Stem Cell Research (2015) 14, 270–282

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scr

REVIEW

CDH2 and CDH11 act as regulators of stem cell fate decisions

Stella Alimperti[a], Stelios T. Andreadis[a,b,*]

[a] Bioengineering Laboratory, Department of Chemical and Biological Engineering, University at Buffalo, State University of New York, Amherst, NY 14260-4200, USA
[b] Center of Excellence in Bioinformatics and Life Sciences, Buffalo, NY 14203, USA

Received 18 September 2014; received in revised form 24 January 2015; accepted 10 February 2015
Available online 19 February 2015

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.

Contents

* Corresponding author at: Bioengineering Laboratory, 908 Furnas Hall, Department of Chemical and Biological Engineering, University at Buffalo, State University of New York, Amherst, NY 14260-4200, USA. Fax: +1 716 645 3822.
E-mail address: sandread@buffalo.edu (S.T. Andreadis).

http://dx.doi.org/10.1016/j.scr.2015.02.002

Introduction

Intercellular adhesion plays important role in tissue architecture and morphogenesis by controlling the assembly of individual cells into the three-dimensional tissues (Cavallaro and Dejana, 2011). 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 (Cavallaro and Dejana, 2011; Niessen and Gumbiner, 2002), embryonic stem cell self-renewal and differentiation (Li et al., 2012) and overall, the maintenance of tissue integrity (Harris and Tepass, 2010).

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 (Kimura et al., 1995; Zeisberg and Neilson, 2009; Tomita et al., 2000). 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 (Redmer et al., 2011; Alimperti et al., 2014a).

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 (Buxboim and Discher, 2010; Buxboim et al., 2010; Engler et al., 2006, 2007; Gao et al., 2010; McBeath et al., 2004; Treiser et al., 2010; Gilbert et al., 2010). 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 the muscle (Redmer et al., 2011; Alimperti et al., 2014a; Hosokawa et al., 2010a). 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.

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 (Brasch et al., 2012) (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 (Boggon et al., 2002; Pokutta et al., 1994; Takeichi, 1991). The outermost EC1 domain regulates cadherin–cadherin interactions between adjacent cells, resulting in formation of adherens junctions between parallel opposing plasma membranes (Shapiro and Weis, 2009). 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 (Thompson et al., 2014).

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 AJ expansion and maturation, further stabilizing and aligning adjacent cell membranes (Harris and Tepass, 2010). In particular, β-catenin binds to the cadherin cytoplasmic tail and interacts with α-catenin, which modulates the actin cytoskeleton (Cavallaro and Dejana, 2011; Stepniak et al., 2009). The intracellular domains of the cadherins also bind to p120 catenin, which links cadherin to microtubules (Harris and Tepass, 2010) and regulates GTPases such as Rho, Rac1 and Cdc42 (Cavallaro and Dejana, 2011; Reynolds and Carnahan, 2004; Yonemura, 2011; Grosheva et al., 2001; Anastasiadis et al., 2000; Anastasiadis and Reynolds, 2000; Noren et al., 2000) (Fig. 1).

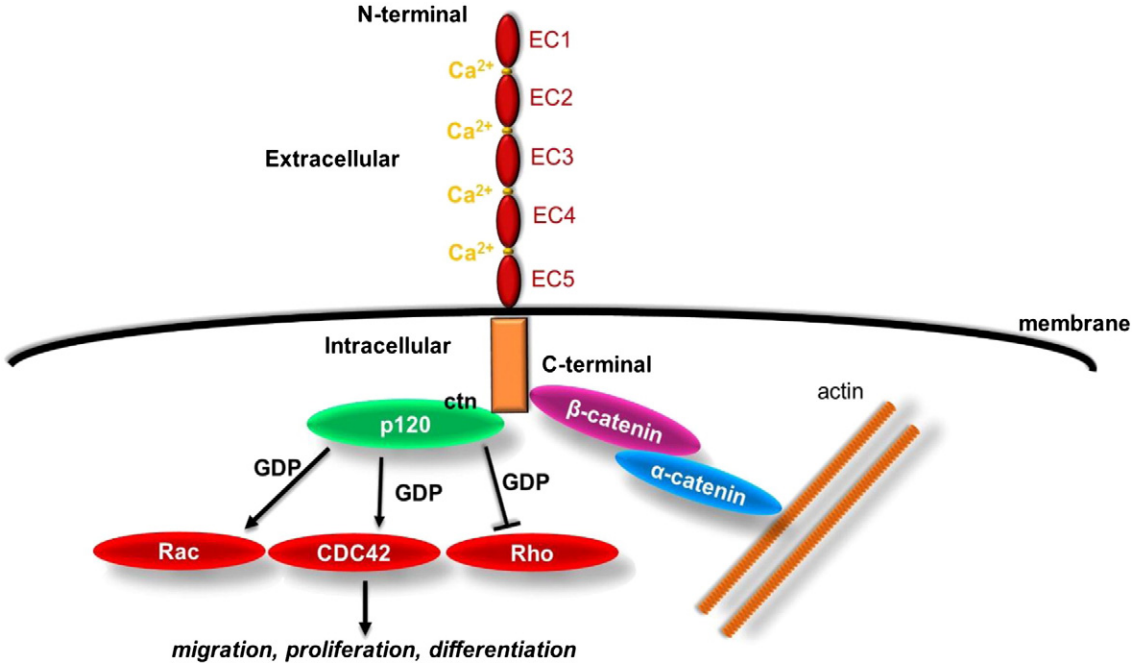

Figure 1 Schematic representation of cadherin structure and downstream signaling. Cadherins contain five extra cellular (EC) domains linked by Ca^{2+} binding sites and one intracellular domain. Classical cadherin partners include to β-catenin, which binds to α-catenin linking the AJ complex to the actin 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.

Disrupting Rac or Rho activity perturbs AJ assembly, while Cdc42 affects AJ maintenance (Yap and Kovacs, 2003; Braga, 2002). The function of GTPases is linked to 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 (Ouyang et al., 2013). Interestingly, CDH2 mediated cell adhesion is important for collective 3D migration (Peglion et al., 2014; Shih and Yamada, 2012a, b), while CDH11 is required for directional migration *in vivo* (Becker et al., 2013).

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 platelet endothelial cell adhesion molecule (PECAM-1) and vascular endothelial growth factor receptor (VEGFR2) and this complex may lead to integrin activation and actin cytoskeleton reorganization (Shay-Salit et al., 2002; Tzima et al., 2005). Epidermal growth factor receptor (EGFR) forms a complex with CDH1, leading to activation of the mitogen-activated protein kinases (MAPK) pathway in epithelial cells (Pece and Gutkind, 2000; Hoschuetzky et al., 1994) with implications for cell survival (Shen and Kramer, 2004) or EMT (Wendt et al., 2010; Jia et al., 2012). Fibroblast growth factor receptors (FGFRs) were shown to stimulate CDH2 during neurite outgrowth (Williams et al., 1994, 2002), while FGF plays a critical role in the maintenance of vascular integrity by enhancing the stability of VE-cadherin at AJ sites (Hatanaka et al., 2012). Hepatocyte growth factor (HGF) modulates the expression of the cell adhesion molecule VE-cadherin and consequently endothelial cell motility, migration and angiogenesis (Martin et al., 2001). Finally, transforming growth factor beta 1 (TGF-β1) increases keratinocyte migration by increasing the levels of CDH2 and this action is counteracted by EGF (Diamond et al., 2008).

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 vivo* and *in vitro* by regulating the phosphorylation levels of the c-Janus N-terminal kinase (JNK) (You et al., 2013). 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 *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 (Trappmann et al., 2012; Wen et al., 2014; Levental et al., 2009), increasing evidence suggests that it may also affect cadherin-mediated intercellular adhesion (Smutny and Yap, 2010; Ladoux et al., 2010). Substrate stiffness was implicated in cadherin-dependent collective cell migration through myosin-II contractility (Ng et al., 2012). CDH2 is considered a mechano-responsive adhesion

receptor, as the forces transmitted through CDH2 junctions are comparable in magnitude to those sustained by integrin–ECM coupling (Chopra et al., 2011). In general, stiffer substrates lead to greater traction forces, larger cell-spread areas and better developed CDH2 junctions (Ladoux et al., 2010).

