulatory events in hESCs, such as cooperation between E-cadherin and the actin cytoskeleton and Rho family GTPases,\textsuperscript{101,102} regulation of E-cadherin expression at the hESC surface by turnover and membrane trafficking\textsuperscript{103} and interplay between E-cadherin and cell signaling.\textsuperscript{104-106}

Cooperation between E-cadherin and the actin cytoskeleton and Rho family GTPases. Increasing evidence has shown that the activity of NM II motor protein, a major component of actin cytoskeleton, is involved in (or regulates) the survival and self-renewal of hESCs, likely through E-cadherin.\textsuperscript{107-110} These studies have demonstrated that NM II exhibits dual functions in hESCs.\textsuperscript{107-110} For hESC colonies where E-cadherin-mediated cell-cell contacts have been established, NM II may help stabilize Otx4-Nanog-Sox2 circuitry possibly by regulating E-cadherin-mediated intercellular adhesion and by stabilizing p120-catenin protein.\textsuperscript{111} For dissociated single hESCs, NM II may accelerate the apoptosis of single hESCs through the Rho-Rock (Rho-associated kinase)-Myosin signaling axis.\textsuperscript{112,113}

For hESC colonies, NM II forms a network with E-cadherin and p120-catenin. This regulatory network helps hESCs remain in close contact and thus maintain their undifferentiated conditions.\textsuperscript{114} NM II, the two-headed conventional myosin, consists of three isoforms, HA, IIB and IIC.\textsuperscript{115} The isoforms IIA and IIB (not IIC) are predominantly localized to plasma membranes, and NM IIA has been observed to colocalize with E-cadherin in undifferentiated hESCs.\textsuperscript{116} Depletion of NM IIA (but not NM IIB) or treatment with Ecadherin inhibitor, a small molecule specifically inhibiting the myosin heavy chain ATPase, reduces E-cadherin accumulation at the junctional sites and impairs the formation of characteristic hESC colonies. As a result, a great number of
E-cadherin dynamics and trafficking is a relatively new topic in hESCs. The interplay between E-cadherin and cell signaling. In addition to Rho family small GTPases, small G protein Rap1 is involved in the regulation of hESC clonogenicity. Thus, hESC dissociation to reseeding should be completed within 30 min to limit Rap1 delivery to the lysosome. It can be concluded that a functional crosstalk between Rap1 and E-cadherin is critical for the self-renewal of hESCs.

In addition, p120-catenin has recently received a great attention in cadherin trafficking. A role of p120-catenin in stabilizing cadherin junctions has been observed previously in epithelial tumor cell lines64-66 and in endothelial cells.67 Loss of p120 results in an increase in turnover rate of E-cadherin has been observed in epithelial cells.64 In hESCs, however, E-cadherin and p120-catenin uniquely work in a positive feedback loop: E-cadherin increases p120-catenin production, while p120-catenin boosts E-cadherin expression. p120-catenin seems to facilitate E-cadherin accumulation at cell-cell junctions, thereby enhancing the self-renewal of hESCs.64 It should be noted that E-cadherin dynamics and trafficking is a relatively new topic in hESCs. The ability for individual E-cadherin molecule or complexes to be continually formed and disassembled is vital for the preservation of hESC property and colony integrity during cell proliferation and colony reorganization and requires further investigation.

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the expression of the apoptotic inhibitory gene Bcl-xL, while suppressing the expression of the pro-apoptotic gene Caspase-3. Consistently, two recent studies showed a significant increase of survival and colonies in the single-cell suspension cultures after overexpression of Bcl-xL and Bcl2 genes in hESCs. Moreover, upregulation of E-cadherin expression leads to high clonogenicity in hESCs while knockdown results in the opposite outcome.63 Interestingly, E-cadherin-expressing feeder cells promote neural lineage restriction of hESCs,1 suggesting that direct E-cadherin engagement have, however, other type of effect on neural specification. It is apparent that the roles of E-cadherin signaling in hESC self-renewal and lineage specification is far from simple and in need of further mechanistic investigation.

