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Notch Signaling and Bone Remodeling

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Abstract

Notch signaling plays context-dependent roles in the development and maintenance of many cell types and tissues in mammals. In the skeleton, both osteoblasts and osteoclasts require Notch signaling for proper differentiation and function, and the specific roles of Notch are dependent on the differentiation status of the cell. The recent discovery of activating NOTCH2 mutations as the cause of Hajdu-Cheney syndrome has highlighted the significance of Notch signaling in human bone physiology.

Keywords

Notch signaling; bone remodeling; osteoblasts; osteoclasts; skeletal development; skeletal diseases; Hajdu-Cheney Syndrome; Alagille syndrome

Introduction

Following completion of skeletal development (also referred to as the modeling phase) the mammalian bones undergo a life-long process of remodeling that is mediated by the reciprocal actions of osteoblasts and osteoclasts. Both the matrix-depositing activity of osteoblasts and the matrix-resorbing activity of osteoclasts must be tightly regulated in order to maintain the homeostasis of bone mass and to prevent disease states. As one might expect, there are a multitude of signaling pathways that control the differentiation, function and cross-talk of osteoblasts and osteoclasts. This review will highlight recent insights into the role of Notch signaling in regulating bone physiology and discuss examples of skeletal diseases caused by genetic mutations in Notch pathway components.

The Notch signaling pathway

Notch signaling requires cell-cell contact, and is initiated when the single-pass transmembrane ligands (Jagged (JAG) 1 and 2 and Delta-like (DLL) 1, 3, and 4 in mammals) engage Notch (1–4 in mammals) receptors expressed on the surface of a neighboring cell¹. Ligand binding leads to proteolytic cleavage of the Notch receptor by the γ -secretase complex, releasing the Notch intracellular domain (NICD). Within the cell, the NICD translocates to the nucleus where it interacts with the DNA-binding protein RBPj κ /CBF1, displacing co-repressors and leading to the assembly of an activator complex that also includes Mastermind-like (MAML) and transcriptional co-activators. Notch target

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genes are thus activated, including the transcriptional repressors hairy and enhancer of split (HES) and HES-related with YRPW motif (HEY)²⁻⁴.

Notch signaling may interact with other signaling pathways such as Wnt and bone morphogenetic protein (BMP) to regulate skeletal development and homeostasis. It should be noted however, that most studies to date have relied on overexpression of NICD from Notch1, which may not faithfully represent physiological Notch signaling. In cultured osteoblast precursors, NICD overexpression opposed the osteoblast-inducing activity of exogenous Wnt, an effect mediated by Hes1⁵. *In vitro* studies examining the relationship between Notch and BMP signaling have provided conflicting results. In one study, NICD overexpression blocked the differentiation of osteoblast precursors in response to BMP2⁶, but others showed that Notch signaling enhanced⁷, whereas inhibition of Notch impaired BMP2-induced osteoblast differentiation⁸. Future studies are necessary to delineate the relationship between Notch and the other signaling pathways under physiological or pathophysiological conditions. Nonetheless, it is expected that Notch signaling does not act in isolation, as cells almost certainly receive and respond to multiple signals simultaneously *in vivo*, resulting in an integrated phenotypic response.

Notch signaling in the osteoblast lineage

Early mouse genetics studies suggested an important role for Notch signaling in skeletal development. Axial skeletal defects were observed in global deletions of presenilin-1, a component of the γ -secretase complex,^{9,10} and Notch ligand Dll3¹¹. However, elucidating the role of Notch signaling specifically in osteoblasts required tissue-specific gene deletions.

Genetic disruption of physiological Notch signaling has revealed a critical role for this pathway in the regulation of osteoblastogenesis. Deletion of Notch1 and Notch2 in the skeletogenic mesenchyme with Prx1-cre caused a massive accumulation of bone within the marrow cavity by 8 weeks of age¹². However, when mutant mice were examined at 26 weeks, the trabecular bone mass was severely reduced to only 10% of control animals¹². Notch signaling was found to play a crucial role in suppressing differentiation of mesenchymal progenitors such that an early bolus of osteoblast differentiation in the Notch-deficient mice produced the initially high bone mass, but also depleted the progenitor pool, preventing later renewal of the osteoblast population¹². Subsequent studies revealed that Notch2, instead of Notch1, plays a predominant role in suppressing osteoblastogenesis, and that the regulation is largely mediated through RBPjk¹³. Downstream of RBPjK, the Hey family of transcriptional suppressors is an important mediator that both inhibits Runx2 activity and suppresses Nfatc1 transcription^{12,14}. The suppressive role of Hey proteins in osteoblastogenesis is also supported by the finding that Hey1-overexpressing mice are osteopenic by 12 weeks of age¹⁵. Interestingly, disruption of RBPjK with Osx-Cre or 2.3Col1-Cre (driven by the 2.3 kb promoter of Col1a1) did not cause an obvious bone phenotype¹⁴. Thus, the suppressive role of Notch-RBPjk signaling in osteoblastogenesis is limited to the early stages of osteoblast differentiation.