Finally, better understanding of cadherin based cell–cell interactions may be useful in development of scaffold-free tissue engineering strategies (Dvir-Ginzberg et al., 2003; Mertsching et al., 2005; Place et al., 2009). 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 (Schiele et al., 2013; Baraniak and McDevitt, 2012; Norotte et al., 2009; Stevens et al., 2009; Napolitano et al., 2007).

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 (Simonneau et al., 1995). During gastrulation, the process in 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 (Gumbiner, 1996; Guillot and Lecuit, 2013; Rossant and Tam, 2009). At later stages as cells undergo EMT, CDH1 is downregulated, while CDH2 is upregulated and is important for proper left–right axis development (Garcia-Castro et al., 2000). In general, gastrulation gives rise to three germ layers: ectoderm, endoderm and mesoderm. CDH2 and CDH11 are absent in cells of the endodermal lineage (Simonneau et al., 1995) 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 (Suzuki et al., 1997; Marthiens et al., 2002a). 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 (Manabe et al., 2000). 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 (Marthiens et al., 2002b; Padilla et al., 1998). CDH2 is present during neuroectoderm formation and is important for nervous system development (Kadowaki et al., 2007; Redies, 2000). 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 (Radice et al., 1997). Others reported that CDH2 is involved in neuronal circuit maturation by contributing to axonal extension (Kimura et al., 1995). Finally, both CDH2 and CDH11 were shown to regulate neurite outgrowth through FGFR (Boscher and Mege, 2008),phosphoinositide phospholipase C (PLC) and CAM kinase pathways (Bixby et al., 1994; Riehl et al., 1996).

Mesodermal lineage

Mesoderm is the middle developmental layer between the ectoderm and endoderm, which gives rise to the skeleton, muscle, heart and bones. In early embryos, both CDH2 and CDH11 are found in the mesoderm (Takeichi, 1991; Hatta and Takeichi, 1986) albeit with different expression patterns. The head mesoderm expresses higher levels of CDH11 comparing to CDH2, while branchial arches express only CDH11 (Kimura et al., 1995). CDH11 is present in all mesenchymal cells throughout the embryo, such as mesenchymal cells of the stomach, intestine, pharynx, lung bud and shaft of the ribs (Simonneau et al., 1995; Kuijpers et al., 2007; Monahan et al., 2007; Halbleib and Nelson, 2006) 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 (Derycke and Bracke, 2004) and its expression is regulated by platelet-derived growth factor (PDGF) and FGF signaling (Yang et al., 2008).

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 (Redmer et al., 2011). Interestingly, CDH2 was implicated in long-term engraftment of hematopoietic stem cells and establishment of hematopoiesis after bone marrow transplantation (Hosokawa et al., 2010a) 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) (Hosokawa et al., 2010b). However, others reported that CDH2 conditional knockout mice do not show defects in HSC number or function (Kiel et al., 2009).

On the other hand, accumulating evidence suggests that both cadherins play important roles in MSC differentiation. MSCs provide an excellent cell source for cellular therapies to treat bone and cartilage disorders (Horwitz et al., 2002; Wakitani et al., 2002), myocardial infarction, stroke (Li et al., 2005; Wang et al., 2002), rheumatoid arthritis (Augello et al., 2007), acute lung injury (Gupta et al., 2007; Ortiz et al., 2007), graft-versus-host disease (Dander et al., 2012) and skin-graft rejection (Bartholomew et al., 2002) among others. The use of MSCs 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 (Xu et al., 2012; Theisen et al., 2007). MSCs have also been shown to have differentiation potential and anti-inflammatory properties (Myers et al., 2010), which are enhanced when cultured as 3D spheroid

aggregates (Bartosh et al., 2010; Alimperti et al., 2014b). Interestingly, both CDH2 and CDH11 were shown to be critical in the response of synovial fibroblasts to inflammation (Agarwal and Brenner, 2006; Chang et al., 2011), suggesting that cadherins may also be important in mediating the anti-inflammatory effects of MSCs. 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).

Osteogenic lineage

CDH2 and CDH11 are highly expressed during MSC osteogenic differentiation (Ferrari et al., 2000) and several pro-osteogenic factors are known to affect their expression. For example, well-known osteogenic inducers, such as bone morphogenetic protein 2 (BMP-2), parathyroid hormone (PTH), bFGF and phorbol ester increased the levels of these cadherins (Cheng et al., 1998; Suva et al., 1994; Debiais et al., 2001). On the other hand, vitamin D decreased expression of CDH2 (Luegmayr et al., 2000) and dexamethasone inhibited the expression of both CDH2 and CDH11 mRNA in human osteoprogenitor marrow stromal cells (BMC) (Lecanda et al., 2000). Interestingly, both CDH2 and CDH11 were downregulated in mature osteocytes (Marie, 2002).

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* (Cheng et al., 1998; Marie, 2002; Kii et al., 2004). In agreement, CDH11 knockout null mice showed modest osteopenia by three months of age as signified by decreased mineralizing surface and trabecular bone volume (Kawaguchi et al., 2001a). 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 (Di Benedetto et al., 2010). This result might explain why overexpression of CDH2 promoted migration but inhibited osteogenesis as evidenced by decreased expression of osteogenic genes osteopontin, osteocalcin, runt-related transcription factor 2 (RUNX2), alkaline phosphatase (ALP) and BMP-2, as well as ALP activity and calcium deposition in BM-MSC (Xu et al., 2012).

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 (Tuan, 2003). CDH2 mediated cell–cell interactions and increased MSC aggregation, which in turn promoted differentiation into the chondrogenic lineage (Goldring et al., 2006; Quintana et al., 2009). CDH2 was

