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CDH2 and CDH11 as Regulators of Stem Cell Fate Decisions

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Abstract

Accumulating evidence suggests that the mechanical and biochemical signals originating from cell-cell adhesion are critical for stem cell lineage specification. In this review, we focus on the role of cadherin mediated signaling in development and stem cell differentiation, with emphasis on two well-known cadherins, cadherin-2 (CDH2) (N-cadherin) and cadherin-11 (CDH11) (OB-cadherin). We summarize the existing knowledge regarding the role of CDH2 and CDH11 during development and differentiation *in vivo* and *in vitro*. We also discuss engineering strategies to control stem cell fate decisions by fine-tuning the extent of cell-cell adhesion through surface chemistry and microtopology. These studies may be greatly facilitated by novel strategies that enable monitoring of stem cell specification in real time. We expect that better understanding of how intercellular adhesion signaling affects lineage specification may impact biomaterial and scaffold design to control stem cell fate decisions in three-dimensional context with potential implications for tissue engineering and regenerative medicine.

Keywords

CDH2; CDH11; adherens junctions; differentiation; mesenchymal stem cells; microfabrication; micropatterning

1. Introduction

Intercellular adhesion plays important role in tissue architecture and morphogenesis by controlling the assembly of individual cells into the three-dimensional tissues[1]. Cell-cell or cell-matrix interactions are mediated by cell adhesion molecules (CAMs) including cadherins, integrins, selectins and immunoglobulin-like CAMs, and regulate multiple aspects of cellular behavior including proliferation, differentiation, apoptosis, cell polarity[1,

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2], embryonic stem cell self-renewal and differentiation[3] and overall, the maintenance of tissue integrity [4].

Cadherins represent one class of CAMs that mediate Ca^{2+} dependent homophilic interactions between cells, through formation of intercellular connections or otherwise known as adherens junctions (AJs). The most well studied cadherins are the classical vertebrate cadherins that have been named based on the tissue in which they are expressed. Neuronal cells mostly express N-Cadherin (CDH2), while epithelial cells highly express E-Cadherin (CDH1). Among the non-classical cadherins, VE-Cadherin (CDH5) is expressed in endothelial cells and OB-Cadherin (CDH11) is expressed in osteoblasts. However, the expression level of cadherins may vary during different cellular processes, especially those that involve transition from one cellular state to another. For example, it is well established that the process of Epithelial to Mesenchymal Transition (EMT) is characterized by augmented expression of CDH2 and CDH11 and diminished expression of CDH1 [5–7]. Recent studies suggest that cadherin expression and cell-cell adhesion may also be critical in other transitions between cellular states such as lineage specification of stem cells or reprogramming of adult cells to a pluripotent state [8, 9].

Stem cell differentiation is affected by many soluble and insoluble signals in their local microenvironment. In addition to soluble growth factors, a number of elegant studies implicated cell-ExtraCellular Matrix (ECM) interactions and substrate mechanics in stem cell lineage commitment[10–17]. However, the mechanical and biochemical signals originating from cell-cell adhesion remain relatively unexplored in this context. Recent studies implicated adherens junctions in the maintenance of embryonic stem cell self-renewal potential, cellular reprogramming, hematopoietic stem cell engraftment and mesenchymal stem cell (MSC) differentiation into muscle [8, 9, 18]. Here we provide a brief review on the role of cadherins, in particular CDH2 and CDH11, in development and stem cell fate decisions. This is a relatively nascent field of stem cell biology that has the potential to guide the development of novel strategies to control stem cell fate decisions as well as to inspire biomimetic design of nanomaterials for tissue engineering and regeneration.