E-cadherin expression enhances the derivation of induced pluripotent stem cells. The active role and function of E-cadherin in the regulation of pluripotent state of ESCs is further demonstrated in the derivation of iPSCs. Using Yamanaka's factors (Oct4, Sox2, Klf4 and c-Myc), Aasen and colleagues have shown that reprogramming efficiency is 100-fold greater and 2-fold faster for the conversion of human epithelial cells in the context of hESCs. (keratinocytes that already express E-cadherin) to hiPSCs than for human fibroblasts. Bypassing a requirement for mesenchymal-to-epithelial transition (MET) has been speculated for the high reprogramming efficiency of epithelial cells, although this hypothesis has yet to be assessed directly. In 2010, several research groups independently demonstrated that undergoing MET is an essential early step of reprogramming34,66 and E-cadherin is required in the initial stages of this process.34,66,92,93 Reprogramming of fibroblasts into iPSCs is a slow (2–3 weeks) and inefficient (< 1%) process in which somatic cells gradually lose their differentiated identity and assume embryonic gene expression patterns and growth behaviors. Several groups have chronologically traced the events that occur during the first 2–3 weeks upon induction of the reprogramming factors in mouse embryonic fibroblasts. The first change in gene expression is the downregulation of somatic markers including key mesenchymal genes such as Snail and N-cadherin.77,78 Consistently, E-cadherin is upregulated when cells undergo a MET and start proliferating.88 Similar observations have been reported in reprogramming of human somatic cells.12 In addition, ectopic expression of human E-cadherin in mouse embryonic fibroblasts results in a 4-fold increase in iPSC colonies.84 In contrast, E-cadherin knockdown or inhibition reduces the reprogramming efficiency of mouse fibroblasts.34,59,92,93,141,142 Human primary fibroblasts and keratinocytes.84 Further mechanistic study has demonstrated that the adhesive binding activity of E-cadherin is essential for the reprogramming process.25 Collectively, these data as well as previous work suggest that E-cadherin is crucial for the self-renewal of human pluripotent stem cells and reprogramming of adult cells to iPSCs.

Most recently, Redmer and colleagues have further demonstrated the importance of E-cadherin in reprogramming.82 They found that forced expression of E-cadherin together with other three Yamanaka factors ( Sox2, Klf4 and c-Myc) was sufficient to reprogram murine fibroblasts to iPSCs, without the need of Oct4.82 Although further testing needs to be done on human cells, their data suggest the spatial and mechanical input provided by E-cadherin alters cell fate. E-cadherin may directly or indirectly tie into the pluripotent transcription factor circuit thereby influencing the master transcription factor Oct4. However, considering their different biological activities, it is unlikely that E-cadherin can functionally replace Oct4. Clearly, molecular mechanism underlying the cross-talk between cell surface molecules and nuclear machinery is key to furthering our understanding of this crucial interaction. Recent findings thus have provided some new insights into this issue. It seems that Oct4 and Sox2 suppress the Snail (an E-cadherin repressor), Klf4 induces E-cadherin expression, and c-Myc downregulates the expression of TGF-β1 and TGF-β receptor 2.85 It is well known that TGF-β signals induce the epithelial-to-mesenchymal transition, at least in part, through the activation of the E-cadherin repressor Snail and thereby negatively regulate the MET. Furthermore, the crucial role and possible mechanism of E-cadherin in reprogramming34,66,92,93 has been further highlighted by failed rescue experiments with β-catenin.89 β-catenin is an adaptors molecule that links to the cytoplasmic tail of E-cadherin mediating cell-cell adhesion and is also a pivotal effector of the canonical Wnt signaling pathway. It is known that β-catenin enhances Oct4 activity and plays an important role in the regulation of self-renewal of ESCs.88 Unexpectedly, β-catenin is incapable of rescuing the poor reprogramming efficiency induced by E-cadherin inhibition,89 suggesting an indispensable role of E-cadherin in reprogramming. Finally, microRNAs (miRNAs) are emerging as critical regulators of cell function within this network. Using a strategy that avoids the caveats associated with transient transfection of chemically synthesized miRNA mimics, Liao and colleagues86 have shown that miRNA clusters 302 and 367 target TGFβ receptor 2, promote E-cadherin expression and accelerate MET necessary for colony formation.87 Together, these observations have provided important biological insights into how E-cadherin plays an overarching role in reprogramming, self-renewal and pluripotency.