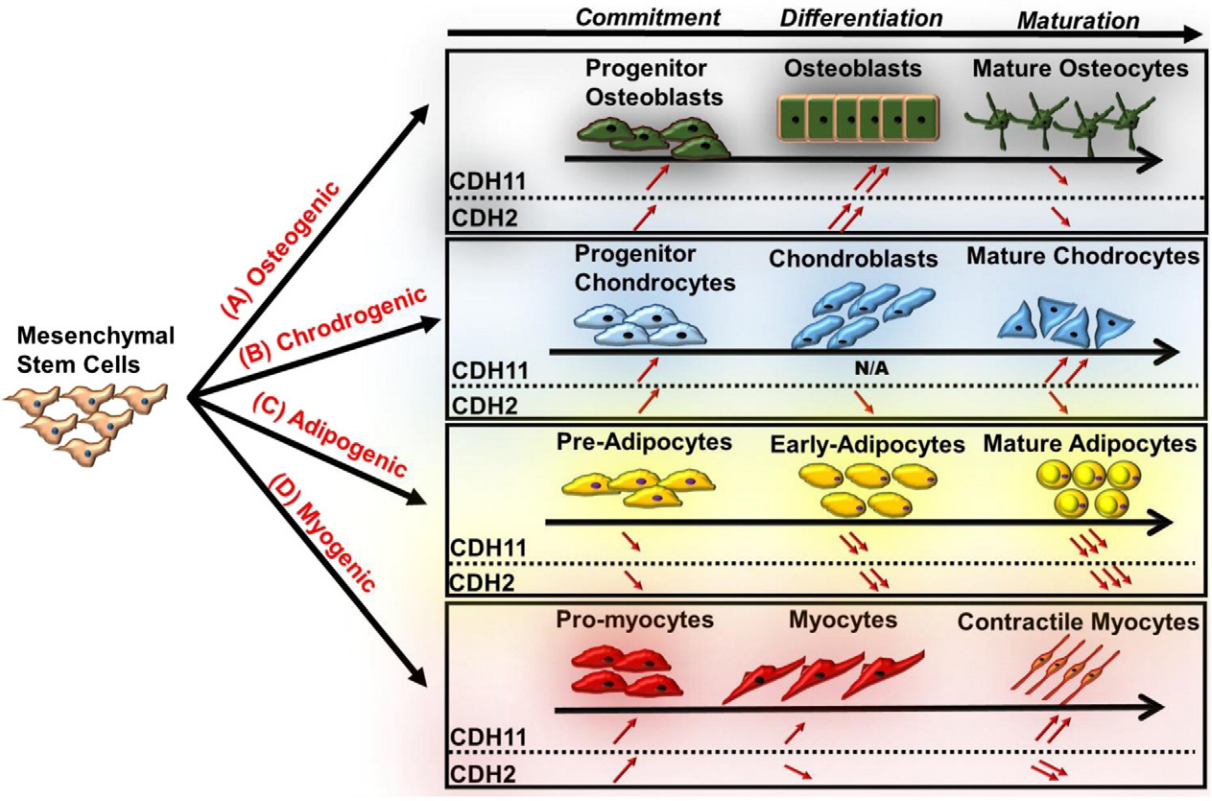

Figure 2 Schematic representation of CDH2 and CDH11 expression during MSC lineage commitment. CDH2 and CDH11 expression levels during MSC commitment, differentiation and maturation towards (A) osteogenic; (B) chondrogenic; (C) adipogenic; or (D) myogenic lineages. Upward or downward pointing arrows indicate increased or decreased expression, respectively.

required for the initial condensation phase but decreased significantly during terminal chondrogenic differentiation (Oberlender and Tuan, 1994; Woods et al., 2007). In agreement, it has been reported that the cleavage of CDH2 was required during chondrogenic differentiation (Nakazora et al., 2010), while inhibition of commitment to chondrogenic lineage by the Wnt7a inhibitor led to enhanced CDH2 expression and stabilization of AJs (Tufan and Tuan, 2001; Tufan et al., 2002a, b). Interestingly, loss of CDH2 led to increased levels of CDH11, suggesting that compensatory mechanisms might be at work (Luo et al., 2005).

Adipogenic lineage

During adipogenesis, CDH2 and CDH11 were downregulated and mature adipocytes did not express either of cadherin (Shin et al., 2000; Kawaguchi et al., 2001b). In addition, CDH11 knockdown induced adipogenic gene expression (e.g. peroxisome proliferator-activated receptor γ (PPARγ)) and differentiation, suggesting that CDH11 might inhibit adipogenesis.

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 (Borghi and James Nelson, 2009; Rougon and Hobert, 2003). CDH2 and CDH11 also play important role in wound healing when fibroblasts turn into myofibroblasts to increase wound contraction and promote wound closure (Kuijpers et al., 2007; Hinz et al., 2001a, b, 2004; Hinz and Gabbiani, 2003). Interestingly, CDH11 was upregulated in vascular smooth muscle cells (SMCs) in response to injury, while its inhibition reduced SMC proliferation and migration (Monahan et al., 2007).

Recently, our group reported that CDH11 but not CDH2 was necessary for MSC differentiation into SMCs (Alimperti et al., 2014a) (Fig. 3). CDH11 engagement regulated MSC to SMC differentiation via two pathways. One pathway was dependent on TGF-β receptor II (TGF-βRII) 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 (Kawaguchi et al., 2001a, b; Shin et al., 2000) and decreased pulmonary fibrosis after lung injury (Schneider et al., 2012). More work is required to understand

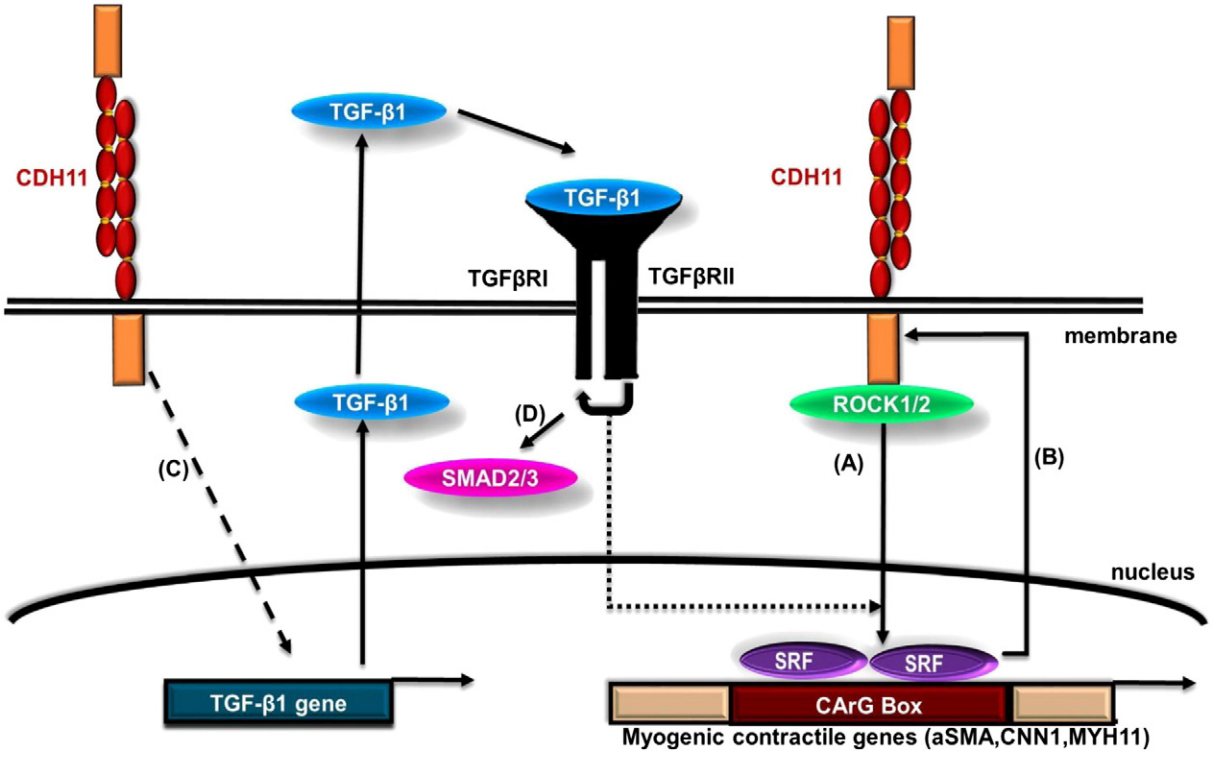

Figure 3 CDH11 mediated AJ formation promotes MSC differentiation into SMC (Alimperti et al., 2014a). (A) Engagement of CDH11 activates the ROCK pathway, which in turn activates SRF leading to increased expression of SMC genes. (B) SRF controls the level of CDH11 expression through a positive feedback 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 myo-genic 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.	Hinz et al. (2004); Schneider et al. (2012); Kovacs et al. (2002a, b); Noren et al. (2001); Brieva and Moghe (2004a, b); Guilak et al. (2009)
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/nano-scales. 2. Cell–cell adhesion independent of cell spreading. 3. Control the extent of cell–cell adhesion through substrate geometry.	Noren et al. (2001); Kovacs et al. (2002b); Brieva and Moghe (2004a, b); Lambert et al. (2000); Charrasse et al. (2002); Gavard et al. (2004); Evans et al. (2013); Pittet et al. (2008); Lira et al. (2008) Engler et al. (2006, 2007); Gao et al. (2010)

the mechanism through which CDH11 affects SMC function and the potential implications of CDH11 loss in cardiovascular, urogenital, gastrointestinal and other SMC containing tissues.

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; and (ii) Engineering surface microtopology to control the extent of cell–cell adhesion and signaling.

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 (Kovacs et al., 2002a).