Similarly, mouse transgenic studies employing overexpression of NICD have revealed different responses depending on the differentiation stage of osteoblast-lineage cells. Overexpression of NICD under control of the 2.3 kb Col1a1 promoter active in committed osteoblastic cells caused a high bone mass phenotype, characterized by excessive immature woven bone and fibrotic cells in the marrow cavity^{16,17}. The effect of NICD was abolished upon deletion of RBPjk¹⁷. At the cellular level, NICD overexpression stimulated proliferation of early-stage osteoblast-lineage cells but prevented progression to fully mature osteoblasts¹⁶. In contrast, when overexpressed from the 3.6 kb Col1a1 promoter active in the osteoblast precursors, NICD caused osteopenia¹⁸. Likewise, when NICD was expressed

from the R26-NICD allele¹⁹ with different Cre drivers targeting various stages of the osteoblast lineage, varying bone phenotypes were observed²⁰. Taken together, these studies support the notion that NICD overexpression suppresses differentiation from early-stage precursors, promotes proliferation of intermediate osteoblast-lineage cells, but impedes formation of mature osteoblasts.

Notch signaling in osteoblast-lineage cells also indirectly regulates osteoclastogenesis by modulating the expression of osteoclastogenic factors by those cells. In particular, removal of Notch signaling in the osteoblast lineage notably reduced the expression of Opg (also known as Tnfrsf11b), a known anti-osteoclastogenic factor^{12,16}. Conversely, overexpression of NICD in osteocytes suppressed bone resorption²¹. Thus, Notch signaling in osteoblast-lineage cells regulates bone remodeling both cell-autonomously and indirectly through regulation of the osteoclast.

Notch signaling in the osteoclast lineage

Notch signaling also directly regulates osteoclastogenesis. Osteoclasts differentiate from macrophage precursors in response to the secreted factors RANKL and macrophage colony-stimulating factor (M-CSF), and the activation of cell surface receptors DAP12 and FcR γ ²². Deletion of Notch receptors in macrophages with Cre expressed from a viral vector enhanced osteoclast differentiation in response to M-CSF and RANKL in vitro²³. Conversely, bone marrow macrophages cultured with an immobilized Notch ligand downregulated the receptor for M-CSF and exhibited suppression of osteoclast differentiation²⁴. However, others reported that Notch2 and Hes1 were upregulated during RANKL-induced osteoclast differentiation in a macrophage cell line, and that treatment with a γ -secretase inhibitor blocked osteoclastogenesis in these cells²⁵. The seemingly contradictory results may reflect different requirements for Notch signaling at distinct stages of osteoclast differentiation. Furthermore, the stage-specific regulation may involve different Notch receptors and ligands, as it was reported that Jag1 and Notch1 blocked but Dll1 and Notch2 promoted osteoclastogenesis²⁶. Thus, the effect of Notch signaling on osteoclastogenesis appears to depend both on the differentiation status of the cell and the expression of particular ligands and receptors.

Notch signaling and human bone diseases

Hajdu-Cheney Syndrome

A series of recent studies identified mutations in Notch2 as the underlying cause of Hajdu-Cheney Syndrome (HCS, OMIM# 102500), a rare disorder with an autosomal dominant pattern of inheritance. The most prominent clinical features of HCS are craniofacial dysmorphism (including hypertelorism, thick eyebrows, low-set ears, malar hypoplasia, prognathism, micrognathia, long philtrum, and abnormal dentition) and radiographic abnormalities, particularly acro-osteolysis, osteoporosis, and Wormian bones. Some other features include short stature, hearing defects, cardiovascular abnormalities, and polycystic kidneys^{27,28}. Remarkably, all of the HCS-causing mutations identified to date occur within the final exon of the Notch2 gene^{28–30}. The mutated gene is predicted to encode a truncated Notch2 protein that contains all of the domains essential for receptor function, cleavage, and nuclear localization but lacks the domain containing conserved proline-glutamate-serine-threonine-rich motifs (PEST domain) which harbors the degradation signal and controls the half-life of the protein¹. Thus, HCS forms of Notch2 are expected to evade normal proteolytic degradation and consequently have increased signaling activity³¹. In addition, similar truncating mutations in Notch2 have been identified in serpentine fibula-polycystic kidney syndrome (SFPKS)^{32,33}. Thus, HCS and SFPKS represent variable phenotypes of the same disease caused by gain-of-function mutations in Notch2.

