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COMMENTARIES

A Notch in Bone, Hey, This Is a Phenotype…

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Commentary on:

- Engin F, Yao Z, Yang T, Zhou G, Bertin T, Jiang MM, Chen Y, Wang L, Zheng H, Sutton RE, Boyce BF, Lee B. Dimorphic effects of Notch signaling in bone homeostasis. *Nat Med*. 2008 Mar;14(3):299-305.
- Hilton MJ, Tu X, Wu X, Bai S, Zhao H, Kobayashi T, Kronenberg HM, Teitelbaum SL, Ross FP, Kopan R, Long F. Notch signaling maintains bone marrow mesenchymal progenitors by suppressing osteoblast differentiation. *Nat Med*. 2008 Mar;14(3):306-14.

NOTCH signaling is well-known as a critical signaling axis during development; however, its involvement in the biology of the mature skeleton was unclear. Two recent studies (1;2) in *Nature Medicine* highlight the importance of NOTCH signaling in adult skeletal homeostasis by demonstrating, using complementary genetic models, that NOTCH signaling regulates osteoblastogenesis and osteoclastogenesis post-natally.

NOTCH signaling is an evolutionarily conserved, intercellular signaling pathway that regulates multiple processes during development, including proliferation, survival and cell fate, in a spatial and temporal-dependent manner. In adult tissues, NOTCH signaling regulates maintenance/renewal of multiple tissues including the gut, skin, hematopoietic system, mammary gland, and central nervous system. NOTCH signaling is also involved in cancer. The major components of this signaling pathway include four NOTCH receptors (NOTCH 1-4) and at least seven NOTCH ligands (JAGGED 1 and 2, DELTA-LIKE 1, 3 and 4, DNER and contactin/F3/NB-3). Both receptors and ligands are single-pass transmembrane proteins expressed by adjacent cells. Activation of this pathway requires conformational changes in the receptor following ligand binding that expose the juxtamembrane region of NOTCH to proteolytic cleavage by ADAM metalloproteases. NOTCH receptors are further cleaved by γ-secretase in their transmembrane domain, releasing the NOTCH intracellular domain (NICD), which is now free to translocate to the nucleus and to associate with RBPSUH (also known as CBF1 and RBP-J) to displace a histone deacetylase (HDAc)/corepressor complex from the RBPSUH protein. This leads to the transcriptional activation of NOTCH target genes, which include HEY/HES (Hairy and Enhancer of Split) family members (3).

The significance of NOTCH signaling in skeletal cells is obvious during early development at a time when somites are formed. Lack of *Presenilin-1*, the catalytic subunit of g-secretase, lack of *Jagged 2*, or mutation in *Delta-like 3* in patients all cause severe skeletal phenotypes including cleft palate, syndactyly or deformities of the axial skeleton (4;5). However, the role of NOTCH signaling in the mature skeleton remained obscure. Two recent studies in *Nature Medicine* bring new insights into the role of NOTCH signaling in osteoblast differentiation and bone remodeling in the adult skeleton. These two studies took advantage of genetic manipulations that modulate NOTCH signaling in the mesenchymal lineage at different time points during development, which proved to be of prime importance for the conclusions of these studies.

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Engin and collaborators at Baylor College of Medicine chose to overexpress NICD in committed osteoblasts, using the osteoblast-specific 2.3kb collagen type I promoter, thus generating a mouse model with activated NOTCH signaling in committed osteoblasts (1). This manipulation led, in young growing mice, to a marked increase in osteoblast number and bone formation, resulting in a pronounced "high bone mass" but poorly organized woven bone, reminiscent of bones built by too many and too immature osteoblasts. In support of this model, osteoblastic gene expression in mutant calvaria osteoblasts was typical of less differentiated osteoblasts compared to control. This group then generated a loss-of-function model lacking both *Presenilin 1* and *Presenilin 2*, based on the same 2.3kb collagen type I promoter-cre system. As a result, all NOTCH signaling was blocked in committed osteoblasts. Surprisingly, this model had no phenotype in young growing mice but displayed a low bone mass phenotype upon aging, which was found to be caused by increased osteoclastogenesis triggered by decreased osteoprotegerin (OPG) levels. This study thus revealed that NOTCH signaling in committed osteoblasts favors their proliferation and inhibits their maturation, but also decreases osteoclastogenesis by regulating OPG expression. What it did not address, however, was the role of NOTCH signaling earlier during the differentiation of the osteoblast lineage.