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 (Kovacs et al., 2002a, b; Noren et al., 2001) and improved hepatocyte DNA synthesis and proliferation (Brieva and Moghe, 2004a, b). 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 (Lambert et al., 2000). CDH2-Fc coated beads triggered myoblast maturation as evidenced by increased expression of myogenic regulators, such as SRF (Charrasse et al., 2002; Gavard et al., 2004). Interestingly, CDH2 in lipid bilayer membranes induced mesenchymal condensation of osteochondrogenic progenitors and suppressed adipogenic differentiation (Evans et al., 2013). Likewise, CDH11-Fc proteins formed dimers that were shown to be functional i.e. engaged in strong homotypic CDH11 interactions (Pittet et al., 2008) and promoted binding of CDH11-expressing L cells (Pittet et al., 2008; Lira et al., 2008). Also, culture of MSCs on surface immobilized fusion protein between a fibronectin domain (rFN) and CDH11 (rFN/CDH11) significantly enhanced osteogenic differentiation (Zhang et al., 2009). 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.

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 nano-meter scale. This approach has been used extensively to control cell–matrix interactions, which have been shown to be critical in stem cell differentiation (Engler et al., 2004, 2006, 2007; Griffin et al., 2004; Discher et al., 2005; Guilak et al., 2009). Fewer studies have used geometric micropatterning to control the extent of cell–cell adhesion and evaluate its effects on stem cell differentiation (Chin et al., 2004).

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 (Gao et al., 2010). Similarly, by controlling the geometry and size of micro-islands it was shown that increased cell contact increased the extent of osteogenic differentiation (Tang et al., 2010; Wang et al., 2013). 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.Thus, the separation of the effects of cell-cell vs. cell-substrate adhesion becomes challenging. Interestingly, novel geometries have been employed to control the extent of cell–cell contact independent of cell density or the cell spreading area (Gray et al., 2008; Nelson et al., 2007; Charest et al., 2009), and therefore, may be used to determine the relationship between the extent of intercellular adhesion and stem cell fate commitment.

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 polymerase chain reaction (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 (Tian and Andreadis, 2009). 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 (Alimperti et al., 2012). In addition, the envelope of lentiviral particles was engineered to bind covalently to fibrin hydrogels during polymerization (Padmashali and Andreadis, 2011; Raut et al., 2010), thereby enabling generation of lentiviral arrays (LVA) that were employed to measure the activity of several Pr/RE participating in the inflammatory response (Tian et al., 2010). 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 (Padmashali et al., 2014; Moharil et al., submitted for publication). 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.

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 reveals 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.

Acknowledgments

This work was supported in part by a grant from the National Science Foundation (CBET-1403086) to S.T.A.

References

Agarwal, S.K., Brenner, M.B., 2006. Role of adhesion molecules in synovial inflammation. Curr. Opin. Rheumatol. 18, 268–276.

Alimperti, S., Lei, P., Tian, J., Andreadis, S.T., 2012. A novel lentivirus for quantitative assessment of gene knockdown in stem cell differentiation. Gene Ther. 19, 1123–1132.

Alimperti, S., You, H., George, T., Agarwal, S.K., Andreadis, S.T., 2014a. Cadherin-11 regulates both mesenchymal stem cell differentiation into smooth muscle cells and the development of contractile function in vivo. J. Cell Sci. 127, 2627–2638.

Alimperti, S., Lei, P., Wen, Y., Tian, J., Campbell, A.M., Andreadis, S.T., 2014b. Serum-free spheroid suspension culture maintains mesenchymal stem cell proliferation and differentiation potential. Biotechnol. Prog. 30, 974–983.

Anastasiadis, P.Z., Reynolds, A.B., 2000. The p120 catenin family: complex roles in adhesion, signaling and cancer. J. Cell Sci. 113 (Pt 8), 1319–1334.

Anastasiadis, P.Z., Moon, S.Y., Thoreson, M.A., Mariner, D.J., Crawford, H.C., Zheng, Y., Reynolds, A.B., 2000. Inhibition of RhoA by p120 catenin. Nat. Cell Biol. 2, 637–644.

Augello, A., Tasso, R., Negrini, S.M., Cancedda, R., Pennesi, G., 2007. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. Arthritis Rheum. 56, 1175–1186.

Baraniak, P.R., McDevitt, T.C., 2012. Scaffold-free culture of mesenchymal stem cell spheroids in suspension preserves multilineage potential. Cell Tissue Res. 347, 701–711.

Bartholomew, A., Sturgeon, C., Siatskas, M., Ferrer, K., McIntosh, K., Patil, S., Hardy, W., Devine, S., Ucker, D., Deans, R., Moseley, A., Hoffman, R., 2002. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp. Hematol. 30, 42–48.

Bartosh, T.J., Ylostalo, J.H., Mohammadipoor, A., Bazhanov, N., Coble, K., Claypool, K., Lee, R.H., Choi, H., Prockop, D.J., 2010. Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. Proc. Natl. Acad. Sci. U. S. A. 107, 13724–13729.

Becker, S.F., Mayor, R., Kashef, J., 2013. Cadherin-11 mediates contact inhibition of locomotion during Xenopus neural crest cell migration. PLoS ONE 8, e85717.

Bixby, J.L., Grunwald, G.B., Bookman, R.J., 1994. Ca2+ influx and neurite growth in response to purified N-cadherin and laminin. J. Cell Biol. 127, 1461–1475.

Boggon, T.J., Murray, J., Chappuis-Flament, S., Wong, E., Gumbiner, B.M., Shapiro, L., 2002. C-cadherin ectodomain structure and implications for cell adhesion mechanisms. Science 296, 1308–1313.

Borghi, N., James Nelson, W., 2009. Intercellular adhesion in morphogenesis: molecular and biophysical considerations. Curr. Top. Dev. Biol. 89, 1–32.

Boscher, C., Mege, R.M., 2008. Cadherin-11 interacts with the FGF receptor and induces neurite outgrowth through associated downstream signalling. Cell. Signal. 20, 1061–1072.

Braga, V.M., 2002. Cell–cell adhesion and signalling. Curr. Opin. Cell Biol. 14, 546–556.

Brasch, J., Harrison, O.J., Honig, B., Shapiro, L., 2012. Thinking outside the cell: how cadherins drive adhesion. Trends Cell Biol. 22, 299–310.

Brieva, T.A., Moghe, P.V., 2004a. Engineering the hepatocyte differentiation–proliferation balance by acellular cadherin micropresentation. Tissue Eng. 10, 553–564.

Brieva, T.A., Moghe, P.V., 2004b. Exogenous cadherin microdisplay can interfere with endogenous signaling and reprogram gene expression in cultured hepatocytes. Biotechnol. Bioeng. 85, 283–292.

Buxboim, A., Discher, D.E., 2010. Stem cells feel the difference. Nat. Methods 7, 695–697.

Buxboim, A., Ivanovska, I.L., Discher, D.E., 2010. Matrix elasticity, cytoskeletal forces and physics of the nucleus: how deeply do cells 'feel' outside and in? J. Cell Sci. 123, 297–308.

Cavallaro, U., Dejana, E., 2011. Adhesion molecule signalling: not always a sticky business. Nat. Rev. Mol. Cell Biol. 12, 189–197.

Chang, S.K., Noss, E.H., Chen, M., Gu, Z., Townsend, K., Grenha, R., Leon, L., Lee, S.Y., Lee, D.M., Brenner, M.B., 2011. Cadherin-11 regulates fibroblast inflammation. Proc. Natl. Acad. Sci. U. S. A. 108, 8402–8407.

Charest, J.L., Jennings, J.M., King, W.P., Kowalczyk, A.P., Garcia, A.J., 2009. Cadherin-mediated cell–cell contact regulates keratinocyte differentiation. J. Investig. Dermatol. 129, 564–572.

Charrasse, S., Meriane, M., Comunale, F., Blangy, A., Gauthier-Rouviere, C., 2002. N-cadherin-dependent cell–cell contact regulates Rho GTPases and beta-catenin localization in mouse C2C12 myoblasts. J. Cell Biol. 158, 953–965.

Cheng, S.L., Lecanda, F., Davidson, M.K., Warlow, P.M., Zhang, S.F., Zhang, L., Suzuki, S., St John, T., Civitelli, R., 1998. Human osteoblasts express a repertoire of cadherins, which are critical for BMP-2-induced osteogenic differentiation. J. Bone Miner. Res. 13, 633–644.