2. Adherens Junctions: signal transduction and mechanosensing

In general, classical cadherins including CDH2 and CDH11 have a common cytoplasmic domain and an ectodomain containing five tandem extracellular cadherin (EC) domains [19] (Fig. 1). The EC domains contain Ca^{2+} binding sites in which three Ca^{2+} ions work as inter-domain linkers, stabilizing the ectodomain structure and protecting it from proteolysis. [20–22] The outermost, EC1 domain regulates cadherin-cadherin interactions between adjacent cells, resulting in formation of adherens junctions between parallel opposing plasma membranes [23]. Specifically, CDH2 and CDH11 are mostly expressed in mesenchymal type cells such as fibroblasts and cardiac cells and mediate intercellular adhesion between cells of the same type e.g. myofibroblasts, or different cell types e.g. between myofibroblasts and cardiac cells[24].

Interaction of cadherin-cadherin may lead to intercellular activation of cellular pathways, initiating through lamellipodial protrusions and is followed by the cadherin-catenin-actin

cluster formation. The association of cadherin with catenin promotes and stabilizes the AJs, while actin polymerization leads to AJs expansion and maturation, further stabilizing and aligning adjacent cell membranes [4]. In particular, β-catenin binds to the cadherin cytoplasmic tail and interacts with α-catenin, which modulates the actin cytoskeleton. [1, 25] The intracellular domains of the cadherins also bind to p120 catenin, which links cadherin to microtubules[4] and regulates GTPases such as RhoA, Rac1 and Cdc42 [1, 26–31] (Fig. 1). Disrupting Rac or Rho activity perturbs AJ assembly, while Cdc42 affects AJ maintenance[32, 33]. The function of GTPases is linked to the cadherins and may control various cellular processes including polarization, migration and apoptosis. Specifically, CDH2 regulates spatially polarized signals through distinct p120 and β-catenin-dependent signaling pathways[34]. Interestingly, CDH2 mediated cell adhesion is important for collective 3D migration[35–37], while CDH11 is required for directional migration in vivo[38].

Several reports showed that cadherins are affected by growth factors and activate signaling pathways as a result of physical interactions with growth factor receptors. On exposure to shear stress, VE-cadherin binds to PECAM and vascular endothelial growth factor receptor (VEGFR2) and this complex may lead to integrin activation and actin cytoskeleton reorganization [39, 40]. Epidermal growth factor receptor (EGFR) forms a complex with CDH1, leading to activation of the mitogen-activated protein kinases (MAPK) pathway in epithelial cells [41, 42] with implications for cell survival [43] or EMT [44, 45]. Fibroblast growth factor receptors (FGFR) were shown to stimulate CDH2 during neurite outgrowth[46, 47], while FGF plays a critical role in the maintenance of vascular integrity by enhancing the stability of VE-cadherin at AJ sites [48]. Hepatocyte Growth Factor (HGF) modulates the expression of the cell adhesion molecule VE-cadherin and consequently endothelial cell motility, migration and angiogenesis[49]. Finally, TGF-β1 increases keratinocyte migration by increasing the levels of CDH2 and this action is counteracted by EGF [50].

Several reports have shown that cadherins are not only chemically but also mechanically regulated. Recently, our laboratory showed that substrate stiffness regulated AJ formation between epithelial cells in two-dimensional (2D) cultures and in three-dimensional (3D) epidermal tissues in vitro and in vivo by regulating the phosphorylation levels of the c-Janus N-terminal kinase (JNK) [51]. Rigid substrates led to JNK activation and AJ disassembly, while soft matrices suppressed JNK activity leading to AJ formation. The results held true in 3D bioengineered epidermis as well as in the epidermis of knockout (*jnk1*$^{-/-}$ or *jnk2*$^{-/-}$) mice. In conclusion, we discovered that the JNK pathway mediated the effects of substrate stiffness on AJ formation in 2D and 3D context in vitro as well as in vivo. These findings shed light into the mechanisms of AJ formation and dissolution during tissue development and may provide novel guiding principles to control cell-cell vs. cell-substrate adhesion in 3D as a therapeutic strategy to promote tissue regeneration or inhibit tumor invasion.

