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Cadherin-Mediated Cell-Cell Adhesion and Signaling in the Skeleton

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Abstract

Direct cell-to-cell interactions via cell adhesion molecules, in particular cadherins, are critical for morphogenesis, tissue architecture, and cell sorting and differentiation. Partially overlapping, yet distinct roles of N-cadherin (cadherin-2) and cadherin-11 in the skeletal system have emerged from mouse genetics and *in vitro* studies. Both cadherins are important for precursor commitment to the osteogenic lineage, and genetic ablation of *Cdh2* and *Cdh11* results in skeletal growth defects and impaired bone formation. While *Cdh11* defines the osteogenic lineage, persistence of *Cdh2* in osteoblasts *in vivo* actually inhibits their terminal differentiation and impairs bone formation. The action of cadherins involves both cell-cell adhesion and interference with intracellular signaling, and in particular the Wnt/ β -catenin pathway. Both cadherin-2 and cadherin-11 bind to β -catenin, thus modulating its cytoplasmic pools and transcriptional activity. Recent data demonstrate that cadherin-2 also interferes with Lrp5/6 signaling by sequestering these receptors in inactive pools via axin binding. These data extend the biologic action of cadherins in bone forming cells, and provide novel mechanisms for development of therapeutic strategies aimed at enhancing bone formation.

Keywords

Cadherins; cell-cell adhesion; osteoblast differentiation; Wnt/ β -catenin signaling; bone formation

Introduction

Bone formation is a complex process involving the commitment of mesenchymal stem cells to osteoprogenitors and their progressive differentiation into mature, matrix producing osteoblasts. Mesenchymal or skeletal stem cells can also give rise to chondrocytes or

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adipocytes under induction by systemic or local factors. Osteogenic differentiation is characterized by sequential up-regulation of specific transcription factors that modulate expression of osteoblast genes [1, 2]. Once new bone is deposited, some osteoblasts become flattened lining cells, a few remain embedded into the mineralized matrix and become osteocytes, while the majority undergo apoptosis [3]. Multiple signaling pathways regulate osteoprogenitor cell commitment, differentiation and survival [4]. Additionally, direct cell-cell interactions via cell adhesion molecules and gap junctions occur during osteoblastogenesis allowing synchronization of cell activity among osteoblasts, and between osteoblasts and osteocytes in the mature skeleton [5, 6, 7].

Cadherins are single chain transmembrane glycoproteins that mediate calcium-dependent homophilic cell-cell adhesion. Based on their molecular structure, “classical” cadherins are classified as Type I, which includes E-cadherin/cadherin-1 (gene name, *Cdh1*), N-cadherin/cadherin-2 (*Cdh2*), P-cadherin/cadherin-3 (*Cdh3*), R-cadherin/cadherin-4 (*Cdh4*); and Type II cadherins [8]. Cadherin-11 (*Cdh11*), previously called “osteoblast cadherin” [9, 10], is a type II cadherin prevalently expressed in mesodermal derived tissues [8, 11]. Other members of this superfamily of cell adhesion molecules include desmosomal cadherins, protocadherins, and other cadherin-related molecules [12-14]. Cadherins associate as homodimers by lateral clustering, and these *cis*-dimers bind to cadherins on opposing cells to form *trans*-dimers, thus allowing cell-cell adhesion. Cadherins are anchored to the actin cytoskeleton via binding to α -, β -, and γ -catenin (plakoglobin) in a complex that forms the adherens junction [15, 16]. Since β -catenin is also a key component of the canonical Wnt signaling system, cadherins can also modulate signal transduction via Wnt/ β -catenin in multiple ways [17, 18]. In this review, we focus on recent advances on the biologic role of direct cell-cell interactions via cadherins in the skeleton, and highlight the concept that cadherins interfere with signaling pathways that are key to osteogenic differentiation, skeletal development, and homeostasis.