Chin, V.I., Taupin, P., Sanga, S., Scheel, J., Gage, F.H., Bhatia, S.N., 2004. Microfabricated platform for studying stem cell fates. Biotechnol. Bioeng. 88, 399–415.

Chopra, A., Tabdanov, E., Patel, H., Janmey, P.A., Kresh, J.Y., 2011. Cardiac myocyte remodeling mediated by N-cadherin-dependent mechanosensing. Am. J. Physiol. Heart Circ. Physiol. 300, H1252–H1266.

Dander, E., Lucchini, G., Vinci, P., Introna, M., Masciocchi, F., Perseghin, P., Balduzzi, A., Bonanomi, S., Longoni, D., Gaipa, G., Belotti, D., Parma, M., Algarotti, A., Capelli, C., Golay, J., Rovelli, A., Rambaldi, A., Biondi, A., Biagi, E., D'Amico, G., 2012. Mesenchymal stromal cells for the treatment of graft-versus-host disease: understanding the in vivo biological effect through patient immune monitoring. Leukemia 26, 1681–1684.

Debiais, F., Lemonnier, J., Hay, E., Delannoy, P., Caverzasio, J., Marie, P.J., 2001. Fibroblast growth factor-2 (FGF-2) increases N-cadherin expression through protein kinase C and Src-kinase pathways in human calvaria osteoblasts. J. Cell. Biochem. 81, 68–81.

Derycke, L.D., Bracke, M.E., 2004. N-cadherin in the spotlight of cell–cell adhesion, differentiation, embryogenesis, invasion and signalling. Int. J. Dev. Biol. 48, 463–476.

Di Benedetto, A., Watkins, M., Grimston, S., Salazar, V., Donsante, C., Mbalaviele, G., Radice, G.L., Civitelli, R., 2010. N-cadherin and cadherin 11 modulate postnatal bone growth and osteoblast differentiation by distinct mechanisms. J. Cell Sci. 123, 2640–2648.

Diamond, M.E., Sun, L., Ottaviano, A.J., Joseph, M.J., Munshi, H.G., 2008. Differential growth factor regulation of N-cadherin expression and motility in normal and malignant oral epithelium. J. Cell Sci. 121, 2197–2207.

Discher, D.E., Janmey, P., Wang, Y.L., 2005. Tissue cells feel and respond to the stiffness of their substrate. Science 310, 1139–1143.

Dvir-Ginzberg, M., Gamlieli-Bonshtein, I., Agbaria, R., Cohen, S., 2003. Liver tissue engineering within alginate scaffolds: effects of cell-seeding density on hepatocyte viability, morphology, and function. Tissue Eng. 9, 757–766.

Engler, A.J., Griffin, M.A., Sen, S., Bonnemann, C.G., Sweeney, H.L., Discher, D.E., 2004. Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. J. Cell Biol. 166, 877–887.

Engler, A.J., Sen, S., Sweeney, H.L., Discher, D.E., 2006. Matrix elasticity directs stem cell lineage specification. Cell 126, 677–689.

Engler, A.J., Sweeney, H.L., Discher, D.E., Schwarzbauer, J.E., 2007. Extracellular matrix elasticity directs stem cell differentiation. J. Musculoskelet Neuronal Interact. 7, 335.

Evans, S.F., Docheva, D., Bernecker, A., Colnot, C., Richter, R.P., Knothe Tate, M.L., 2013. Solid-supported lipid bilayers to drive stem cell fate and tissue architecture using periosteum derived progenitor cells. Biomaterials 34, 1878–1887.

Ferrari, S.L., Traianedes, K., Thorne, M., Lafage-Proust, M.H., Genever, P., Cecchini, M.G., Behar, V., Bisello, A., Chorev, M., Rosenblatt, M., Suva, L.J., 2000. A role for N-cadherin in the development of the differentiated osteoblastic phenotype. J. Bone Miner. Res. 15, 198–208.

Gao, L., McBeath, R., Chen, C.S., 2010. Stem cell shape regulates a chondrogenic versus myogenic fate through Rac1 and N-cadherin. Stem Cells 28, 564–572.

Garcia-Castro, M.I., Vielmetter, E., Bronner-Fraser, M., 2000. N-Cadherin, a cell adhesion molecule involved in establishment of embryonic left–right asymmetry. Science 288, 1047–1051.

Gavard, J., Marthiens, V., Monnet, C., Lambert, M., Mege, R.M., 2004. N-Cadherin activation substitutes for the cell contact