One piece of evidence gave the authors an insight into this question: NICD interacts directly with RUNX2 and represses its function, including transactivation of the *Osteocalcin* gene. This result suggested that early loss of function of NOTCH in mesenchymal cells may relieve RUNX2 repression and thus lead to increased commitment to the osteoblast lineage, and possibly depletion of the osteoprogenitors cell pool. This hypothesis is supported by the results of the second study of this series by Hilton and collaborators at Washington University (2). This group generated a similar NOTCH loss-of-function model by producing mice lacking *Presenilin 1* and *Presenilin 2* in the limb mesenchyme, using Prx-cre promoter transgenic mice. In contrast to the 2.3kb collagen type I-cre *Presenilin 1/2* mutants from the study by Engin *et al*. (lacking NOTCH signaling in committed osteoblasts), Prx-cre *Presenilin 1/2* mutant mice (lacking NOTCH signaling in osteoblast progenitors and committed osteoblasts, and called "PPS" mice) displayed a high bone mass phenotype at 8 weeks of age. Deleting NOTCH1/2 receptors by the same strategy to generate "PNN" mice had the same effect on bone mass. Close examination of cellular distribution in the bones of PNN mice suggested that the total number of osteoblasts per bone area was increased compared to control (but density, *i.e*., number per surface, was normal), whereas osteoclast density was increased. The fact that bone marrow mesenchymal progenitors from the PNN mice *in vitro* produced less Cfu-f, less Cfu-ob and less differentiated adipocytes led the authors to the conclusion that PNN mice had a deficit in bone marrow mesenchymal progenitors, and therefore that the role of NOTCH signaling in early osteoblast differentiation stages is to maintain the pool of undifferentiated/uncommitted mesenchymal precursors and to decrease osteoblast differentiation, in agreement with the conclusions reached by Engin and collaborators. Another important similarity with the study by Engin *et al*. is the striking loss by the PNN mice of the high bone mass phenotype upon aging. Mice at 26 weeks of age had only 10% of the bone mass observed in WT littermates, which was accompanied by a reduction in the density of osteoblasts and increased bone resorption.

The evidence presented by both groups together leads to the following interpretation: NOTCH signaling in early mesenchymal progenitors maintains the bone marrow mesenchymal cell pool and paces the rate of commitment and differentiation to the osteoblast lineage. As a result, blocking NOTCH signaling in mesenchymal osteoblast progenitors releases this brake and pushes these multi-potent cells to the osteoblast lineage, increasing the active bone-building work force, bone formation and bone mass, as observed in young Prx-cre *Presenilin 1/2* mutants. However, this depletes the pool of osteoblast progenitors over time, which has negative functional

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consequences upon aging, since a continuous pool of progenitors is required to maintain bone formation. As a consequence, PNN mutants cannot recruit osteoblasts and exhibit progressive bone loss upon aging. During the process of osteoblast differentiation, at the time bone marrow mesenchymal progenitors become committed to osteoblasts, NOTCH signaling favors osteoblast proliferation and inhibits osteoblast maturation, maintaining a pool of active "young" osteoblasts. As a result, forced activation of NOTCH signaling in committed osteoblasts, as observed in the 2.3kb collagen type I-NICD transgenic mice, led to a high bone mass caused by exuberant bone formation.

NOTCH signaling appears to regulate another important function of osteoblasts, *i.e*., the formation of osteoclasts. The decrease in *Opg* and increase in *Rankl* expression observed in PNN mutant osteoblasts and the decrease in *Opg* expression in 2.3kb collagen type I-cre *Presenilin 1/2* mutants suggests that NOTCH signaling regulates *Rankl* expression in early osteoblast progenitors and *Opg* in more mature osteoblasts. These effects of NOTCH signaling on osteoclastogenic genes in bone-forming cells may be one primary mechanism whereby osteoclastogenesis is regulated during development and aging. It should be mentioned here that NOTCH signaling regulates osteoclast precursor differentiation in a cell-autonomous fashion as well, as recently demonstrated by Bai *et al*. (6).

The mechanism whereby NOTCH signaling maintains the pool of mesenchymal progenitors, favors committed osteoblast proliferation and keeps a brake on osteoblast maturation involves several transcription factors. Hilton's and Engin's studies lead to a model whereby NOTCH activation in osteoblasts induces cleavage of NICD, and expression of *Hey1, Hes,* and *HeyL*, which is followed by the interaction between NICD and HES proteins with RUNX2, leading to inhibition of its function and to a subsequent brake on osteoblast differentiation (Figure 1). The negative effect of NOTCH blockade on *Hey* and *Hes* expression, the inhibitory effect of these later factors on RUNX2 activity, and the effect of RUNX2 on *Opg* and *Rankl* expression (7) also suggest the existence of a pathway whereby NOTCH signaling, via HEY/HES and RUNX2 in osteoblasts, regulates the RANKL/OPG ratio and osteoclastogenesis. On the other hand, the activation of the *Osx* promoter in BMP2-differentiated mesenchymal cells by NICD suggests that NOTCH signaling favors the proliferation of committed osteoblasts via OSX. Whether changes in WNT signaling occurred in these mouse models, as suggested by previous *in vitro* studies by the Canalis group (8), was not reported.