Even though substrate stiffness and tethering is mostly known to affect focal adhesions [52–54], increasing evidence suggests that it may also affect cadherin-mediated intercellular adhesion [55, 56]. Substrate stiffness was implicated in cadherin-dependent collective cell migration through myosin-II contractility [57]. CDH2 is considered a mechanoresponsive

adhesion receptor, as the forces transmitted through CDH2 junctions are comparable in magnitude to those sustained by integrin-ECM coupling[58]. In general, stiffer substrates lead to greater traction forces, larger cell-spread areas and better developed CDH2 junctions[56].

Finally, better understanding of cadherin based cell-cell interactions may be useful in development of scaffold-free tissue engineering strategies [59–61]. These strategies rely on directed cellular self-assembly using scaffold-free techniques including formation of spheroids or bioprinting, instead of biomaterial scaffolds to guide tissue formation, 3D organization and structure [62–66].

3. The role of CDH2 and CDH11 during development and morphogenesis

In the early stages of embryogenesis, the trophoblast giant cells are devoid of CDH2 or CDH11.[67] During gastrulation, the process generating the three germ cell layers, CDH11 is highly expressed enabling spatial recognition and segregation of cells as they move to generate primitive tissue structures[68–70]. At later stages as cells undergo EMT, CDH1 is downregulated, while CDH2 is upregulated and is important for proper left-right axis development [71]. In general, gastrulation gives rise to three germ layers: ectoderm, endoderm and mesoderm. CDH2 and CDH11 are absent in cells of the endodermal lineage [67] but play important roles in the development of ectodermal and mesodermal lineages as described below.

Ectodermal lineage

The ectoderm is the first germ layer to emerge during gastrulation. In vertebrates, the ectoderm is responsible for the formation of the nervous system and spinal cord. The nervous system is formed during neurulation, when the neural tube is transformed into a primitive structure and eventually into the central nervous system. Early in neural tube development, the notochord and the dorsal aorta do not express CDH11, which is expressed during the later stages of neural tube formation and is important for brain and spinal cord development[72, 73]. CDH11 is expressed in the limbic system of the brain, particularly in the hippocampus where it is thought to participate in the organization and stabilization of synaptic connections [74]. It is also expressed in the peripheral nervous system and, in particular, in motor and sensory axons during the period of active nerve elongation and path finding. [75, 76] CDH2 is present during neuroectoderm formation and is important for nervous system development. [77, 78] CDH2 knockout mice die on day 10 of gestation due to heart defects and malformed neural tubes, although tissue development appears normal up to this stage [79]. Others reported that CDH2 is involved in neuronal circuit maturation by contributing to axonal extension [5]. Finally, both CDH2 and CDH11 were shown to regulate neurite outgrowth through FGFR [80], PLCγ and CAM kinase pathways [81, 82].

Mesodermal lineage

Mesoderm is the middle developmental layer between the ectoderm and endoderm, which gives rise to skeleton, muscle, heart and bones. In early embryos, both CDH2 and CDH11 are found in the mesoderm [22, 83] albeit with different expression patterns. The head

mesoderm expresses higher levels of CDH11 comparing to CDH2, while branchial arches express only CDH11.[5] CDH11 is present in all mesenchymal cells throughout the embryo such as mesenchymal cells of the stomach, intestine, pharynx, lung bud and shaft of ribs [67, 84–86] as well as mesenchymal stem cells originating from the pre-chondal and paraxial mesoderm and from neuroectodermal neural crest cells. CDH2 is also expressed in all mesenchymal and mesothelial tissues [87] and its expression is regulated by PDGF and FGF signaling [88].

4. The role of CDH2 and CDH11 in mesenchymal stem cell differentiation

Recently cadherins were found to regulate stem cell maintenance and differentiation. CDH1 was necessary for maintaining pluripotency of embryonic stem cells as well as for cellular reprogramming, where ectopic expression of CDH1 could substitute for the pluripotency factor Oct4 [8]. Interestingly, CDH2 was implicated in long-term engraftment of hematopoietic stem cells and establishment of hematopoiesis after bone marrow transplantation [18] but its exact role remains controversial. Some studies suggested that it might be necessary as inhibition of cadherin-mediated homophilic and heterophilic adhesion reduced the long-term repopulation activity of Hematopoietic Stem Cells (HSCs) [89]. However, others reported that CDH2 conditional knockout mice do not show defects in HSC number or function [90].