Alagille Syndrome

Alagille syndrome is an autosomal dominant disorder that occurs in at least 1 in 70,000 live births³⁴. Haploinsufficiency in the JAG1 gene accounts for approximately 97% of Alagille syndrome cases^{35,36}, designated ALGS1 (OMIM# 118450). ALGS2 (OMIM# 610205) is caused by mutations in NOTCH2³⁷ and accounts for <1% of total cases. Unlike HCS, the mutations causing ALGS2 lead to Notch2 proteins that are predicted to exhibit compromised activity. The most common skeletal malformation found in ALGS patients is known as butterfly vertebrae, which is caused by incomplete fusion of the anterior arch^{34,38}. Other reported skeletal defects include hand and pelvic anomalies, shortened ulna, and absence of the 12th rib³⁹. Characteristic facial dysmorphology can include a prominent forehead, deep-set eyes with moderate hypertelorism, pointed chin, and straight nose with bulbous tip⁴⁰. Although none of the common skeletal malformations are functionally compromising, there have been reports of increased fracture incidence in young children with ALGS^{41,42}. While the increased fracture risk could be secondary to decreased vitamin and mineral absorption caused by the cholestasis^{34,42}, recent evidence has implicated JAG1 in regulating bone mineral density. A genome-wide association study identified a single nucleotide polymorphism in the JAG1 gene correlated with higher BMD across multiple ethnic groups, and further analyses demonstrated a higher level of JAG1 transcript in individuals with the “high BMD” genotype⁴³. Given that ALGS1 results from haploinsufficiency of JAG1, compromised Notch signaling may contribute to skeletal fragility in young ALGS patients.

Concluding remarks

Mouse genetic studies in recent years have uncovered important roles for Notch signaling in both the osteoblast and osteoclast lineages. A common feature for Notch regulation in both cell lineages has emerged that the Notch effect is highly dependent on the cell context represented by various differentiation stages. Studies to date employing overexpression of NICD have provided important clues to the potential effect of hyperactive Notch signaling in pathophysiological conditions. However, a mouse model recapitulating the Notch2 mutations causing HCS and SFPKS in humans is needed to elucidate the cellular basis for the disease. The fact that physiological Notch signaling suppresses osteoblast differentiation in the early progenitors provides the hope that inhibition of the pathway may be explored as a means to stimulate bone formation. However, given that Notch signaling affects both osteoblast and osteoclast lineages and that the effects are dependent on the cell context, it may be critical to develop therapeutic options that can preferentially target the desired cell types.

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References

Papers of particular interest, published recently, have been highlighted as:

- Of importance

1. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell*. 2009; 137:216–33. [PubMed: 19379690]
2. Fortini ME. Notch signaling: the core pathway and its posttranslational regulation. *Dev Cell*. 2009; 16:633–47. [PubMed: 19460341]
3. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. *Development*. 2011; 138:3593–612. [PubMed: 21828089]