The growth defect observed in the 2.3kb collagen type I-NICD mutants is surprising since recombination occurred in theory in committed osteoblasts, and not chondrocytes. The most plausible explanation is that cre-combination occurred in some osteochondroprogenitors due to "leaky" activity of the 2.3kb collagen type I promoter in mesenchymal cells, which may have caused to some extent sufficient extra NOTCH signaling in chondrocytes to induce the observed growth defects. This is supported by previous work by Hardingham and collaborators demonstrating the requirement of JAG-1-mediated NOTCH signaling in the regulation of chondrogenesis (9), and by the fact that NICD seems to act at very low concentration (10). The fact that the PPS and PNN mutants display a growth defect is more expected due to the early recombination event driven by the Prx promoter. The expansion of the hypertrophic chondrocyte zone and abnormalities in bone modeling in PPS mice suggest that NOTCH signaling negatively regulates chondrocyte differentiation, and also regulates the coupling between chondrogenesis and bone formation.

The intercellular mode of NOTCH signaling presents an intriguing analogy to the recently described two-way communication system between osteoblasts and osteoclasts, involving the ephrinB2/EphB4 receptor system (11). The two studies by the Engin and Hilton groups demonstrate the involvement of NOTCH signaling in the regulation of *Opg* and *Rankl* expression in osteoprogenitors and osteoblasts, but

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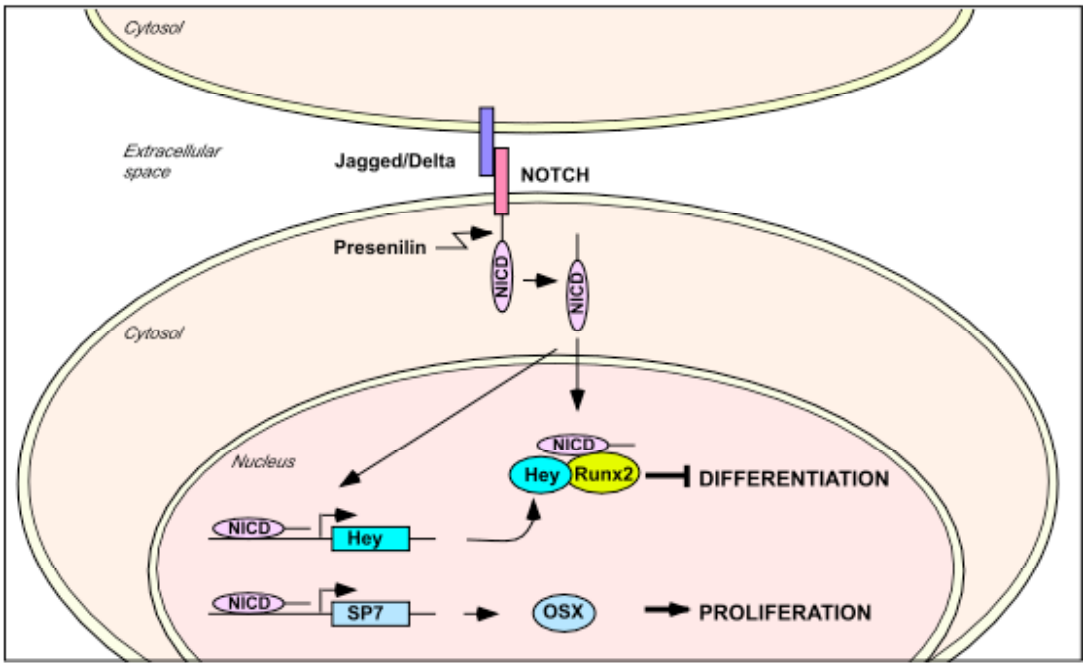

Figure 1. NOTCH regulates osteoblast proliferation and differentiation. Upon binding of Notch ligand to NOTCH receptor, the intracellular NICD domain of NOTCH receptor is cleaved and translocates to the nucleus where it promotes transcription of Hey and Sp7 genes. Both HEY and NICD interact with RUNX2 and repress its function, leading to decreased osteoblast differentiation.

whether or not the NOTCH axis is the molecular basis by which cross-talk and coupling occurs between osteoclasts and osteoblasts is still an open question. A recent study by Bai *et al*. demonstrated that NOTCH1 inhibits osteoclast precursor commitment to the osteoclast lineage and differentiation in a cell-autonomous manner, via ligand-mediated receptor activation. Since osteoblasts express NOTCH receptors and their ligands (12), it seems that the required machinery exists for such cross-talk to occur. Furthermore, cross-talk might not be limited to osteoclasts-osteoblasts and coupling between these two cell types, and may be relevant to coupling between osteoblasts and hematopoietic cells as well, as suggested by studies by Calvi *et al*. (12-14).