On the other hand, accumulating evidence suggests that both cadherins play important roles in MSC differentiation. MSC provide an excellent cell source for cellular therapies to treat bone and cartilage disorders [91, 92], myocardial infarction, stroke [93, 94], rheumatoid arthritis [95], acute lung injury [96, 97], graft-versus-host disease [98] and skin-graft rejection [99] among others. The use of MSC for tissue repair requires the migration and homing to the site of damaged tissue and it has been shown that both the migratory and proliferation potential of these cells are affected by CDH2 and CDH11 [100, 101]. MSCs have also been shown not only to have differentiation potential but also potent anti-inflammatory effects [102], which are enhanced when cultured as 3D spheroid aggregates [103, 104]. Interestingly, both CDH2 and CDH11 were shown to be critical in the response of synovial fibroblasts to inflammation [105, 106], suggesting that cadherins may also be important in mediating the anti-inflammatory effects of MSC. Finally, CDH2 and CDH11 have been shown to be critical for MSC differentiation and their expression levels are regulated differently in osteogenic, chondrogenic or myogenic lineages as described below (Fig. 2).

I. Osteogenic Lineage

CDH2 and CDH11 are highly expressed during MSC osteogenic differentiation [107] and several pro-osteogenic factors are known to affect their expression. For example, well-known osteogenic inducers, such as BMP-2, parathyroid hormone (PTH), bFGF and phorbol ester increased the levels of these cadherins [108–110]. On the other hand, Vitamin D decreased expression of CDH2 [111] and dexamethasone inhibited the expression of both CDH2 and CDH11 mRNA in human osteoprogenitor marrow stromal cells (BMC) [112]. Interestingly, both CDH2 and CDH11 were downregulated in mature osteocytes [113].

Loss-of-function studies provided definitive data supporting the role of both cadherins in bone formation. Blocking of CDH2 or CDH11 with inhibitory peptides prevented osteoblastic differentiation in vitro [108, 113, 114]. In agreement, CDH11 knockout null mice showed modest osteopenia by three months of age as signified by decreased mineralizing surface and trabecular bone volume [115]. The role of each cadherin in osteogenesis was further dissected by using double knockout mice ($Chd2^{+/-}$;$Cdh11^{-/-}$) and showed that although both CDH2 and CDH11 are important for osteogenesis, their contributions were mediated by distinct mechanisms. Specifically, CDH11 was pro-osteogenic but dispensable for postnatal skeletal growth; on the other hand, CDH2 was necessary for maintaining the precursor osteoblast pool [116]. This result might explain why overexpression of CDH2 promoted migration but inhibited osteogenesis as evidenced by decreased expression of osteogenic genes osteopontin, osteocalcin, RunX2, alkaline phosphatase (ALP) and BMP-2, as well as ALP activity and calcium deposition in BM-MSC [100].

II. Chondrogenic Lineage

During chondrogenesis CDH2 and Sox9 were upregulated by the action of paracrine factors like TGF-β, FGFs, or BMPs, and the transcription factor Sox9 further increased the CDH2 promoter activity [117]. CDH2 mediated cell-cell interactions and increased MSC aggregation, which in turn promoted differentiation into the chondrogenic lineage [118, 119]. CDH2 was required for the initial condensation phase but decreased significantly during terminal chondrogenic differentiation. [120, 121] In agreement, it has been reported that the cleavage of CDH2 was required during chondrogenic differentiation [122], while inhibition of commitment to chondrogenic lineage by the Wnt7a inhibitor led to enhanced CDH2 expression and stabilization of AJs. [123–125] Interestingly, loss of CDH2 led to increased levels of CDH11, suggesting that compensatory mechanisms might be at work [126].

III. Adipogenic Lineage

During adipogenesis, CDH2 and CDH11 were downregulated and mature adipocytes did not express either of cadherin [127, 128]. In addition, CDH11 knockdown induced adipogenic gene expression (e.g. PPARγ) and differentiation, suggesting that CDH11 might inhibit adipogenesis.