4. Canalis E. Notch signaling in osteoblasts. *Sci Signal*. 2008; 1:pe17. [PubMed: 18445833]
5. Deregowski V, Gazzero E, Priest L, Rydziel S, Canalis E. Notch 1 overexpression inhibits osteoblastogenesis by suppressing Wnt/beta-catenin but not bone morphogenetic protein signaling. *J Biol Chem*. 2006; 281:6203–10. [PubMed: 16407293]
6. Sciaudone M, Gazzero E, Priest L, Delany AM, Canalis E. Notch 1 impairs osteoblastic cell differentiation. *Endocrinology*. 2003; 144:5631–9. An early in vitro study demonstrating a suppressive role for Notch signaling in osteoblast differentiation. [PubMed: 12960086]
7. Tezuka K, Yasuda M, Watanabe N, et al. Stimulation of osteoblastic cell differentiation by Notch. *J Bone Miner Res*. 2002; 17:231–9. [PubMed: 11811553]
8. Nobta M, Tsukazaki T, Shibata Y, et al. Critical regulation of bone morphogenetic protein-induced osteoblastic differentiation by Delta1/Jagged1-activated Notch1 signaling. *J Biol Chem*. 2005; 280:15842–8. [PubMed: 15695512]
9. Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S. Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell*. 1997; 89:629–39. [PubMed: 9160754]
10. Wong PC, Zheng H, Chen H, et al. Presenilin 1 is required for Notch1 and Dll1 expression in the paraxial mesoderm. *Nature*. 1997; 387:288–92. [PubMed: 9153393]
11. Dunwoodie SL, Clements M, Sparrow DB, Sa X, Conlon RA, Beddington RS. Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene *Dll3* are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development*. 2002; 129:1795–806. [PubMed: 11923214]
12. Hilton MJ, Tu X, Wu X, et al. Notch signaling maintains bone marrow mesenchymal progenitors by suppressing osteoblast differentiation. *Nat Med*. 2008; 14:306–14. This mouse genetic study establishes that physiological Notch signaling suppresses bone formation in vivo. [PubMed: 18297083]
13. Tu X, Chen J, Lim J, et al. Physiological notch signaling maintains bone homeostasis via RBPjk and Hey upstream of NFATc1. *PLoS Genet*. 2012; 8:e1002577. This study delineates the mechanism through which physiological Notch signaling suppresses bone formation, and identifies Notch2 as a critical regulator. [PubMed: 22457635]
14. Tu X, Chen J, Lim J, et al. Physiological Notch signaling maintains bone homeostasis via RBPjk and Hey upstream of NFATc1. *PLoS Genet*. 2012; 8:e1002577. [PubMed: 22457635]
15. Salie R, Kneissel M, Vukevic M, et al. Ubiquitous overexpression of Hey1 transcription factor leads to osteopenia and chondrocyte hypertrophy in bone. *Bone*. 2010; 46:680–94. [PubMed: 19857617]
16. Engin F, Yao Z, Yang T, et al. Dimorphic effects of Notch signaling in bone homeostasis. *Nat Med*. 2008; 14:299–305. [PubMed: 18297084]
17. Tao J, Chen S, Yang T, et al. Osteosclerosis owing to Notch gain of function is solely Rbpj-dependent. *J Bone Miner Res*. 2010; 25:2175–83. These two mouse genetic studies demonstrate that hyperactivation of Notch signaling through RBPj impairs bone homeostasis. [PubMed: 20499347]
18. Zanotti S, Smerdel-Ramoya A, Stadmeier L, Durant D, Radtke F, Canalis E. Notch inhibits osteoblast differentiation and causes osteopenia. *Endocrinology*. 2008; 149:3890–9. [PubMed: 18420737]
19. Murtaugh LC, Stanger BZ, Kwan KM, Melton DA. Notch signaling controls multiple steps of pancreatic differentiation. *Proc Natl Acad Sci U S A*. 2003; 100:14920–5. [PubMed: 14657333]
20. Canalis E, Parker K, Feng JQ, Zanotti S. Osteoblast Lineage-Specific Effects of Notch Activation in the Skeleton. *Endocrinology*. 2012 This study highlights the stage-specific effects of hyperactive Notch signaling in the osteoblast lineage.
21. Canalis E, Parker K, Feng JQ, Zanotti S. Osteoblast lineage-specific effects of notch activation in the skeleton. *Endocrinology*. 2013; 154:623–34. [PubMed: 23275471]
22. Novack DV, Teitelbaum SL. The osteoclast: friend or foe? *Annu Rev Pathol*. 2008; 3:457–84. [PubMed: 18039135]
23. Bai S, Kopan R, Zou W, et al. NOTCH1 regulates osteoclastogenesis directly in osteoclast precursors and indirectly via osteoblast lineage cells. *J Biol Chem*. 2008; 283:6509–18. This

study demonstrates both direct and osteoblast-mediated regulation of osteoclastogenesis by Notch. [PubMed: 18156632]

