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PERSPECTIVES

N-Cadherin-Wnt Connections and the Control of Bone Formation

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Abstract

Cadherins are calcium-dependent cell adhesion molecules that play major roles during morphogenesis and tissue formation. Notably, the cell adhesion N-cadherin is an important regulator of chondrogenesis and osteogenesis. One recognized mechanism by which N-cadherin may promote osteoblast differentiation is by increasing cell-cell adhesion, resulting in activation of signals that promote osteoblast phenotypic gene expression. Cadherins can also trigger intracellular signals by interacting with the Wnt signaling pathway. In particular, cadherins can interact with β-catenin at the cell membrane, resulting in β-catenin sequestration, reduction of the cytosolic β-catenin pool and inhibition of Wnt signaling. Recent data provide another mechanism by which N-cadherin may control osteoblast function: N-cadherin was found to interact with the Wnt-co-receptors LRP5 or LRP6 in osteoblasts *in vitro* and *in vivo*. This interaction promotes β-catenin degradation, resulting in reduced Wnt signaling, decreased osteoblast differentiation and bone formation, and delayed bone accrual in mice. These data highlight the important crosstalk between cell adhesion and Wnt signaling molecules that impact osteoblast function, bone formation and bone mass. *IBMS BoneKEy.* 2009 April;6(4):150-156.

Wnt Signaling and Osteoblastogenesis

Osteoblast commitment, differentiation and function are all governed by multiple factors that in turn regulate bone formation and bone mass. In recent years, canonical Wnt signaling has emerged as an important regulator of bone formation and bone mass (1-5). Genetic evidence for the important role of Wnt signaling in bone formation originated from the altered bone mass induced by gain-of-function or loss-of-function mutations in the Wnt co-receptor LRP5 in humans (6;7). Genetic manipulations in mice have confirmed that Wnt signaling mediated by LRP5 is of major importance in the regulation of bone formation and bone mass (8). The positive effects of Wnt signaling on osteoblastogenesis appear to be mediated in part through the Wnt/β-catenin canonical pathway. The current model of the Wnt canonical pathway implies that Wnt binding to LRP5 and Frizzled leads to the recruitment of an axin/Frat1/APC/glycogen synthase kinase 3β/β-catenin complex to LRP5, resulting in inhibition of GSK-3β, decreased phosphorylation of β-catenin and its subsequent translocation into the nucleus where it binds TCF/LEF transcription factors to activate target genes (9). As expected from its important role in cells and tissues, Wnt canonical signaling is tightly regulated by several antagonists that interact with Wnt proteins or Wnt signaling partners (9;10). An important finding is that changes in the expression or function of Wnt antagonists result in alteration of Wnt signaling and bone formation. Indeed, several experimental studies have revealed that inactivation of Wnt antagonists causes increased bone mass whereas Wnt antagonist overexpression induces low bone mass in mice (8). The identification of molecules that act as Wnt partners in osteoblasts may therefore help in the development of novel targeted strategies for promoting bone formation and bone mass in clinical situations where osteoblastogenesis is compromised (10).

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Cadherins as Cell Adhesion Molecules

Cadherins are calcium-dependent cell adhesion molecules that play major roles during embryonic development, morphogenesis and tissue formation (11). During skeletal development, N-cadherin is a key component of mesenchymal condensation and chondrogenesis (12). In bone, osteoblasts express N-cadherin among other cadherins and the expression profile of N-cadherin in osteoblasts during bone formation suggests a role for this molecule in osteogenesis (13). Consistent with this observation, functional studies have revealed that N-cadherin is involved in cell-cell aggregation and in the development of the osteoblast phenotype through activation of intracellular signals (14;15). Furthermore, N-cadherin is upregulated by several anabolic molecules, which suggests a potential role for N-cadherin in osteoblast differentiation (16-18). This concept is also supported by the finding that over-expression of a dominant-negative N-cadherin inhibits osteoblast differentiation (19). Consistently, targeted expression of a dominant-negative N-cadherin was found to alter bone formation and bone mass (20;21). All these studies indicate that N-cadherin is an important regulator of osteoblast differentiation and osteogenesis (22;23). The molecular mechanisms by which N-cadherin controls intracellular signals regulating osteoblasts are beginning to be understood and are summarized below.