Cadherins in chondrogenesis

Since cadherin-mediated cell-cell adhesion is essential for tissue development [19], regulation of cell proliferation, differentiation, and survival [20], it is not surprising that cadherins are intimately involved in skeletal development. Both *Cdh2* and *Cdh11* are expressed during early mesenchyme condensation [21, 22]. Specifically, *Cdh2* increases during early chondrogenic differentiation and progressively decreases at late stages of chondrogenesis [23, 24, 25]. Substantiating a role in early chondroblast differentiation, *in vitro* perturbation of cadherin-2 mediated cell-cell contacts inhibits mesenchymal condensation as well as chondrogenesis in micromass cultures [26-28] and in embryonic chick limbs [29]. However contrary to these *in vitro* data, *ex vivo* limb bud cultures from *Cdh2* null embryos partially rescued by transgenic expression of *Cdh1* undergo cartilage condensation and develop into structured limbs [30], suggesting that cadherin-2 is not essential for limb morphogenesis. Resolution of this discrepancy would require models of conditional *Cdh2* inactivation in chondrocytes. Intriguingly, persistence of *Cdh2* prevents further progression from precartilaginous condensation to chondrocyte development [31, 32], implying that while *Cdh2* may be involved in early stages of chondrogenesis [26] it may restrain progression of chondrogenic differentiation. As noted later, this concept also applies

to osteogenesis (Figure 1). Cadherin-2 control of chondrogenesis involves both modulation of cell-cell adhesion, and interplay with Wnt and bone morphogenetic protein (BMP) signaling [26, 33]. Specifically, BMP-2 increases *Cdh2* expression during chondrogenic differentiation; and a dominant negative *Cdh2* mutant inhibits BMP-2-stimulated chondrogenesis *in vitro* [27].

Cadherin-mediated cell-cell adhesion in osteoblastogenesis

Osteogenic precursors express a repertoire of cadherins, including *Cdh1*, *Cdh2*, *Cdh3* and *Cdh11* [34, 35, 36, 37-41]. These multiple cadherins are present at low levels in undifferentiated cells, but as cells commit to the different lineages the relative expression of cadherins changes. Specifically, R-cadherin/cadherin-4 is rapidly down regulated as cells undergo osteogenic differentiation, whereas *Cdh11* is up regulated and it is present throughout the osteoblast differentiation program [34, 41, 42]. On the other hand, *Cdh2* initially increases with osteogenic commitment [37, 43, 44-47], but as differentiation progresses it is down-regulated and it is barely detectable in fully differentiated osteoblasts, *in vitro* [42, 48, 49]. In sections of adult mouse bone, cadherin-2 is abundant in cells lining the bone surfaces, primarily in trabecular bone, but it is not detectable in the bone marrow, or in periosteal cells or osteocytes [49]. Accordingly, neither *Cdh2* nor *Cdh11* expression was found in the osteocytic cells line MLO-Y4 [41]. *In vivo*, the only place where cadherins (or any cell adhesion molecule) would be present are the tips of the osteocyte processes; thus, their abundance would be rather low. These findings underscore partially overlapping but distinct functions of *Cdh2* and *Cdh11* at different stages of osteogenesis. However, the mechanisms involved in the differential regulation of cadherins during osteogenesis are unknown.

On the contrary, both *Cdh2* and *Cdh11* are down-regulated with commitment to adipogenesis, and they are absent in adipocytes [41, 42], suggesting that coexpression of *Cdh2* and *Cdh11* may be permissive of commitment to osteogenic differentiation and oppose adipogenesis. Indeed, teratomas originating from cells overexpressing *Cdh11* form preferentially bone and cartilage tissue [41]; and interference with cadherin-2-mediated cell-cell adhesion using either a HAV inhibitory peptide (specific for type I cadherins, thus not interfering with cadherin-11 adhesion), neutralizing antibodies, antisense oligonucleotides, or transfection of a *Cdh2* mutant with dominant negative action reduces osteoblast differentiation *in vitro* [37, 44, 45]. However, bone marrow stromal cells isolated from *Cdh2* haploinsufficient mice or from conditionally osteoblast/osteocyte *Cdh2* deleted mice differentiate faster *in vitro* [48, 49], and the number of osteoprogenitors is increased in *Cdh2* haploinsufficient or osteoblast *Cdh2* deficient mice [48, 50], suggesting that *Cdh2* may inhibit later stages of differentiation. The accumulated data support a model whereby both *Cdh2* and *Cdh11* contribute to osteogenic commitment, but for the osteogenic program to proceed to terminal differentiation *Cdh2* must be down-regulated, leaving *Cdh11* as the most abundant cadherin present in fully differentiated bone forming cells (Figure 1). This model is also borne out of *in vivo* data in *Cdh2* mutant mice (see below).