24. Yamada T, Yamazaki H, Yamane T, et al. Regulation of osteoclast development by Notch signaling directed to osteoclast precursors and through stromal cells. *Blood*. 2003; 101:2227–34. [PubMed: 12411305]
25. Fukushima H, Nakao A, Okamoto F, et al. The association of Notch2 and NF-kappaB accelerates RANKL-induced osteoclastogenesis. *Mol Cell Biol*. 2008; 28:6402–12. [PubMed: 18710934]
26. Sekine C, Koyanagi A, Koyama N, Hozumi K, Chiba S, Yagita H. Differential regulation of osteoclastogenesis by Notch2/Delta-like 1 and Notch1/Jagged1 axes. *Arthritis Res Ther*. 2012; 14:R45. [PubMed: 22390640]
27. Brennan AM, Pauli RM. Hajdu–Cheney syndrome: evolution of phenotype and clinical problems. *Am J Med Genet*. 2001; 100:292–310. [PubMed: 11343321]
28. Isidor B, Lindenbaum P, Pichon O, et al. Truncating mutations in the last exon of NOTCH2 cause a rare skeletal disorder with osteoporosis. *Nat Genet*. 2011; 43:306–8. [PubMed: 21378989]
29. Simpson MA, Irving MD, Asilmaz E, et al. Mutations in NOTCH2 cause Hajdu–Cheney syndrome, a disorder of severe and progressive bone loss. *Nat Genet*. 2011; 43:303–5. [PubMed: 21378985]
30. Majewski J, Schwartzentruber JA, Caqueret A, et al. Mutations in NOTCH2 in families with Hajdu–Cheney syndrome. *Hum Mutat*. 2011; 32:1114–7. These studies identify NOTCH2 mutations as the cause for Hajdu–Cheney Syndrome. [PubMed: 21681853]
31. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*. 2004; 306:269–71. [PubMed: 15472075]
32. Isidor B, Le Merrer M, Exner GU, et al. Serpentine fibula-polycystic kidney syndrome caused by truncating mutations in NOTCH2. *Hum Mutat*. 2011; 32:1239–42. [PubMed: 21793104]
33. Gray MJ, Kim CA, Bertola DR, et al. Serpentine fibula polycystic kidney syndrome is part of the phenotypic spectrum of Hajdu–Cheney syndrome. *Eur J Hum Genet*. 2012; 20:122–4. [PubMed: 21712856]
34. Turnpenny PD, Ellard S. Alagille syndrome: pathogenesis, diagnosis and management. *Eur J Hum Genet*. 2012; 20:251–7. [PubMed: 21934706]
35. Oda T, Elkahoul AG, Pike BL, et al. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. *Nat Genet*. 1997; 16:235–42. [PubMed: 9207787]
36. Li L, Krantz ID, Deng Y, et al. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet*. 1997; 16:243–51. [PubMed: 9207788]
37. McDaniel R, Warthen DM, Sanchez-Lara PA, et al. NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. *Am J Hum Genet*. 2006; 79:169–73. These studies discover the role of impaired Notch signaling in Alagille syndrome. [PubMed: 16773578]
38. Sanderson E, Newman V, Haigh SF, Baker A, Sidhu PS. Vertebral anomalies in children with Alagille syndrome: an analysis of 50 consecutive patients. *Pediatr Radiol*. 2002; 32:114–9. [PubMed: 11819079]
39. Berrocal T, Gamo E, Navalón J, et al. Syndrome of Alagille: radiological and sonographic findings. A review of 37 cases. *Eur Radiol*. 1997; 7:115–8. [PubMed: 9000411]
40. Krantz ID, Piccoli DA, Spinner NB. Alagille syndrome. *J Med Genet*. 1997; 34:152–7. [PubMed: 9039994]
41. Hoffenberg EJ, Narkewicz MR, Sondheimer JM, Smith DJ, Silverman A, Sokol RJ. Outcome of syndromic paucity of interlobular bile ducts (Alagille syndrome) with onset of cholestasis in infancy. *J Pediatr*. 1995; 127:220–4. [PubMed: 7636645]
42. Bales CB, Kamath BM, Munoz PS, et al. Pathologic lower extremity fractures in children with Alagille syndrome. *J Pediatr Gastroenterol Nutr*. 2010; 51:66–70. [PubMed: 20453673]
43. Kung AW, Xiao SM, Cherny S, et al. Association of JAG1 with bone mineral density and osteoporotic fractures: a genome-wide association study and follow-up replication studies. *Am J Hum Genet*. 2010; 86:229–39. This study Links JAG1 polymorphism with bone mineral density in a diverse human population. [PubMed: 20096396]