Cadherin Interactions With Wnt Signaling

Besides being transmembrane cell adhesion molecules, cadherins mediate cellular signaling by interacting with Wnt signaling (24-26). Classic cadherins are connected to the cytoskeleton by association with cytoplasmic proteins such as α-, β-, and γ-catenin (11). The interactions between cadherins and catenins are known to play essential roles in cell adhesion and normal development (27-31). At cell junctions, E-cadherin can associate with β-catenin and the association of β-catenin with E-cadherin and the actin microfilament cytoskeletal network controls cell adhesion and cell shape (33-36). Although these cadherin-catenin interactions are known to modulate key intracellular signaling pathways such as the Wnt signaling pathway, the downstream signals emanating from cadherin-mediated contacts are both cadherin-specific and cell-context-specific (27).

One generally recognized mechanism by which E-cadherin may negatively modulate Wnt-dependent gene expression is by sequestering β-catenin at the plasma membrane (9). β-catenin can bind to the cadherin cytoplasmic tail in the membrane, which reduces the β-catenin cytosolic pool, β-catenin nuclear translocation and subsequent transcription in conjunction with LEF/TCF transcription factors. In addition to E-cadherin, N-cadherin was also found to interact directly with β-catenin and thereby may decrease Wnt signaling (37;38). N-cadherin may therefore have a dual functional role in cell signaling both through a cell adhesion-dependent mechanism and by repressing canonical Wnt signaling. This suggests that concerted actions or crosstalk between the Wnt and cadherin pathways are key modulators of cytoplasmic β-catenin protein levels and Wnt signaling. Given the important role of Wnt signaling in the control of bone formation, the possibility exists that mechanisms linking cadherins and Wnt partners may control Wnt signaling and thereby osteoblastogenesis. Indeed, recent data indicate that Wnt signaling is controlled by N-cadherin in osteoblasts by a mechanism involving a Wnt partner other than β-catenin, namely, the Wnt co-receptors LRP5/6. This provides evidence for a previously unrecognized link between a cell adhesion molecule and Wnt co-receptors, and emphasizes the important role of the signaling network linking cell adhesion and Wnt signaling molecules in osteoblasts.

N-Cadherin-LRP5 Interaction Impacts Wnt Signaling and Osteoblast Differentiation

The hypothesis that cadherins may interact with Wnt signaling independently of β-catenin binding has been tested recently. Recent data revealed a novel link between LRP5, a major co-receptor of Wnt proteins, and N-cadherin (39). This concept came

from the observation that LRP5 (or LRP6) can bind to N-cadherin, as revealed by immunoprecipitation analyses *in vitro*, and is supported by the finding that forced expression of N-cadherin in murine osteoblasts increases N-cadherin-LRP5/6 interaction. This interaction has functional consequences since increasing N-cadherin-LRP5 interaction negatively regulates Wnt/β-catenin signaling, resulting in alteration of expression of osteoblast phenotypic genes. Furthermore, this interaction has significant implications *in vivo* since transgenic expression of N-cadherin targeted to osteoblasts in mice results in reduced osteoblast activity and bone formation, resulting in delayed bone mass accrual (39). Interestingly, a similar phenotype was found in mice exhibiting N-cadherin loss-of-function, presumably because of blockade of N-cadherin-mediated intracellular signaling (20). These novel findings suggest that N-cadherin is a novel LRP5 antagonist that negatively regulates Wnt/β-catenin signaling in osteoblasts. This concept is highly relevant to the regulation of osteoblastogenesis since both N-cadherin and LRP5 are strongly expressed in osteoblasts and Wnt signaling is a major modulator of osteoblast function and bone mass (1-5).

Several downstream mechanisms come into play as the result of N-cadherin-LRP5 interactions. Both *in vitro* studies and analysis of osteoblasts from N-cadherin transgenic mice show that increasing N-cadherin/LRP5 interaction results in increased β-catenin ubiquitination, decreased β-catenin cytosol levels and nuclear translocation in response to Wnt3a and subsequent downregulation of Wnt/β-catenin signaling in osteoblasts (39). The mechanism by which N-cadherin-LRP5 interaction negatively regulates Wnt/β-catenin signaling does not appear to result from direct interaction of N-cadherin with β-catenin and subsequent β-catenin sequestration. Indeed, raising N-cadherin levels fails to increase β-catenin membrane sequestration or to prevent the release of β-catenin by Wnt3a stimulation (39). Thus, β-catenin sequestration by N-cadherin is not primarily responsible for the defective Wnt signaling in N-cadherin over-expressing osteoblasts. Rather, both *in vitro* and *in vivo* data show that N-cadherin interacts with the intracellular domain of LRP5. This action of N-cadherin as a Wnt antagonist therefore differs from the effect of other Wnt antagonists that act by binding to Wnt ligands (SFRPs, Cerberus, WIF1) or by interacting with LRP5 extracellular domains (DKK1, CTGF, Wise, sclerostin). Further studies have revealed that the interaction of N-cadherin with LRP5 or LRP6 in osteoblasts is mediated by the formation of an axin-LRP5 complex implicating axin-binding sites in the cytoplasmic tail of LRP5. However, other proteins may also be partners of the β-catenin-axin-N-cadherin-LRP5 complex.