Regulation of cadherins in osteoblastic cells

Several factors that regulate osteoblast differentiation and survival modulate cadherin expression and/or function. Notably, the stimulatory effects of parathyroid hormone (PTH) and BMP-2 on early stages of osteoblastogenesis are linked to increased *Cdh2* expression [34, 37, 45]. In contrast, dexamethasone inhibits *Cdh2* and *Cdh11* expression in long term culture of bone marrow stromal cells and human trabecular osteoblasts [51]. Both tumor necrosis factor- α (TNF α) and interleukin-1 (IL-1) also suppress *Cdh2* but not *Cdh11* cadherin expression, resulting in decreased cadherin-mediated cell-cell adhesion and osteoblast differentiation [52]. On the other hand, fibroblast growth factor-2 (FGF-2) increases *Cdh2* expression in human osteoblasts via protein kinase C α (PKC α) and Src activation, resulting in increased cell-cell adhesion [53]. Corroborating a role for PKC α in cadherin-2 modulation of osteoblast differentiation, direct activation of PKC α increases *Cdh2*, cell-cell adhesion, and ALP activity [54]. These findings are consistent with observations of increased *Cdh2* expression and osteoblast differentiation in cells derived from subjects with Apert syndrome, a craniosynostosis linked to constitutive activation of FGF receptor-2 (FGFR2) [55]. This observation provides further demonstration that cadherin-2-mediated cell-cell adhesion favors osteogenesis [56]. Other signaling and downstream molecular mechanisms are likely to control *Cdh2* expression in osteoblasts, as indicated by analysis of the human *Cdh2* promoter [57]. Cadherin-2 may also be involved in modulation of osteoblast survival, as suggested by reduced cadherin-2-mediated cell-cell adhesion during apoptosis, and by induction of osteoblast apoptosis upon disruption of cell-cell adhesion by an anti-cadherin-2 antibody [58]. Finally, data also suggest that IL-1 and TNF α may induce osteoblast apoptosis [59] by downregulating *Cdh2* expression [58].

Cadherins in bone development and homeostasis

Definitive proof of the biologic role of cadherins in bone development and homeostasis has emerged from mouse genetics studies. Germline *Cdh2* ablation is embryonic lethal [60]. However, transgenic mice expressing a dominant-negative *Cdh2* mutant – lacking most of the extracellular adhesion domain – in osteoblasts/osteocytes driven by the *Bglap* (osteocalcin) promoter exhibit delayed acquisition of peak bone mass, reduced bone formation, and impaired osteoblast differentiation [61]. Likewise, targeted overexpression of *Cdh2* in osteoblasts driven by the *Col1A1* promoter also results in decreased bone formation and low bone mass in young mice [62]. The similarity of these phenotypes underlines similar mechanisms causative of the osteoblast defect, related to interference with Wnt/ β -catenin signalling by the overabundant wild type or mutant cadherin proteins (see below). However, conditional *Cdh2* ablation in osteoblast/osteocytes driven by either the *Col1A1* or the more broadly expressed *Osx* promoter also results in smaller bones, age-dependent osteopenia and decreased bone strength [49, 50]. These apparently contrasting results likely reflect the different functions of cadherin-2 at different stages of osteoblast differentiation (Figure 1). While persistence of *Cdh2* in mature osteoblasts retards differentiation (thus resulting in low bone mass in *Cdh2* overexpressing mice), lack of *Cdh2* in bone forming cells is associated with decreased number of stromal cell precursors [50], thus resulting in decreased availability of new osteoblasts. This may explain the low bone mass and developmental defects in conditional *Cdh2* knockout mice. Notably, decrease in bone

marrow stromal precursors occurs in mice where *Cdh2* deletion is restricted to mature osteoblasts and osteocytes [50]; and transgenic expression of a dominant-negative *Cdh2* mutant in differentiated osteoblasts results in an osteoblast to adipocyte shift in bone marrow precursors [61]. Hence, cadherin-2 may possibly permit direct cell-cell interactions between osteoblasts and stromal stem cells, ultimately regulating their self-renewal and fate. Lack of cadherin-2 in supporting cells (differentiated osteoblasts) could lead to loss of stem cells or precursors, and decreased osteoblastogenesis potential [63]. Examples of such stem cell-niche cells interactions via cadherin-2 exist in other systems [64, 65], and a similar mechanism has been proposed for osteoblast support of hematopoietic stem cells [66]. However, this concept has been recently challenged [49, 67], and it is the subject of another review in this series.