The two studies by Engin and Hilton and collaborators clearly demonstrate the relevance of NOTCH signaling in osteoprogenitor commitment and osteoblast function, but whether the age-related osteopenia characterized in the study by Hilton *et al*. is really due to a depletion of the pool of mesenchymal progenitors is difficult to demonstrate experimentally and can also be discussed. Even though aging may negatively affect mesenchymal stem cell frequency, proliferation or differentiation potential (15), one can argue that, by definition, the self-renewal capacity of mesenchymal stem cells should continuously provide mesenchymal progenitors, whether or not NOTCH signaling is blocked in osteoprogenitors. In fact, aging by itself may directly involve NOTCH signaling. In a recent study using a model of premature aging like the Hutchinson-Gilford Progeria syndrome, nuclear defects associated with aging were shown to activate NOTCH signaling (16). It is thus clear that NOTCH signaling is a major signaling axis in not only embryonic development but also in the mature skeleton, and these two studies should foster new exciting basic and clinical research directions.

Conflict of Interest: None reported.

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References

1. Engin F, Yao Z, Yang T, Zhou G, Bertin T, Jiang MM, Chen Y, Wang L, Zheng H, Sutton RE, Boyce BF, Lee B. Dimorphic effects of Notch signaling in bone homeostasis. *Nat Med*. 2008 Mar;14(3):299-305.

2. Hilton MJ, Tu X, Wu X, Bai S, Zhao H, Kobayashi T, Kronenberg HM, Teitelbaum SL, Ross FP, Kopan R, Long F. Notch signaling maintains bone marrow mesenchymal progenitors by suppressing osteoblast differentiation. *Nat Med*. 2008 Mar;14(3):306-14.

3. Fiúza UM, Arias AM**.** Cell and molecular biology of Notch. *J Endocrinol*. 2007 Sep;194(3):459-74.

4. Bulman MP, Kusumi K, Frayling TM, McKeown C, Garrett C, Lander ES, Krumlauf R, Hattersley AT, Ellard S, Turnpenny PD**.** Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. *Nat Genet*. 2000 Apr;24(4):438-41.

5. Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S. Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell*. 1997 May 16;89(4):629-39.

6. Bai S, Kopan R, Zou W, Hilton MJ, Ong CT, Long F, Ross FP, Teitelbaum SL. NOTCH1 regulates osteoclastogenesis directly in osteoclast precursors and indirectly via osteoblast lineage cells. *J Biol Chem*. 2008 Mar 7;283(10):6509-18.

7. Enomoto H, Shiojiri S, Hoshi K, Furuichi T, Fukuyama R, Yoshida CA, Kanatani N, Nakamura R, Mizuno A, Zanma A, Yano K, Yasuda H, Higashio K, Takada K, Komori T. Induction of osteoclast differentiation by Runx2 through receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegerin regulation and partial rescue of osteoclastogenesis in Runx2-/- mice by RANKL transgene. *J Biol Chem*. 2003 Jun 27;278(26):23971-7.

8. Deregowski V, Gazzerro 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 Mar 10;281(10):6203-10.

9. Oldershaw RA, Tew SR, Russell AM, Meade K, Hawkins R, McKay TR, Brennan KR, Hardingham TE. Notch signaling through Jagged-1 is necessary to initiate chondrogenesis in human bone marrow stromal cells but must be switched off to complete chondrogenesis. *Stem Cells*. 2008 Mar;26(3):666-74.

10. Schroeter EH, Kisslinger JA, Kopan R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature*. 1998 May 28;393(6683):382-6.

11. Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T, Suda T, Matsuo K. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab*. 2006 Aug;4(2):111-21.

12. Weber JM, Forsythe SR, Christianson CA, Frisch BJ, Gigliotti BJ, Jordan CT, Milner LA, Guzman ML, Calvi LM. Parathyroid hormone stimulates expression of the Notch ligand Jagged1 in osteoblastic cells. *Bone*. 2006 Sep;39(3):485-93.

13. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003 Oct 23;425(6960):841-6.

14. Calvi LM. Osteoblastic activation in the hematopoietic stem cell niche. *Ann N Y Acad Sci*. 2006 Apr;1068:477-88.

15. Roobrouck VD, Ulloa-Montoya F, Verfaillie CM. Self-renewal and differentiation capacity of young and

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aged stem cells. *Exp Cell Res*. 2008 Jun 10;314(9):1937-44.

16. Scaffidi P, Misteli T. Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. *Nat Cell Biol*. 2008 Apr;10(4):452-9.

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