The role of N-cadherin and VE-cadherin in human pluripotent stem cells. Another two members of classic cadherin subfamily studied in hESCs are N-cadherin and VE-cadherin. Similar to E-cadherin, both family members are single-pass transmembrane glycoproteins that form Ca2+-dependent homophilic cis- and trans-interactions with their extracellular regions and also link to catenins through their cytoplasmic tails. Unlike E-cadherin, however, they are not expressed by undifferentiated hESCs.75,93 The appearance of N-cadherin and VE-cadherin often represent a specific cell lineage transition differentiated from hESCs.

N-cadherin is expressed by a variety of cell types, including neuromesenchymal cells, neurons, mesenchymal cells,75 as well as fetal and adult hepatocytes,80 but is not expressed by undifferentiated hESCs.93,95 N-cadherin has therefore been used as a neuromesenchymal marker or a mesenchymal marker in the studies of hESC differentiation, depending on the status of other co-markers. During neural induction, hESCs and hiPSCs change their morphology into compactly assembled cells and then into tubular rosette-like structures expressing neural precursor cell specific markers such as Pax6, nestin and Sox2.80 Expression of
N-cadherin is asymmetrically localized on the luminal side of the rosette, a characteristic feature of primitive neuroepithelial rosette structures. A switch from E-cadherin expression in undifferentiated hESCs to N-cadherin expression is retained in rosette-stage neural stem cells. This transition recapitulates embryonic development in vivo. For instance, during the formation of the neural tube, E-cadherin is switched off in a subset of cells, whereas N-cadherin expression is turned on in those cells. Additionally, N-cadherin is also expressed in mesodermal tissues. A switch from E-cadherin to N-cadherin expression, indicating epithelial-to-mesenchymal transition, is observed in hESC differentiation. Recently, N-cadherin has also been reported as a surface marker for the enrichment of hepatic endodermal cells from differentiated hESCs.

VE-cadherin, an endothelial-specific cell-cell adhesion protein of the adherens junction complex, plays a key role in endothelial barrier function and angiogenesis. VE-cadherin is absent in undifferentiated hESCs but is upregulated prior to hematopoietic emergence between days 3 and 10 of human embryoid body (hEB) development. Several studies have identified a population of intermediate-stage precursors defined, in part, by their expression of VE-cadherin and other specific surface markers that possess primitive endothelial properties during hESC differentiation. These precursors are capable of giving rise to endothelial and hematopoietic cells. Additionally, screens using green fluorescent protein driven by VE-cadherin promoter to identify factors that promote vascular commitment have revealed that the expansion and maintenance of hESC-derived endothelial cells by TGFβ inhibition is dependent on Id1 (an inhibitor of a group of basic helix-loop-helix transcription factors), providing a further correlative link between VE-cadherin and hESC fate determination.

Cell-Cell Adhesion Molecules in Human Pluripotent Stem Cells: L1-CAM, NCAM and PECAM-1

The immunoglobulin superfamily (IgSF) is another class of CAMs. IgSF CAMs are either homophilic or heterophilic and bind integrins or different IgSF CAMs. IgSF CAMs contain one or more of the extracellular Ig-like domains characteristic of antibody molecules. Analysis of the human genome reveals that this Ig-like domain has the widest representation of any protein domain, being encoded by 765 genes. Expression and function of IgSF CAMs in undifferentiated hESCs and hiPSCs have not been extensively studied. Our knowledge of the expression patterns or levels and the roles or functions of IgSF CAMs on hESCs and hiPSCs remain limited. For example, a molecule called L1-CAM (CD171) that belongs to IgSF CAM family has been shown to be displayed by undifferentiated hESCs but little is known about its function. Other IgSF CAM molecules, if detected, most often appear first during hESC differentiation into a specific lineage and are thus used as surface markers to fractionate hESC-derived stage-specific subpopulations. These molecules include NCAM (Neural Cell Adhesion Molecule/CD56) and PECAM-1 (Platelet-Endothelial Cell Adhesion Molecule-1/CD31).