IV. Myogenic Lineage

CDH2 and CDH11 are also important during myogenic differentiation. High cell density was shown to promote myoblast differentiation, suggesting that cadherin mediated cell–cell contact might affect myogenesis [129, 130]. CDH2 and CDH11 also play important role in wound healing when fibroblasts turn into myofibroblasts to increase wound contraction and promote wound closure [84, 131–134]. Interestingly, CDH11 was upregulated in vascular smooth muscle cells (SMCs) in response to injury, while its inhibition reduced SMC proliferation and migration [85].

Recently, our group reported that CDH11 but not CDH2 was necessary for MSC differentiation into SMCs [9] (Fig. 3). CDH11 engagement regulated MSC to SMC

differentiation via two pathways. One pathway was dependent on TGFβ receptor II (TGFβRII) but independent of SMAD2/3. The second pathway involved activation of Rho-associated protein kinase (ROCK), which in turn induced expression of serum response factor (SRF) and SMC proteins such as alpha smooth muscle actin (αSMA), calponin and myosin heavy chain (MYH11). Increased expression of SRF resulted in increased expression of CDH11, indicating the presence of a positive feedback loop that led to increased CDH11 engagement and subsequent commitment of MSC to the SMC fate (Fig. 3). Experiments with CDH11-null ($Cdh11^{-/-}$) mice verified the role of CDH11 in SMC function as vascular and urogenital tissues of these animals exhibited significantly reduced levels of SMC proteins and most notably, diminished contractility as compared to wild-type controls. These findings are novel and surprising as $Cdh11^{-/-}$ mice develop normally, are fertile and display no obvious phenotype other than modest osteopenia[115, 127, 128] and decreased pulmonary fibrosis after lung injury[135]. More work is required to understand the mechanism through which CDH11 affects SMC function and the potential implications of CDH11 loss in cardiovascular, urogenital, gastrointestinal and other SMC containing tissues.

5. Engineering cell-cell adhesion to direct stem cell fate decisions

The findings that we described above show that CDH2 and CDH11 play important roles in stem cell lineage specification, and therefore, could be used to develop technologies to control stem cell differentiation by exploiting cell-cell interactions. To this end, we propose the following strategies (Table 1) to capitalize on the effects of cadherin-mediated intercellular adhesion: (i) Engineering cadherin surfaces to control stem cell differentiation; (ii) Engineering surface microtopology to control the extent of cell-cell adhesion and signaling.

(i) Engineering cadherin surfaces to control stem cell differentiation

It has been shown that immobilized cadherins induced similar signaling cascades in epithelial cells as CDH1 engagement during cell-cell contact. Cadherin immobilization was facilitated by generating fusion proteins between cadherins with the Fc antibody fragment that enables protein immobilization to the surface. In addition, to generating functional surfaces, immobilized cadherins can be used to distinguish cadherin-mediated signaling pathways from pathways activated by the engagement of other junctional proteins e.g. connexins, which usually follows AJ formation during cell-cell contact [136].

This approach has been used to immobilize several cadherins including CDH1, CDH2 and CDH11 to regulate cellular behavior. Specifically, CDH1-Fc activated Rac1 and decreased RhoA activity in epithelial cells [136–138] and improved hepatocyte DNA synthesis and proliferation [139, 140]. Similarly, immobilization of CDH2-Fc retained the adhesive properties of native CDH2, resulting in recruitment of β-catenin, α-catenin and p120 at the cell-cell contact sites [141]. CDH2-Fc coated beads triggered myoblast maturation as evidenced by increased expression of myogenic regulators such as SRF [142, 143]. Interestingly, CDH2 in lipid bilayer membranes induced mesenchymal condensation of osteochondrogenic progenitors and suppressed adipogenic differentiation. [144] Likewise, CDH11-Fc proteins formed dimers that were shown to be functional i.e. engaged in strong