Despite considerable *in vitro* evidence for a role of cadherin-11 in osteoblastogenesis (discussed earlier), germline deletion of *Cdh11* leads to only modest osteopenia in young mice [61]. Indeed, adult *Cdh11* knockout mice show no obvious skeletal defects, although they have increased number of bone marrow adipogenic precursors and spontaneous emergence of adipocytes in calvaria cell cultures [50]. These data confirm an anti-adipogenic action of *Cdh11*, and it is likely that *in vivo* *Cdh2* may partially compensate for lack of *Cdh11* in regulating osteoblastogenesis and bone development [50]. Supporting this hypothesis, double *Cdh2*;*Cdh11* null embryos exhibit a more severe disruption of somite structure than single cadherin gene deletion [68]; and ablation of one *Cdh2* allele in a *Cdh11* null background in mice leads to overt skeletal growth defect, severe osteopenia and reduced resistance to fractures [50]. Intriguingly, a recent report indicates that *Cdh1* may also control osteoblast function via induction of Smurf1 E3 ubiquitin ligase activity, leading to reduced MEKK2 activation and osteogenic differentiation of calvaria cell cultures [69]. It remains to be determined whether this mechanism may be targeted for promoting osteogenesis *in vivo*. Notably, *Cdh1* is not expressed by human trabecular bone osteoblasts [34].

Earlier work had shown that E-cadherin is important for osteoclast precursor fusion [70], and that cadherin-6, the murine homologue of human K-cadherin, and its splice variant, cadherin-6/2, mediate heterophilic interactions between osteoclast precursors and stromal supporting cells [71]. More recently, another group reported that N-cadherin in osteogenic cells modulates osteoclastogenesis via β -catenin dependent regulation of RANKL expression [72]. Finally, cadherins have been shown in the actin ring of the osteoclast sealing zone, suggesting a possible role in osteoclast attachment to the matrix [73]. However, *in vivo* corroboration of cadherin role in osteoclastogenesis and bone resorption is still missing.

Cadherin-Wnt/ β -catenin signalling interactions in osteoblastogenesis

In addition to their function in the adherens junction, cadherins interfere with intracellular signaling pathways in several cell types [74, 75]. Indeed, cadherins may retain signalling properties even after modifications that abrogate their adhesive properties by cleavage, shedding, or mutations [76]. It is rather intuitive that cadherins may affect the Wnt pathway simply because cadherins bind to β -catenin, a key component of the canonical Wnt signaling [77, 78]. The Wnt/ β -catenin pathway is activated by binding of Wnt ligands to LRP5/6 and

Frizzled co-receptors, followed by recruitment of a complex including axin/Frat1/APC/ glycogen synthase kinase 3 β (GSK3 β). This in turn leads to inhibition of GSK-3, decreased β -catenin phosphorylation at specific sites near its N-terminus, and subsequent β -catenin translocation to the nucleus where it binds TCF/LEF transcription factors to activate gene transcription [79]. Association of cadherin with β -catenin contributes to stabilize the adhesion structure [80]. Conversely, release of β -catenin from cadherins destabilizes the adhesion complex, thus facilitating cell movement [81]. On the other hand, cell-cell adhesion via cadherins promotes β -catenin phosphorylation and inactivation [82]. Thus, an inverse relationship exists between cell adhesion and Wnt/ β -catenin signaling, though the interplay between cadherins and signaling is more complex than such simple notion may predict. Indeed, increased cadherin abundance on the cell surface has a sequestration effect on β -catenin, resulting in decreased β -catenin nuclear translocation and transcriptional activity [81, 83-85] (Figure 2). Considering the important role of Wnt signaling in osteoblastogenesis and bone formation [86-88], the potential impact of such interaction on bone homeostasis is easily predictable. As already noted, overexpression of a dominant-negative *Cdh2* mutant in differentiated osteoblasts leads to β -catenin sequestration at the cell surface and decreased bone formation [61]. A negative effect on bone formation has also been shown by *Cdh2* overexpression in differentiated osteoblasts [89]; whereas deletion of either *Cdh2*, *Cdh11*, or both reduces β -catenin abundance at cell-cell contacts and cell-cell adhesion with a negative effect on osteoblastogenesis and bone growth [50]. *In vitro* evidence also suggests that modulation of cadherin-2/ β -catenin association may be involved in transduction of mechanical signals. Oscillatory fluid flow releases β -catenin from adherens junctions, resulting in β -catenin nuclear translocation and transcriptional activation, in turn contributing to osteogenic differentiation [90].