NCAM/CD56 is a homophilic binding glycoprotein. It is the first member of IgSF CAM family described in the central nervous system although its expression is also found in other cell types and not restricted to neural cells. NCAM/CD56 has been used to isolate hESC-derived neurons by fluorescence activated cell sorter (FACS) at late stage of neural differentiation since NCAM does not present on undifferentiated hESCs, neural stem cells, or neural precursor cells. FACS-sorted hESC-derived neurons survive in vivo after transplantation into the rodent brain. Furthermore, there is evidence that NCAM/CD56-positive and CD326 (epithelial cell adhesion/activating molecule, EpCAM)-negative populations may represent more lineage-restricted mesodermal progenitors differentiated from hESCs.

PECAM-1/CD31 has been considered to be a marker associated with cells with early hematopoietic potential in the human embryo. PECAM-1/CD31 is undetectable in undifferentiated hESCs but upregulated prior to hematopoietic emergence between days 3 and 10 of hESC differentiation. A subset of embryonic endothelia lacking the common leukocyte marker CD45 but expressing surface markers PECAM-1/CD31, CD34, VEGFR2 (vascular endothelial growth factor receptor 2) and VE-cadherin are first identified within 3–10 d of hEB development. These cells have the ability to give rise to both endothelial and hematopoietic cells. Subsequent in vivo studies have shown that these hESC-derived hematopoietic cells possess hematopoietic stem/progenitor properties after transplantation into immunodeficient mice. A recent study further reveals PECAM-CD31-positive cells purified from differentiating EBs express high levels of ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1). They also express multiple endothelial genes and form lumened vessels when seeded onto porous poly(2-hydroxyethyl methacrylate) scaffolds and subcutaneously implanted in athymic rats. Thus, it is likely that, during early hESC differentiation, the PECAM/CD31-positive cells may represent primitive endothelium capable of further development into committed hematopoietic and endothelial cells.

Cell-Matrix Adhesion Molecules in Human Pluripotent Stem Cells: Integrins

In addition to cadherin mediated cell-cell adhesion, the maintenance of hESCs and hiPSCs also requires contact with extracellular matrix components. The requirement for basement membrane formation in human pluripotent stem cells is consistent with their cellular origin. During embryonic development, cells not only interact with each other but also with the extracellular matrix composed of proteins such as collagen, fibronectin and laminin that interface with proteoglycans. hESCs interact with extracellular matrix via cell-surface receptors including integrins, which allow for mechanically anchoring to the extracellular substrates as well as intracellular signal transduction. Integrins are heterodimeric surface glycoproteins and constitute a large and widespread family of cell-matrix adhesion molecules.
There are 24 known integrin heterodimers comprised of combinations of one of 18 α subunits and one of 8 β subunits.\(^9,25\) The integrin dimers bind to an array of different extracellular matrix molecules with overlapping binding affinities.\(^26\)

Genomic data (microarray, expressed sequence tags analysis and massively parallel signature sequencing)\(^27,117,118\) have provided the expression profiles of different integrin subunits in hESCs. Integri expression and function in hESCs have also been examined by studying cell interactions with various extracellular matrix components,\(^12,119-122\) including vitronectin.\(^33,123,124\) Of 18 α subunits and 8 β subunits, α1, α2, α3, α5, α7, αE, α11, αV, and α6β1 have been detected in undifferentiated hESCs in the aforementioned reports, albeit with some controversy. These controversial results have been attributed to inter-line variation, heterogeneity and/or highly plastic phenotype of hESCs. Additionally, culture methodologies (e.g., feeder layer vs. feeder free) and analysis methods vary significantly among different research groups.

