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Review Article

Hylonome

The high bone mass family – the role of Wnt/Lrp5 signaling in the regulation of bone mass

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Abstract

A G171V mutation in the low-density lipoprotein receptor-related protein 5 (LRP5) was identified as causal for an autosomal dominant high bone mass trait in a single human family. A transgenic mouse line was produced that carries this mutation and develops a high bone mass phenotype that recapitulates the human phenotype. LRP5 is a co-receptor for Wnt and we have investigated the potential role of this gene/protein and the Wnt signaling pathway in mediating the bone formation response to mechanical loading. The G171V mutation results in an increased responsiveness of bone to mechanical load and reduces the threshold of load required to elicit a response. Our studies have shown that the Wnt signaling pathway is activated in response to mechanical loading and this response is greatly enhanced in the presence of the G171V mutation. Additionally, this mutation results in increased transcription of osteoprotegerin (OPG) in response to loading. Thus, the mutation appears to have direct effects at the level of the osteoblast and may also result in a reduction in osteoclastogenesis. The identification of LRP5/Wnt signaling in bone mechanosensation has resulted in a new paradigm for understanding bone formation. Hopefully, knowledge gained from these studies will result in new therapies for treating osteoporosis.

Keywords: High Bone Mass, Lrp5, Wnt Signaling, Mechanosensation

Studies of single gene traits inherited within families have proven to be invaluable entries into understanding basic, fundamental mechanisms of biology and disease. Often these explorations lead to discoveries that were not intuitively obvious based on existing knowledge and result in the convergence of different fields of study. The identification of mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) that give rise to the high bone mass (HBM) kindred[1] and the families with osteoporosis pseudoglioma syndrome (OPPG)[2] are another example of how new paradigms and molecular mechanisms can emerge from unexpected places. Since those initial reports, several other groups have identified mutations in LRP5 that give rise to increased bone mass phenotypes[3,4] or occurs in other patients with OPPG[5]. At the same time that these mutations were being identified in LRP5, work in the Wnt signaling field demonstrated that LRP5 and its close homolog, LRP6, served as co-receptors to bind Wnt[6,7]. The connection between LRP5 and the Wnt pathway as an explanation for the molecular basis of the human phenotypes was immediately obvious. These combined discoveries have brought the focus of attention of many bone biologists to the role of the Wnt signaling pathway in the regulation of bone mass and only time will reveal where this will ultimately lead.

We ascertained a single, large kindred with an autosomal dominant high bone mass trait[8] that was subsequently shown to be due to a mutation in the low-density lipoprotein receptor-related protein 5 (LRP5) gene that gives rise to a G171V substitution in the protein[1]. As noted above, there have been a number of mutations currently described in LRP5 that give rise to a wide variety of bone phenotypes. LRP5 is known to function as a co-receptor with the protein *frizzled* to bind Wnt which functions through the canonical β–catenin signaling pathway[9]. Upon binding of Wnt to the LRP5-*frizzled* complex several intracellular events occur that ultimately lead to an increase in the intracellular concentration of β-catenin. β–catenin is free to translocate to the nucleus where it interacts with the TCF family of transcription factors to

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promote transcription. There are several downstream target genes whose expression is known to be regulated by the Wnt signaling pathway[9] including cyclo-oxygenase-2[10], c-jun[11], and Connexin 43[12].

In our early phenotypic characterization of the kindred members, we were struck by the normal looking appearance of the bones (by radiography) from affected members of the family. This led to our initial hypothesis that the gene responsible for this phenotype was an integral component of the mechanism by which bone senses mechanical load. Alternatively, and just as attractive a hypothesis, is that the gene somehow regulates osteoblast proliferation and activity. These two hypotheses are by no means mutually exclusive, and I will briefly describe evidence that supports both.

In order to dissect the molecular basis for the increased bone mass that results from the G171V mutation a transgenic mouse line was constructed[13]. This mouse develops a high bone mass phenotype that is indistinguishable from the affected members of our kindred. These HBM transgenic mice have increased bone mineral density (BMD), increased strength and resistance to fracture, increased BV/TV, increased connectivity and trabecular number and thickness.

Osteoblast differentiation is mainly controlled by Cbfa1[14]. Studies with the Lrp5 knockout (Lrp5 KO) mouse have demonstrated decreased osteoblast proliferation and function, despite normal expression of Cbfa1[15]. Furthermore, the Lrp5 KO mice develop a pseudoglioma syndrome similar to patients with inactivating mutations in LRP5 that give rise to osteoporosis pseudoglioma (OPPG)[2]. A molecular explanation for the pseudoglioma syndrome that came from studies of the Lrp5 KO mice is the failure of macrophages to induce apoptosis in the hyaloid-artery system. A very interesting and important additional finding from the initial characterization of the LRP5 G171V transgenic mice was that osteoblast and osteocyte apoptosis were decreased in these transgenic mice. Thus, in both the knockout and transgenic HBM mice phenotype a connection between apoptosis and LRP5 has been observed.