homotypic CDH11 interactions [145] and promoted binding of CDH11-expressing L cells. [145, 146] Also, culture of MSCs on surface immobilized fusion protein between a fibronectin domain (rFN) and CDH11 (rFN/CDH11) significantly enhanced osteogenic differentiation.[147] Finally, preliminary experiments in our laboratory showed that immobilized cadherins promoted MSC differentiation into SMC cells in a dose dependent manner, thereby providing control of differentiation by surface presentation and density. Collectively, these studies suggest the cadherin immobilization can be employed to direct and/or fine tune stem cell fate decisions and therefore, can be a useful strategy enabling functionalization of biomaterial scaffolds for tissue engineering and regenerative medicine.

(ii) Engineering surface microtopology to control the extent of cell-cell adhesion and signaling

Microfabrication technology offers the possibility to control the extent cell-cell adhesion at the micro- or nanometer scale. This approach has been used extensively to control cell-matrix interactions, which have been shown to be critical in stem cell differentiation [12, 13, 148–151]. Fewer studies have used geometric micropatterning to control the extent of cell-cell adhesion and evaluate its effects on stem cell differentiation [152].

It was shown that the size of micro-islands correlated with the level of cell spreading and CDH2 expression leading to MSC differentiation into the myogenic or chondrogenic lineages on the larger islands but adipogenic lineage on the small ones.[14] Similarly, by controlling the geometry and size of micro-islands it was shown that increased cell contact increased the extent of osteogenic differentiation [153, 154]. However, attempts to control cell-cell interactions by varying the size of micropatterns are compounded by the fact that cell density and therefore the degree of cell spreading change with island size, making it difficult to separate the effects of cell-cell vs. cell-substrate adhesion. Interestingly, novel geometries have been employed to control the extent of cell-cell contact independent of cell density or the cell spreading area [155–157], and therefore, may be used to determine the relationship between the extent of intercellular adhesion and stem cell fate commitment.

6. Monitoring intercellular adhesion mediated stem cell lineage specification in real time

Understanding how intercellular adhesion affects stem cell fate decisions requires methods to interrogate stem cell differentiation in real time and in a quantitative manner. In particular, methods to monitor individual cells may be particularly useful in experiments that involve small numbers of cells on micropatterned surfaces, thereby making traditional assays such as Western Blot and PCR challenging. In addition, monitoring single cells is useful in addressing issues of heterogeneity in embryonic, induced pluripotent or adult stem cells populations and therefore, in distinguishing between cells with varying differentiation potential.

To this end, our laboratory developed LentiViral Arrays (LVA) to monitor gene or pathway activation during stem cell differentiation. We designed a novel lentiviral dual promoter vector (LVDP) vector that enables quantitative measurements of the activity of a gene

promoter (Pr) or a transcription factor (TF) binding site (Response Element, RE) independent of the number of gene copies per cell [158]. We also designed a second lentiviral vector (shLVDP) that enables dynamic monitoring of Pr/RE activity with concomitant gene knockdown in a doxycycline (Dox)-regulatable manner, thereby enabling discovery of genes that may be involved in stem cell differentiation.[159] In addition, the envelope of lentiviral particles was engineered to bind covalently to fibrin hydrogels during polymerization [160, 161], thereby enabling generation of lentiviral arrays (LVA) that were employed to measure the activity of several Pr/RE participating in the inflammatory response[162]. More recently, we generated a library of Pr/RE to monitor MSC differentiation towards adipogenic, osteogenic, chondrogenic or myogenic lineages and used it to identify novel pathways that may be involved in lineage specification[163, 164]. Potentially, this technology may be combined with novel microfabrication methods to determine how the extent of intercellular adhesion influences stem cell specification decisions of adult stem cells, cancer stem cells or hiPSC and potentially also the pluripotency networks that are critical for cellular reprogramming.