These data support the concept that interaction between N-cadherin and the Wnt co-receptors LRP5/6 negatively regulates Wnt/β-catenin signaling and is critical in the regulation of osteoblast function and bone formation *in vitro* and *in vivo*. Thus, N-cadherin appears to control osteoblasts by at least three mechanisms: a positive mechanism involving increased cell-cell adhesion and downstream signals, and two negative mechanisms involving β-catenin sequestration and reduction of Wnt signaling mediated by its interaction with LRP5/6 (Fig. 1). These three regulatory mechanisms are likely to play important roles in the control of osteoblasts where both cadherins and Wnt receptors are highly expressed and play a significant role in the control of cell differentiation and function.

Implications for Osteoblast Biology and Pathology

There is emerging evidence that crosstalk between N-cadherin and Wnt signaling controls osteoblast function *in vitro* and *in vivo*. In addition to the previously recognized homophilic N-cadherin links and N-cadherin-β-catenin interaction, there is now evidence that N-cadherin-LRP5/6 interaction controls bone formation and bone mass, which provides new insight into the mechanisms involved in N-cadherin-mediated osteoblast differentiation. This highlights the key role of N-cadherin in the control of Wnt signal transduction and osteoblastogenesis and emphasizes the important crosstalk between

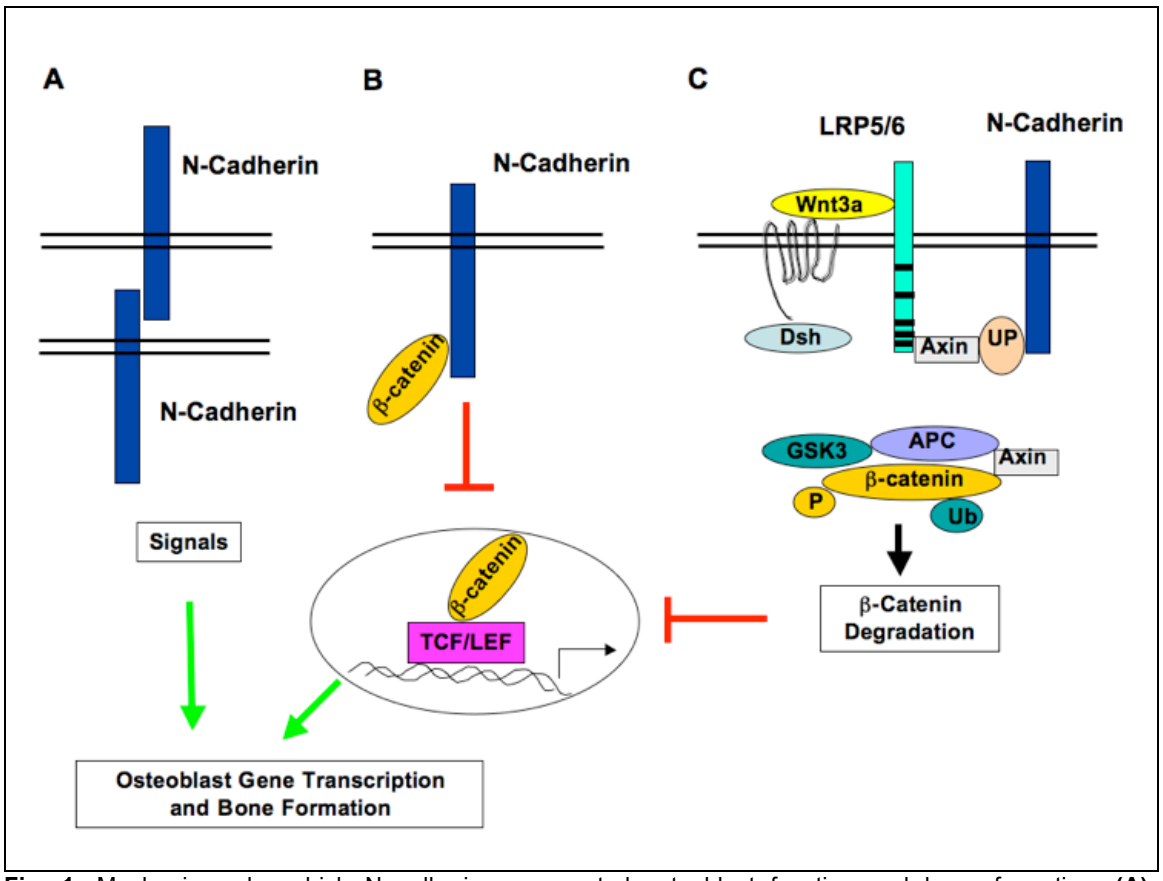

Fig. 1. Mechanisms by which N-cadherin may control osteoblast function and bone formation. **(A).** Homophilic N-cadherin interactions promote cell-cell adhesion, downstream signals and osteoblast differentiation. **(B).** N-cadherin-β-catenin interaction results in β-catenin sequestration at the membrane and reduced Wnt signaling. **(C).** The interaction between N-cadherin and the Wnt co-receptors LRP5/6 results in β-catenin degradation, inhibition of Wnt/β-catenin signaling, reduced osteoblast function and bone formation. (UP: unknown protein; Ub: ubiquitin).

cell adhesion and Wnt signaling molecules in osteoblast biology. This also opens the possibility that targeting N-cadherin, now considered as a Wnt antagonist, may be of therapeutic value to promote osteoblast function in disorders where bone formation is compromised.

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References

1. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. *Gene*. 2004 Oct 27;341:19-39.

2. Glass DA 2nd, Karsenty G. Molecular bases of the regulation of bone remodeling by the canonical Wnt signaling pathway. *Curr Top Dev Biol*. 2006;73:43-84.