control in cell cycle arrest and myogenic differentiation: involvement of p120 and beta-catenin. J. Biol. Chem. 279, 36795–36802.
Gilbert, P.M., Havenstrite, K.L., Magnusson, K.E., Sacco, A., Leonardi, N.A., Kraft, P., Nguyen, N.K., Thrun, S., Lutolf, M.P., Blau, H.M., 2010. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science 329, 1078–1081.
Goldring, M.B., Tsuchimochi, K., Ijiri, K., 2006. The control of chondrogenesis. J. Cell. Biochem. 97, 33–44.
Gray, D.S., Liu, W.F., Shen, C.J., Bhadriraju, K., Nelson, C.M., Chen, C.S., 2008. Engineering amount of cell–cell contact demonstrates biphasic proliferative regulation through RhoA and the actin cytoskeleton. Exp. Cell Res. 314, 2846–2854.
Griffin, M.A., Sen, S., Sweeney, H.L., Discher, D.E., 2004. Adhesion–contractile balance in myocyte differentiation. J. Cell Sci. 117, 5855–5863.
Grosheva, I., Shtutman, M., Elbaum, M., Bershadsky, A.D., 2001. p120 catenin affects cell motility via modulation of activity of Rho-family GTPases: a link between cell–cell contact formation and regulation of cell locomotion. J. Cell Sci. 114, 695–707.
Guilak, F., Cohen, D.M., Estes, B.T., Gimble, J.M., Liedtke, W., Chen, C.S., 2009. Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell 5, 17–26.
Guillot, C., Lecuit, T., 2013. Mechanics of epithelial tissue homeostasis and morphogenesis. Science 340, 1185–1189.
Gumbiner, B.M., 1996. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell 84, 345–357.
Gupta, N., Su, X., Popov, B., Lee, J.W., Serikov, V., Matthay, M.A., 2007. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J. Immunol. 179, 1855–1863.
Halbleib, J.M., Nelson, W.J., 2006. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. Genes Dev. 20, 3199–3214.
Harris, T.J., Tepass, U., 2010. Adherens junctions: from molecules to morphogenesis. Nat. Rev. Mol. Cell Biol. 11, 502–514.
Hatanaka, K., Lanahan, A.A., Murakami, M., Simons, M., 2012. Fibroblast growth factor signaling potentiates VE-cadherin stability at adherens junctions by regulating SHP2. PLoS ONE 7, e37600.
Hatta, K., Takeichi, M., 1986. Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development. Nature 320, 447–449.
Hinz, B., Gabbiani, G., 2003. Mechanisms of force generation and transmission by myofibroblasts. Curr. Opin. Biotechnol. 14, 538–546.
Hinz, B., Celetta, G., Tomasek, J.J., Gabbiani, G., Chaponnier, C., 2001a. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Mol. Biol. Cell 12, 2730–2741.
Hinz, B., Mastrangelo, D., Iselin, C.E., Chaponnier, C., Gabbiani, G., 2001b. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am. J. Pathol. 159, 1009–1020.
Hinz, B., Pittet, P., Smith-Clerc, J., Chaponnier, C., Meister, J.J., 2004. Myofibroblast development is characterized by specific cell–cell adherens junctions. Mol. Biol. Cell 15, 4310–4320.
Horwitz, E.M., Gordon, P.L., Koo, W.K., Marx, J.C., Neel, M.D., McNall, R.Y., Muul, L., Hofmann, T., 2002. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc. Natl. Acad. Sci. U. S. A. 99, 8932–8937.
Hoschuetzky, H., Aberle, H., Kemler, R., 1994. Beta-catenin mediates the interaction of the cadherin–catenin complex with epidermal growth factor receptor. J. Cell Biol. 127, 1375–1380.
Hosokawa, K., Arai, F., Yoshihara, H., Iwasaki, H., Nakamura, Y., Gomei, Y., Suda, T., 2010a. Knockdown of N-cadherin suppresses the long-term engraftment of hematopoietic stem cells. Blood 116, 554–563.
Hosokawa, K., Arai, F., Yoshihara, H., Iwasaki, H., Hembree, M., Yin, T., Nakamura, Y., Gomei, Y., Takubo, K., Shiama, H., Matsuoka, S., Li, L., Suda, T., 2010b. Cadherin-based adhesion is a potential target for niche manipulation to protect hematopoietic stem cells in adult bone marrow. Cell Stem Cell 6, 194–198.
Jia, J., Zhang, W., Liu, J.Y., Chen, G., Liu, H., Zhong, H.Y., Liu, B., Cai, Y., Zhang, J.L., Zhao, Y.F., 2012. Epithelial mesenchymal transition is required for acquisition of anoikis resistance and metastatic potential in adenoid cystic carcinoma. PLoS ONE 7, e51549.
Kadowaki, M., Nakamura, S., Machon, O., Krauss, S., Radice, G.L., Takeichi, M., 2007. N-Cadherin mediates cortical organization in the mouse brain. Dev. Biol. 304, 22–33.
Kawaguchi, J., Azuma, Y., Hoshi, K., Kii, I., Takeshita, S., Ohta, T., Ozawa, H., Takeichi, M., Chisaka, O., Kudo, A., 2001a. Targeted disruption of cadherin-11 leads to a reduction in bone density in calvaria and long bone metaphyses. J. Bone Miner. Res. 16, 1265–1271.
Kawaguchi, J., Kii, I., Sugiyama, Y., Takeshita, S., Kudo, A., 2001b. The transition of cadherin expression in osteoblast differentiation from mesenchymal cells: consistent expression of cadherin-11 in osteoblast lineage. J. Bone Miner. Res. 16, 260–269.
Kiel, M.J., Acar, M., Radice, G.L., Morrison, S.J., 2009. Hematopoietic stem cells do not depend on N-cadherin to regulate their maintenance. Cell Stem Cell 4, 170–179.
Kii, I., Amizuka, N., Shimomura, J., Saga, Y., Kudo, A., 2004. Cell–cell interaction mediated by cadherin-11 directly regulates the differentiation of mesenchymal cells into the cells of the osteolineage and the chondro-lineage. J. Bone Miner. Res. 19, 1840–1849.
Kimura, Y., Matsunami, H., Inoue, T., Shimamura, K., Uchida, N., Ueno, T., Miyazaki, T., Takeichi, M., 1995. Cadherin-11 expressed in association with mesenchymal morphogenesis in the head, somite, and limb bud of early mouse embryos. Dev. Biol. 169, 347–358.
Kovacs, E.M., Goodwin, M., Ali, R.G., Paterson, A.D., Yap, A.S., 2002a. Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. Curr. Biol. 12, 379–382.
Kovacs, E.M., Ali, R.G., McCormack, A.J., Yap, A.S., 2002b. E-cadherin homophilic ligation directly signals through Rac and phosphatidylinositol 3-kinase to regulate adhesive contacts. J. Biol. Chem. 277, 6708–6718.
Kuijpers, K.A., Heesakkers, J.P., Jansen, C.F., Schalken, J.A., 2007. Cadherin-11 is expressed in detrusor smooth muscle cells and myofibroblasts of normal human bladder. Eur. Urol. 52, 1213–1221.
Ladoux, B., Anon, E., Lambert, M., Rabodzey, A., Hersen, P., Buguin, A., Silberzan, P., Mege, R.M., 2010. Strength dependence of cadherin-mediated adhesions. Biophys. J. 98, 534–542.
Lambert, M., Padilla, F., Mege, R.M., 2000. Immobilized dimers of N-cadherin-Fc chimera mimic cadherin-mediated cell contact formation: contribution of both outside-in and inside-out signals. J. Cell Sci. 113 (Pt 12), 2207–2219.
Lecanda, F., Cheng, S.L., Shin, C.S., Davidson, M.K., Warlow, P., Avioli, L.V., Civitelli, R., 2000. Differential regulation of cadherins by dexamethasone in human osteoblastic cells. J. Cell. Biochem. 77, 499–506.
Levental, K.R., Yu, H., Kass, L., Lakins, J.N., Egeblad, M., Erler, J.T., Fong, S.F., Csiszar, K., Giaccia, A., Weninger, W., Yamauchi, M., Gasser, D.L., Weaver, V.M., 2009. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 139, 891–906.
Li, Y., Chen, J., Zhang, C.L., Wang, L., Lu, D., Katakowski, M., Gao, Q., Shen, L.H., Zhang, J., Lu, M., Chopp, M., 2005. Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. Glia 49, 407–417.

Li, L., Bennett, S.A., Wang, L., 2012. Role of E-cadherin and other cell adhesion molecules in survival and differentiation of human pluripotent stem cells. Cell Adhes. Migr. 6, 59–70.

Lira, C.B., Chu, K., Lee, Y.C., Hu, M.C., Lin, S.H., 2008. Expression of the extracellular domain of OB-cadherin as an Fc fusion protein using bicistronic retroviral expression vector. Protein Expr. Purif. 61, 220–226.

Luegmayr, E., Glantschnig, H., Varga, F., Klaushofer, K., 2000. The organization of adherens junctions in mouse osteoblast-like cells (MC3T3-E1) and their modulation by triiodothyronine and 1,25-dihydroxyvitamin D3. Histochem. Cell Biol. 113, 467–478.

Luo, Y., Kostetskii, I., Radice, G.L., 2005. N-Cadherin is not essential for limb mesenchymal chondrogenesis. Dev. Dyn. 232, 336–344.

Manabe, T., Togashi, H., Uchida, N., Suzuki, S.C., Hayakawa, Y., Yamamoto, M., Yoda, H., Miyakawa, T., Takeichi, M., Chisaka, O., 2000. Loss of cadherin-11 adhesion receptor enhances plastic changes in hippocampal synapses and modifies behavioral responses. Mol. Cell. Neurosci. 15, 534–546.

Marie, P.J., 2002. Role of N-cadherin in bone formation. J. Cell. Physiol. 190, 297–305.

Marthiens, V., Gavard, J., Lambert, M., Mege, R.M., 2002a. Cadherin-based cell adhesion in neuromuscular development. Biol. Cell. 94, 315–326.

Marthiens, V., Padilla, F., Lambert, M., Mege, R.M., 2002b. Complementary expression and regulation of cadherins 6 and 11 during specific steps of motoneuron differentiation. Mol. Cell. Neurosci. 20, 458–475.

Martin, T.A., Mansel, R., Jiang, W.G., 2001. Hepatocyte growth factor modulates vascular endothelial-cadherin expression in human endothelial cells. Clin. Cancer Res. 7, 734–737.

McBeath, R., Pirone, D.M., Nelson, C.M., Bhadriraju, K., Chen, C.S., 2004. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev. Cell 6, 483–495.

Mertsching, H., Walles, T., Hofmann, M., Schanz, J., Knapp, W.H., 2005. Engineering of a vascularized scaffold for artificial tissue and organ generation. Biomaterials 26, 6610–6617.

Moharil, J., Lei, P., Gaile, D., Andreadis, S.T., 2015. Lentiviral microarrays for high throughput monitoring MSC differentiation along the myogenic lineage (submitted for publication).

Monahan, T.S., Andersen, N.D., Panossian, H., Kalish, J.A., Daniel, S., Shrikhande, G.V., Ferran, C., Logerfo, F.W., 2007. A novel function for cadherin 11/osteoblast-cadherin in vascular smooth muscle cells: modulation of cell migration and proliferation. J. Vasc. Surg. 45, 581–589.

Myers, T.J., Granero-Molto, F., Longobardi, L., Li, T., Yan, Y., Spagnoli, A., 2010. Mesenchymal stem cells at the intersection of cell and gene therapy. Expert. Opin. Biol. Ther. 10, 1663–1679.