A recent study shows that hiPSC lines reprogrammed by Ocs4, Sox2, Nanog and Lin28 and cultured on Matrigel and vitronectin express a repertoire of integrins similar to that of hESCs, although hiPSCs prominently express subunits α5, α6, αV, β1 and β5.\(^33\) It appears that β1 integrin is required for hiPSC adhesion and proliferation on Matrigel, while integrin αVβ3 is required for the initial attachment to vitronectin.\(^26\) Furthermore, integrins αVβ3 and αVβ5 (also mediating hiPSC adhesion to vitronectin) have been found to contribute to self-renewal and pluripotency\(^18\) and to the clonal growth\(^121\) of hESCs. Integri-related pathways, such as PI3K-Akt\(^24,125\) and MeK/Erk\(^126\) also play important role in hESC survival. The PI3K-Akt pathway has been shown to inhibit apoptosis and attenuate cell death after hESC dissociation and constitutive overexpression of Akt partially improves hESC survival.\(^69\)

**Cell-Matrix Adhesion Molecules in Human Pluripotent Stem Cells: Heparan Sulfate Proteoglycans**

Heparan sulfate proteoglycans (HSPGs) reside on the plasma membrane of all animal cells studied to date and are a major component of extracellular matrices.\(^126\) HSPGs are composed of a core protein and one or more heparan sulfate glycosaminoglycan (GAG)-chains. There are three subfamilies of HSPGs: the membrane-spanning proteoglycans (namely syndecans, betaglycan and CD44v3), the glycosphathidylinositol (GPI)-linked proteoglycans (namely glypican) and the secreted extracellular matrix proteoglycans (namely agrin, collagen XVIII and perlecan). HSPGs contain long polysaccharide side chains that bind signal proteins and immobilize them, by which HSPGs help localize the action of secreted signal proteins.\(^127\) HSPGs may also control the stability of signal proteins, transport them through the extra-cellular space, or make them interact with cell-surface receptors. For example, the stability, movement and reception of diffusible growth factors such as FGF, BMP, TGFβ, Indian hedgehog,\(^128\) and Wnt\(^129\),\(^130\) have been reported to be controlled by HSPGs. HSPGs are present throughout embryonic development\(^131\),\(^132\) and potentially mediate signals essential for the maintenance of self-renewal or for the initiation of differentiation. Most recently, Shimokawa et al. revealed that cell surface-tethered heparan sulfate chains (key components of HSPGs) play pivotal roles in the local retention of FGF ligands and moreover, can “spread” FGF signaling to adjacent cells within a short-distance in heparan sulfate-deficient mouse embryos.\(^133\) However, the functional role of cell surface-HSPGs in hESCs and hiPSCs has not been elucidated until recently.

New data showed that secreted HSPGs produced by mouse embryonic fibroblast feeder cells coordinated hESC proliferation.\(^134\) It is well known that hESCs can be maintained in an undifferentiated state if the culture medium is first conditioned on a layer of mouse embryonic fibroblast feeder. Using column chromatography, immunoblotting and mass spectrometry-based proteomic analysis, multiple secreted HSPG species have been identified in mouse embryonic fibroblast-conditioned medium.\(^136\) These HSPGs and other heparinoids can stabilize basic fibroblast growth factor (bFGF) and also directly mediate binding of bFGF to the hESC surface, promoting hESC maintenance. In contrast, removal of HSPGs from conditioned medium impairs hESC proliferation,\(^137\) suggesting that HSPGs might be key signaling cofactors in hESC maintenance. One potential mechanism, inferred from studies of mouse ESCs, could be that heparan sulfate chains mediate autocrine/paracrine Wnt/β-catenin signal- ing to regulate Nanog expression,\(^138\) although further validation will be required in human pluripotent stem cells.

**Concluding Remarks**

CAMs provide two-way communication links from hESCs (or hiPSCs) to their surrounding environment and from one cell to another. CAMs also mediate/regulate the transduction initiated by many chemical signals across cell membrane and enable communications with important intracellular signaling pathways. The molecular and cellular biology of CAMs and their functions in hESCs and hiPSCs represents a very exciting area of discovery with emerging links to self-renewal, pluripotency, reprogramm- ing and differentiation. Further understanding the underlying mechanisms will greatly facilitate the maintenance and direct differentiation of hESCs and hiPSCs into a given cell type for potential applications necessary to validate their regenerative potential. There is little doubt that research into CAMs and human pluripotent stem cells will continue to produce interesting and surprising results key to the future cell replacement strategies.

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