7. Conclusion and future perspectives

Although many studies have focused on the effects of substrate stiffness on stem cell biology, the role of intercellular adhesion forces in guiding stem cell self-renewal or differentiation has been relatively unexplored. In this review, we focused on CDH2 and CDH11 as regulators of stem cell fate decisions. Although evidence that cadherins are important has been surfacing, more work is necessary to understand how intercellular adhesion affects MSC differentiation and reveal some of the molecular pathways guiding this process. These studies may also provide design parameters for guiding MSC fate by controlling the extent of cadherin-mediated adhesion with implications for tissue engineering and regenerative medicine.

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Highlights

- The role of CDH11 and CDH2 in development and morphogenesis.
- The role of CDH2 and CDH11 during MSC lineage commitment.
- CDH11 mediated AJ formation promotes MSC differentiation into SMC.
- Directing stem cell fate by controlling intracellular adhesion.
- Engineering cell-cell adhesion to direct stem cell fate decisions.

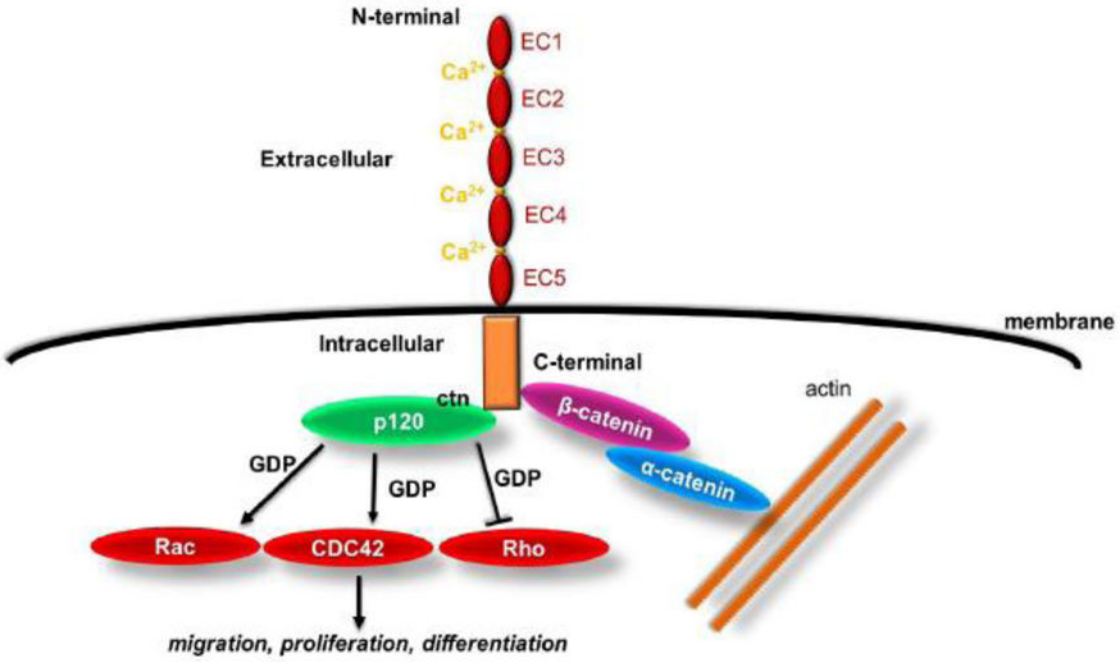

Figure 1. Schematic representation of cadherin structure and downstream signaling

Cadherins contain five extra cellular (EC) domains linked by Ca^{2+} binding sites and one intracellular domain. Classical cadherin partners include to β-catenin, which binds to α-catenin linking the AJ complex to the actin cytoskeleton, as well as p120 catenin, which regulates small GTPases such as Rho, Rac, and Cdc42. Ultimately, cadherin engagement regulates many cellular processes including proliferation, migration and stem cell differentiation.