3. Baron R, Rawadi G, Roman-Roman S. Wnt signaling: a key regulator of bone mass. *Curr Top Dev Biol*. 2006;76:103-27.

4. Bodine PV, Komm BS. Wnt signaling and osteoblastogenesis. *Rev Endocr Metab Disord*. 2006 Jun;7(1-2):33-9.

5. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest*. 2006 May;116(5):1202-9.

6. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang

H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Jüppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML; Osteoporosis-Pseudoglioma Syndrome Collaborative Group. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell*. 2001 Nov 16;107(4):513-23.

7. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP. High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med*. 2002 May 16;346(20):1513-21.

8. Glass DA 2nd, Karsenty G. In vivo analysis of Wnt signalling in bone. *Endocrinology*. 2007 Jun;148(6):2630-4.

9. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*. 2004;20:781-810.

10. Rawadi G, Roman-Roman S. Wnt signalling pathway: a new target for the treatment of osteoporosis. *Expert Opin Ther Targets*. 2005 Oct;9(5):1063-77.

11. Gumbiner BM. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell*. 1996 Feb 9;84(3):345-57.

12. Tuan RS. Cellular signaling in developmental chondrogenesis: N-cadherin, Wnts, and BMP-2. *J Bone Joint Surg Am*. 2003;85-A Suppl 2:137-41.

13. Cheng SL, Lecanda F, Davidson MK, Warlow PM, Zhang SF, Zhang L, Suzuki S, St John T, Civitelli R. Human osteoblasts express a repertoire of cadherins, which are critical for BMP-2-induced osteogenic differentiation. *J Bone Miner Res*. 1998 Apr;13(4):633-44.

14. Ferrari SL, Traianedes K, Thorne M, Lafage-Proust MH, Genever P, Cecchini MG, Behar V, Bisello A, Chorev M, Rosenblatt M, Suva LJ. A role for N-cadherin in the development of the differentiated osteoblastic phenotype. *J Bone Miner Res*. 2000 Feb;15(2):198-208.

15. Haÿ E, Lemonnier J, Modrowski D, Lomri A, Lasmoles F, Marie PJ. N- and E-cadherin mediate early human calvaria osteoblast differentiation promoted by bone morphogenetic protein-2. *J Cell Physiol*. 2000 Apr;183(1):117-28.

16. Debiais F, Lemonnier J, Hay E, Delannoy P, Caverzasio J, Marie PJ. 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*. 2001;81(1):68-81.

17. Delannoy P, Lemonnier J, Haÿ E, Modrowski D, Marie PJ. Protein kinase C-dependent upregulation of N-cadherin expression by phorbol ester in human calvaria osteoblasts. *Exp Cell Res*. 2001 Sep 10;269(1):154-61.