An alternative mechanism of cadherin interaction with Wnt signaling components has recently emerged from observations that cadherin-2 can sequester Lrp5 or Lrp6 in inactive pools, thus resulting in decreased Wnt/ β -catenin signaling in osteoblasts [62]. Specifically, cadherin-2 forms a complex with Lrp5/6 via an axin “bridge”, thus preventing Lrp5/6 activation of β -catenin, which is instead targeted for proteasomal degradation (Figure 3A). The result is defective canonical Wnt signaling, decreased osteogenic differentiation, reduced osteoblast function and bone formation [62]. Furthermore, cadherin-2-mediated adhesion has been linked to activation of PI3K signaling in osteoblastic cells [91]. Consistently, cadherin-2/Lrp5/6 interaction negatively controls osteoblast growth and survival by reducing endogenous Wnt3a expression as well as Wnt-dependent PI3K/Akt signaling [92]. The emerging evidence that cadherins control osteogenic differentiation and bone formation may conceptually be used to devise new therapeutic approaches for promoting bone formation and increasing bone mass [6, 63]. Along these lines, a strategy has been developed to inhibit cadherin-2/Lrp5/6 interaction via axin [62], with the aim of facilitating canonical Wnt signaling and bone formation. Proof-of-principle of this strategy is provided by the finding that deletion of the cadherin-2 domain interacting with axin and Lrp5/6 promotes Wnt/ β -catenin signaling and osteoblast differentiation without affecting cell-cell adhesion [89]. Consequently, peptides that bind to the cadherin-2/axin interacting domain of Lrp5 and disrupt cadherin-2-Lrp5/6 interaction enhance Wnt/ β -catenin signaling, stimulate osteoblast function *in vitro*, and promote calvaria bone formation *in vivo* [89].

(Figure 3B). Such competitor peptide based strategy represents a novel approach that could be applied to conditions characterized by insufficient bone formation relative to bone resorption [93]. Recent preliminary data, also suggest that cadherin-2 may restrain the bone anabolic action of Lrp5/6 signaling activators [94], and even of intermittent PTH [95]. If these data hold, they will further expand cadherin-2 biologic role in bone forming cells, and strengthen the notion that cadherins represent legitimate targets for pharmacological intervention.

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Figure 1. Homophilic cadherin interactions promote cell-cell adhesion and osteoblast differentiation during the early stages of bone formation

Mesenchymal stem cells express many cadherins at low abundance. Both *Cdh2* and *Cdh11* increase with commitment to the osteogenic lineage, but as differentiation progresses *Cdh2* expression decreases whereas *Cdh11* persists. Terminally differentiated bone forming cells lose cadherin-2 and cell-cell adhesion; many undergo apoptosis, a few become osteocytes, matrix embedded cells with very limited physical contacts with other cells. *Cdh2* is also up-regulated during early steps of chondrogenic differentiation, whereas *Cdh11* is not present in condensed growth plate chondrocytes. Differentiated chondrocytes do not express either cadherin. Adipogenic differentiation is associated with loss of both *Cdh2* and *Cdh11*. Indeed, *Cdh11* inhibits adipogenesis, while favoring chondro-osteogenesis.

Figure 2. Interaction between cadherins and Wnt/β-catenin signaling

Cadherin *cis*-dimers are connected to the actin cytoskeleton via binding to β-catenin and γ-catenin (plakoglobin), which in turn bind α-catenin and through it, actin. Cytoplasmic β-catenin – not bound to cadherins – can translocate to the nucleus to induce gene transcription, and its levels are kept in homeostatic balance by glucose synthase kinase-3β (GSK3β) phosphorylation and targeting for ubiquitination and proteosomal degradation. GSK3β is inhibited by binding to an APC/axin/GSK3β complex, which forms in response to Wnt activation of Frizzled receptors. Since β-catenin bound to cadherins is kept away from transcriptionally active pools, increased cadherin expression results in β-catenin sequestration at the plasma membrane and reduced β-catenin availability for transcriptional activation.

Figure 3. Cadherin-2 is a negative regulator of full osteoblast differentiation

Cadherin-2 (via the 62 amino-acids at the C-terminus) interacts with the Wnt co-receptors LRP5/6 via binding to axin through its C-terminus domain. Such interaction prevents activation of β -catenin, ERK1/2 and PI3K/Akt signalling, resulting in inhibition of cell proliferation, reduced osteoblast differentiation and survival (left panel). Disruption of cadherin-2-axin-LRP5/6 interaction by a small competitor peptide (28AA) removes this inhibitory interaction on LRP5/6, thus allowing activation of signalling cascades leading to cell proliferation, osteogenic differentiation and bone formation (right panel).