Nakazora, S., Matsumine, A., Iino, T., Hasegawa, M., Kinoshita, A., Uemura, K., Niimi, R., Uchida, A., Sudo, A., 2010. The cleavage of N-cadherin is essential for chondrocyte differentiation. Biochem. Biophys. Res. Commun. 400, 493–499.

Napolitano, A.P., Dean, D.M., Man, A.J., Youssef, J., Ho, D.N., Rago, A.P., Lech, M.P., Morgan, J.R., 2007. Scaffold-free three-dimensional cell culture utilizing micromolded nonadhesive hydrogels. Biotechniques 43 (494), 496–500.

Nelson, C.M., Liu, W.F., Chen, C.S., 2007. Manipulation of cell–cell adhesion using bowtie-shaped microwells. Methods Mol. Biol. 370, 1–10.

Ng, M.R., Besser, A., Danuser, G., Brugge, J.S., 2012. Substrate stiffness regulates cadherin-dependent collective migration through myosin-II contractility. J. Cell Biol. 199, 545–563.

Niessen, C.M., Gumbiner, B.M., 2002. Cadherin-mediated cell sorting not determined by binding or adhesion specificity. J. Cell Biol. 156, 389–399.

Noren, N.K., Liu, B.P., Burridge, K., Kreft, B., 2000. p120 catenin regulates the actin cytoskeleton via Rho family GTPases. J. Cell Biol. 150, 567–580.

Noren, N.K., Niessen, C.M., Gumbiner, B.M., Burridge, K., 2001. Cadherin engagement regulates Rho family GTPases. J. Biol. Chem. 276, 33305–33308.

Norotte, C., Marga, F.S., Niklason, L.E., Forgacs, G., 2009. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials 30, 5910–5917.

Oberlender, S.A., Tuan, R.S., 1994. Spatiotemporal profile of N-cadherin expression in the developing limb mesenchyme. Cell Adhes. Commun. 2, 521–537.

Ortiz, L.A., Dutreil, M., Fattman, C., Pandey, A.C., Torres, G., Go, K., Phinney, D.G., 2007. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc. Natl. Acad. Sci. U. S. A. 104, 11002–11007.

Ouyang, M., Lu, S., Kim, T., Chen, C.E., Seong, J., Leckband, D.E., Wang, F., Reynolds, A.B., Schwartz, M.A., Wang, Y., 2013. N-cadherin regulates spatially polarized signals through distinct p120ctn and beta-catenin-dependent signalling pathways. Nat. Commun. 4, 1589.

Padilla, F., Broders, F., Nicolet, M., Mege, R.M., 1998. Cadherins M, 11, and 6 expression patterns suggest complementary roles in mouse neuromuscular axis development. Mol. Cell. Neurosci. 11, 217–233.

Padmashali, R.M., Andreadis, S.T., 2011. Engineering fibrinogen-binding VSV-G envelope for spatially- and cell-controlled lentivirus delivery through fibrin hydrogels. Biomaterials 32, 3330–3339.

Padmashali, R.M., Mistriotis, P., Liang, M.S., Andreadis, S.T., 2014. Lentiviral arrays for live-cell dynamic monitoring of gene and pathway activity during stem cell differentiation. Mol. Ther. 22, 1971–1982.

Pece, S., Gutkind, J.S., 2000. Signaling from E-cadherins to the MAPK pathway by the recruitment and activation of epidermal growth factor receptors upon cell–cell contact formation. J. Biol. Chem. 275, 41227–41233.

Peglion, F., Llense, F., Etienne-Manneville, S., 2014. Adherens junction treadmilling during collective migration. Nat. Cell Biol. 16, 639–651.

Pittet, P., Lee, K., Kulik, A.J., Meister, J.J., Hinz, B., 2008. Fibrogenic fibroblasts increase intercellular adhesion strength by reinforcing individual OB-cadherin bonds. J. Cell Sci. 121, 877–886.

Place, E.S., Evans, N.D., Stevens, M.M., 2009. Complexity in biomaterials for tissue engineering. Nat. Mater. 8, 457–470.

Pokutta, S., Herrenknecht, K., Kemler, R., Engel, J., 1994. Conformational changes of the recombinant extracellular domain of E-cadherin upon calcium binding. Eur. J. Biochem. 223, 1019–1026.

Quintana, L., zur Nieden, N.I., Semino, C.E., 2009. Morphogenetic and regulatory mechanisms during developmental chondrogenesis: new paradigms for cartilage tissue engineering. Tissue Eng. B Rev. 15, 29–41.

Radice, G.L., Rayburn, H., Matsunami, H., Knudsen, K.A., Takeichi, M., Hynes, R.O., 1997. Developmental defects in mouse embryos lacking N-cadherin. Dev. Biol. 181, 64–78.

Raut, S.D., Lei, P., Padmashali, R.M., Andreadis, S.T., 2010. Fibrin-mediated lentivirus gene transfer: implications for lentivirus microarrays. J. Control. Release 144, 213–220.

Redies, C., 2000. Cadherins in the central nervous system. Prog. Neurobiol. 61, 611–648.

Redmer, T., Diecke, S., Grigoryan, T., Quiroga-Negreira, A., Birchmeier, W., Besser, D., 2011. E-cadherin is crucial for embryonic stem cell pluripotency and can replace OCT4 during somatic cell reprogramming. EMBO Rep. 12, 720–726.

Reynolds, A.B., Carnahan, R.H., 2004. Regulation of cadherin stability and turnover by p120ctn: implications in disease and cancer. Semin. Cell Dev. Biol. 15, 657–663.

Riehl, R., Johnson, K., Bradley, R., Grunwald, G.B., Cornel, E., Lilienbaum, A., Holt, C.E., 1996. Cadherin function is required

for axon outgrowth in retinal ganglion cells in vivo. Neuron 17, 837–848.

Rossant, J., Tam, P.P., 2009. Blastocyst lineage formation, early embryonic asymmetries and axis patterning in the mouse. Development 136, 701–713.

Rougon, G., Hobert, O., 2003. New insights into the diversity and function of neuronal immunoglobulin superfamily molecules. Annu. Rev. Neurosci. 26, 207–238.

Schiele, N.R., Koppes, R.A., Chrisey, D.B., Corr, D.T., 2013. Engineering cellular fibers for musculoskeletal soft tissues using directed self-assembly. Tissue Eng. A 19, 1223–1232.

Schneider, D.J., Wu, M., Le, T.T., Cho, S.H., Brenner, M.B., Blackburn, M.R., Agarwal, S.K., 2012. Cadherin-11 contributes to pulmonary fibrosis: potential role in TGF-beta production and epithelial to mesenchymal transition. FASEB J. 26, 503–512.

Shapiro, L., Weis, W.I., 2009. Structure and biochemistry of cadherins and catenins. Cold Spring Harb. Perspect. Biol. 1, a003053.

Shay-Salit, A., Shushy, M., Wolfovitz, E., Yahav, H., Breviario, F., Dejana, E., Resnick, N., 2002. VEGF receptor 2 and the adherens junction as a mechanical transducer in vascular endothelial cells. Proc. Natl. Acad. Sci. U. S. A. 99, 9462–9467.

Shen, X., Kramer, R.H., 2004. Adhesion-mediated squamous cell carcinoma survival through ligand-independent activation of epidermal growth factor receptor. Am. J. Pathol. 165, 1315–1329.

Shih, W., Yamada, S., 2012a. N-Cadherin as a key regulator of collective cell migration in a 3D environment. Cell Adhes. Migr. 6, 513–517.

Shih, W., Yamada, S., 2012b. N-Cadherin-mediated cell–cell adhesion promotes cell migration in a three-dimensional matrix. J. Cell Sci. 125, 3661–3670.

Shin, C.S., Lecanda, F., Sheikh, S., Weitzmann, L., Cheng, S.L., Civitelli, R., 2000. Relative abundance of different cadherins defines differentiation of mesenchymal precursors into osteogenic, myogenic, or adipogenic pathways. J. Cell. Biochem. 78, 566–577.