18. Lemonnier J, Haÿ E, Delannoy P, Lomri A, Modrowski D, Caverzasio J, Marie PJ. Role of N-cadherin and protein kinase C in osteoblast gene activation induced by the S252W fibroblast growth factor receptor 2 mutation in Apert craniosynostosis. *J Bone Miner Res*. 2001 May;16(5):832-45.

19. Cheng SL, Shin CS, Towler DA, Civitelli R. A dominant negative cadherin inhibits

osteoblast differentiation. *J Bone Miner Res*. 2000 Dec;15(12):2362-70.

20. Castro CH, Shin CS, Stains JP, Cheng SL, Sheikh S, Mbalaviele G, Szejnfeld VL, Civitelli R. Targeted expression of a dominant-negative N-cadherin in vivo delays peak bone mass and increases adipogenesis. *J Cell Sci*. 2004 Jun 1;117(Pt 13):2853-64.

21. Lai CF, Cheng SL, Mbalaviele G, Donsante C, Watkins M, Radice GL, Civitelli R. Accentuated ovariectomy-induced bone loss and altered osteogenesis in heterozygous N-cadherin null mice. *J Bone Miner Res*. 2006 Dec;21(12):1897-906.

22. Marie PJ. Role of N-cadherin in bone formation. *J Cell Physiol*. 2002 Mar;190(3):297-305.

23. Mbalaviele G, Shin CS, Civitelli R. Cell-cell adhesion and signalling through cadherins: connecting bone cells in their microenvironment. *J Bone Miner Res*. 2006 Dec;21(12):1821-7.

24. Wheelock MJ, Johnson KR. Cadherin-mediated cellular signaling. *Curr Opin Cell Biol*. 2003 Oct;15(5):509-14.

25. Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. *Science*. 2004 Mar 5;303(5663):1483-7.

26. Conacci-Sorrell M, Zhurinsky J, Ben-Ze'ev A. The cadherin-catenin adhesion system in signaling and cancer. *J Clin Invest*. 2002 Apr;109(8):987-91.

27. Kemler R. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet*. 1993 Sep;9(9):317-21.

28. Hinck L, Nelson WJ, Papkoff J. Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing beta-catenin binding to the cell adhesion protein cadherin. *J Cell Biol*. 1994 Mar;124(5):729-41.

29. Schambony A, Kunz M, Gradl D. Cross-regulation of Wnt signaling and cell adhesion. *Differentiation*. 2004 Sep;72(7):307-18.

30. Bienz M. beta-Catenin: a pivot between cell adhesion and Wnt signalling. *Curr Biol*. 2005 Jan 26;15(2):R64-7.

31. Brembeck FH, Rosário M, Birchmeier W. Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. *Curr Opin Genet Dev*. 2006 Feb;16(1):51-9.

32. Sadot E, Simcha I, Shtutman M, Ben-Ze'ev A, Geiger B. Inhibition of beta-catenin-mediated transactivation by cadherin derivatives. *Proc Natl Acad Sci U S A*. 1998 Dec 22;95(26):15339-44.

33. Barth AI, Näthke IS, Nelson WJ. Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways. *Curr Opin Cell Biol*. 1997 Oct;9(5):683-90.

34. Behrens J. Cadherins and catenins: role in signal transduction and tumor progression. *Cancer Metastasis Rev*. 1999;18(1):15-30.

35. Huber AH, Weis WI. The structure of the beta-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by beta-catenin. *Cell*. 2001 May 4;105(3):391-402.

36. Simcha I, Kirkpatrick C, Sadot E, Shtutman M, Polevoy G, Geiger B, Peifer M, Ben-Ze'ev A. Cadherin sequences that inhibit beta-catenin signaling: a study in yeast and mammalian cells. *Mol Biol Cell*. 2001 Apr;12(4):1177-88.

37. Shoval I, Ludwig A, Kalcheim C. Antagonistic roles of full-length N-cadherin and its soluble BMP cleavage product in neural crest delamination. *Development*. 2007 Feb;134(3):491-501.

38. Xu, G, Arregui C, Lilien J, Balsamo J. PTP1B modulates the association of beta-catenin with N-cadherin through binding to an adjacent and partially overlapping target site. *J Biol Chem*. 2002 Dec 20;277(51):49989-97.

39. Haÿ E, Laplantine E, Geoffroy V, Frain M, Kohler T, Müller R, Marie PJ. N-cadherin interacts with axin and LRP5 to negatively regulate Wnt/beta-catenin signaling, osteoblast function, and bone formation. *Mol Cell Biol*. 2009 Feb;29(4):953-64.