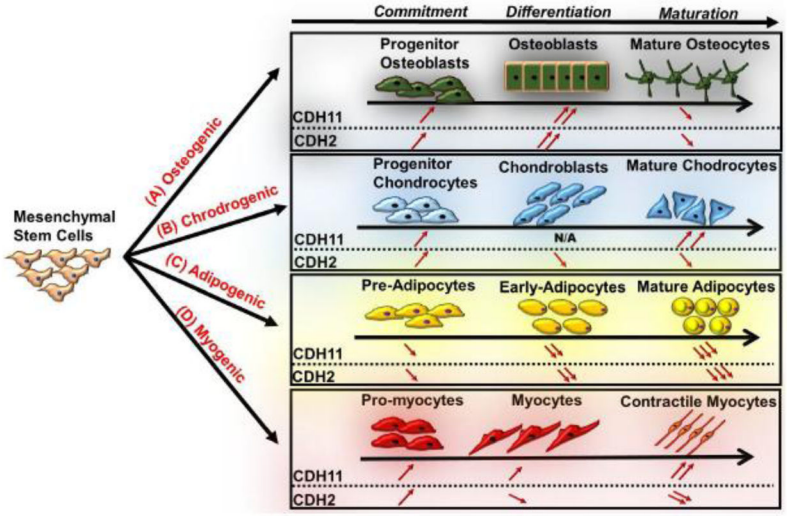

Figure 2. Schematic representation of CDH2 and CDH11 expression during MSC lineage commitment

CDH2 and CDH11 expression levels during MSC commitment, differentiation and maturation towards (A) Osteogenic; (B) Chondrogenic; (C) Adipogenic; or (D) Myogenic Lineages. Upward or downward pointing arrows indicate increased or decreased expression, respectively.

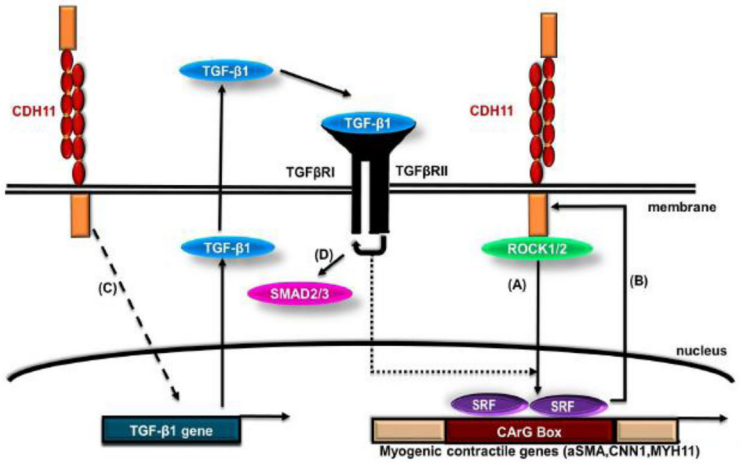

Figure 3. CDH11 mediated AJ formation promotes MSC differentiation into SMC [9]

(A) Engagement of CDH11 activates the ROCK pathway, which in turn activates SRF leading to increased expression of SMC genes. (B) SRF controls the level of CDH11 expression through a positive feedback loop further promoting intercellular adhesion. (C) CDH11 engagement also increases TGF-β1 expression further promoting SMC differentiation (D) through a Smad2/3 independent pathway.

Table 1

Engineering cell adhesion strategies to direct stem cell fate decision

Strategy	Approach	MSC Differentiation	Advantages	Ref.
Engineering cadherin surfaces	Cadherin immobilization to surfaces	*CDH2-CDH2 interactions:* Increased osteo-, chondro- and myogenic differentiation Decreased adipogenic differentiation *CDH11-CDH11 interactions:* Increased myogenic and osteogenic differentiation	**1** The extent of cell-cell adhesion is independent of cell density. **2** Enables single cell analysis. **3** Isolate the effects of cadherins from other CAMs.	[134–140, 151]
Engineering surface microtopology	Microfabrication/Micropatterning	*Large micro-island:* Upregulate chondrogenic and myogenic differentiation *Small micro-island:* Increase adipogenic fate	**1** Control cell adhesion at the micro/ nanoscales. **2** Cell-cell adhesion independent of cell spreading. **3** **4** Control the extent of cell-cell adhesion through substrate geometry.	[137–146] [12–14]