Simonneau, L., Kitagawa, M., Suzuki, S., Thiery, J.P., 1995. Cadherin 11 expression marks the mesenchymal phenotype: towards new functions for cadherins? Cell Adhes. Commun. 3, 115–130.

Smutny, M., Yap, A.S., 2010. Neighborly relations: cadherins and mechanotransduction. J. Cell Biol. 189, 1075–1077.

Stepniak, E., Radice, G.L., Vasioukhin, V., 2009. Adhesive and signaling functions of cadherins and catenins in vertebrate development. Cold Spring Harb. Perspect. Biol. 1, a002949.

Stevens, K.R., Kreutziger, K.L., Dupras, S.K., Korte, F.S., Regnier, M., Muskheli, V., Nourse, M.B., Bendixen, K., Reinecke, H., Murry, C.E., 2009. Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. Proc. Natl. Acad. Sci. U. S. A. 106, 16568–16573.

Suva, L.J., Towler, D.A., Harada, S., Gaub, M.P., Rodan, G.A., 1994. Characterization of retinoic acid- and cell-dependent sequences which regulate zif268 gene expression in osteoblastic cells. Mol. Endocrinol. 8, 1507–1520.

Suzuki, S.C., Inoue, T., Kimura, Y., Tanaka, T., Takeichi, M., 1997. Neuronal circuits are subdivided by differential expression of type-II classic cadherins in postnatal mouse brains. Mol. Cell. Neurosci. 9, 433–447.

Takeichi, M., 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251, 1451–1455.

Tang, J., Peng, R., Ding, J., 2010. The regulation of stem cell differentiation by cell–cell contact on micropatterned material surfaces. Biomaterials 31, 2470–2476.

Theisen, C.S., Wahl 3rd, J.K., Johnson, K.R., Wheelock, M.J., 2007. NHERF links the N-cadherin/catenin complex to the platelet-derived growth factor receptor to modulate the actin cytoskeleton and regulate cell motility. Mol. Biol. Cell 18, 1220–1232.

Thompson, S.A., Blazeski, A., Copeland, C.R., Cohen, D.M., Chen, C.S., Reich, D.M., Tung, L., 2014. Acute slowing of cardiac conduction in response to myofibroblast coupling to cardiomyocytes through N-cadherin. J. Mol. Cell. Cardiol. 68, 29–37.

Tian, J., Andreadis, S.T., 2009. Independent and high-level dual-gene expression in adult stem-progenitor cells from a single lentiviral vector. Gene Ther. 16, 874–884.

Tian, J., Alimperti, S., Lei, P., Andreadis, S.T., 2010. Lentiviral microarrays for real-time monitoring of gene expression dynamics. Lab Chip 10, 1967–1975.

Tomita, K., van Bokhoven, A., van Leenders, G.J., Ruijter, E.T., Jansen, C.F., Bussemakers, M.J., Schalken, J.A., 2000. Cadherin switching in human prostate cancer progression. Cancer Res. 60, 3650–3654.

Trappmann, B., Gautrot, J.E., Connelly, J.T., Strange, D.G., Li, Y., Oyen, M.L., Cohen Stuart, M.A., Boehm, H., Li, B., Vogel, V., Spatz, J.P., Watt, F.M., Huck, W.T., 2012. Extracellular-matrix tethering regulates stem-cell fate. Nat. Mater. 11, 642–649.

Treiser, M.D., Yang, E.H., Gordonov, S., Cohen, D.M., Androulakis, I.P., Kohn, J., Chen, C.S., Moghe, P.V., 2010. Cytoskeleton-based forecasting of stem cell lineage fates. Proc. Natl. Acad. Sci. U. S. A. 107, 610–615.

Tuan, R.S., 2003. Cellular signaling in developmental chondrogenesis: N-cadherin, Wnts, and BMP-2. J. Bone Joint Surg. Am. 85-A (Suppl. 2), 137–141.

Tufan, A.C., Tuan, R.S., 2001. Wnt regulation of limb mesenchymal chondrogenesis is accompanied by altered N-cadherin-related functions. FASEB J. 15, 1436–1438.

Tufan, A.C., Daumer, K.M., DeLise, A.M., Tuan, R.S., 2002a. AP-1 transcription factor complex is a target of signals from both WnT-7a and N-cadherin-dependent cell–cell adhesion complex during the regulation of limb mesenchymal chondrogenesis. Exp. Cell Res. 273, 197–203.

Tufan, A.C., Daumer, K.M., Tuan, R.S., 2002b. Frizzled-7 and limb mesenchymal chondrogenesis: effect of misexpression and involvement of N-cadherin. Dev. Dyn. 223, 241–253.

Tzima, E., Irani-Tehrani, M., Kiosses, W.B., Dejana, E., Schultz, D.A., Engelhardt, B., Cao, G., DeLisser, H., Schwartz, M.A., 2005. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature 437, 426–431.

Wakitani, S., Imoto, K., Yamamoto, T., Saito, M., Murata, N., Yoneda, M., 2002. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthr. Cartil. 10, 199–206.

Wang, L., Li, Y., Chen, X., Chen, J., Gautam, S.C., Xu, Y., Chopp, M., 2002. MCP-1, MIP-1, IL-8 and ischemic cerebral tissue enhance human bone marrow stromal cell migration in interface culture. Hematology 7, 113–117.

Wang, X., Song, W., Kawazoe, N., Chen, G., 2013. The osteogenic differentiation of mesenchymal stem cells by controlled cell–cell interaction on micropatterned surfaces. J. Biomed. Mater. Res. A 101, 3388–3395.

Wen, J.H., Vincent, L.G., Fuhrmann, A., Choi, Y.S., Hribar, K.C., Taylor-Weiner, H., Chen, S., Engler, A.J., 2014. Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat. Mater. 13, 979–987.

Wendt, M.K., Smith, J.A., Schiemann, W.P., 2010. Transforming growth factor-beta-induced epithelial–mesenchymal transition facilitates epidermal growth factor-dependent breast cancer progression. Oncogene 29, 6485–6498.

Williams, E.J., Furness, J., Walsh, F.S., Doherty, P., 1994. Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. Neuron 13, 583–594.

Williams, G., Williams, E.J., Doherty, P., 2002. Dimeric versions of two short N-cadherin binding motifs (HAVDI and INPISG) function as N-cadherin agonists. J. Biol. Chem. 277, 4361–4367.

Woods, A., Wang, G., Dupuis, H., Shao, Z., Beier, F., 2007. Rac1 signaling stimulates N-cadherin expression, mesenchymal condensation, and chondrogenesis. J. Biol. Chem. 282, 23500–23508.

Xu, L., Meng, F., Ni, M., Lee, Y., Li, G., 2012. N-Cadherin regulates osteogenesis and migration of bone marrow-derived mesenchymal stem cells. Mol. Biol. Rep.

Yang, X., Chrisman, H., Weijer, C.J., 2008. PDGF signalling controls the migration of mesoderm cells during chick gastrulation by regulating N-cadherin expression. Development 135, 3521–3530.

Yap, A.S., Kovacs, E.M., 2003. Direct cadherin-activated cell signaling: a view from the plasma membrane. J. Cell Biol. 160, 11–16.

Yonemura, S., 2011. Cadherin–actin interactions at adherens junctions. Curr. Opin. Cell Biol. 23, 515–522.

You, H., Padmashali, R.M., Ranganathan, A., Lei, P., Girnius, N., Davis, R.J., Andreadis, S.T., 2013. JNK regulates compliance-induced adherens junctions formation in epithelial cells and tissues. J. Cell Sci. 126, 2718–2729.

Zeisberg, M., Neilson, E.G., 2009. Biomarkers for epithelial–mesenchymal transitions. J. Clin. Invest. 119, 1429–1437.

Zhang, Y., Zhou, Y., Zhu, J., Dong, S., Li, C., Xiang, Q., 2009. Effect of a novel recombinant protein of fibronectinIII7-10/cadherin 11 EC1-2 on osteoblastic adhesion and differentiation. Biosci. Biotechnol. Biochem. 73, 1999–2006.