Bone volume is a product of osteoblast formation activity and osteoclast resorption activity and having an osteocyte population available to maintain bone once it is formed. In addition, the osteocytes within bone function to regulate these two processes. Decreased osteoblast and osteocyte apoptosis would drive the system in favor of increased bone formation and maintenance. Presumably a longer osteoblast life span would result in the osteoblast forming more total bone during its life. The unanswered question that remains is whether the LRP5 G171V osteoblast is also more efficient, e.g., does the HBM mutation make an osteoblast that forms more bone per unit time. Studies to evaluate osteoblast activity are currently underway.

The LRP5 G171V transgenic mouse has proven invaluable in terms of exploring the connection between LRP5 and mechanosensation. The phenotype of this mouse recapitulates that of the human kindred. Consistent with "mechanostat" theory[16] the normal shape of the skeleton in these transgenic mice suggests an alteration in the sensitivity and/or response of the skeleton to mechanical loading. Bone formation response studies were performed using mechanical loading of the tibia in a 4-point bending device. Both periosteal and endosteal bone formation responses in the HBM transgenic mice were greater (~2-fold) compared to non-transgenic mice. Furthermore, the threshold for activation of the response was lower (~ 2-fold) in the HBM transgenic mice. These results are consistent with our hypothesis that the HBM mutation results in an alteration in the mechanosensation system in bone. Preliminary results from disuse studies using sciatic neurectomy suggest that the HBM mutation protects against disuse-associated bone loss[17]. Studies with ovariectomy-induced bone loss indicate no difference in the rate of bone loss in HBM transgenic mice compared to non-transgenic littermates[17]. These data suggest that the effects of the HBM mutation on bone may be restricted to the mechanosensation pathway. Further studies are warranted and are in progress.

As mentioned previously, LRP5 is thought to mainly act through the canonical Wnt signaling pathway. Several pathways have been previously implicated in mediating the response of bone to mechanical loading, including the integrins and their associated pathways[18], Ca^{+2} channels[19], and MAP kinase (ERK1/2 and p38) pathways[20]. The integrin linked kinase (ILK) pathway has an obvious intersection with the canonical Wnt signaling pathway in that ILK has been shown to phosphorylate GSK-β[21], which would lead to increased levels of cytoplasmic β–catenin and initiate transcription of target genes.

Molecular studies have been conducted that were aimed at trying to understand if the effects of the LRP5 G171V mutation were mediated through primarily the Wnt signaling pathway, or if other pathways were also involved. Transcriptional profiling studies of cells obtained from bone biopsies of affected and unaffected members of the kindred and from cells and bones from the transgenic mice identified increased activity of a number of Wnt pathway target genes, bone matrix proteins and stress response genes. Mechanical loading studies in the mice tibia have demonstrated activation of these genes in both the normal mouse and the LRP5 G171V transgenic mouse. The activation in the LRP5 G171V transgenic mouse was several fold greater than the normal mouse, consistent with increased sensitivity and responsiveness of bone cells in the presence of the mutation. One unique aspect of the transgenic mouse response was a significant increase in the OPG:RANKL mRNA ratio, which was not observed in the normal, wild-type mouse. This would have the potential additional effect of shutting down osteoclastogenesis in the transgenic mice, which would theoretically result in increased bone mass, and may provide a partial explanation for the phenotype observed in the presence of the mutation. We also observed an increase in secreted *frizzled* (sFRP1) production. The secreted frizzleds are another class of molecules that can act as a decoy receptor for RANKL[22] and reduce osteoclastogenesis. All of these

gene expression studies have confirmed a role for LRP5/Wnt signaling in the response to mechanical loading.

Fluid flow culture studies with the MC3T3-E1 mouse osteoblast cell line have demonstrated a critical role for β–catenin in the response of the osteoblast to mechanical loading. These studies have shown that activation of the Wnt signaling pathway enhances the sensitivity of these cells to mechanical loading[23]. Thus, data from both *in vivo* and *in vitro* studies consistently demonstrate a critical role of this pathway in the mechanosensation mechanism. Much more needs to be learned, however, before we have a full understanding of the molecular mechanism mediating the response of bone to mechanical loading.

In conclusion, what began as an interesting clinical case, a single female with high bone mass, has led us to appreciate the importance of a previous unsuspected pathway in the regulation of bone mass. Where this will lead remains to be proven. Clearly, as our understanding of the molecular basis of osteoblast, osteoclast and osteocyte biology increases, new paradigms for treating osteoporosis have the potential to emerge, and that is what makes this an exciting time in our field.

References

1. Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recke SM, Van Eerdewegh P, Recker RR, Johnson ML. A mutation in the LDL receptor-related protein 5 gene results in the autosomal sominant high-bone-mass trait. Am J Hum Genet 2002; 70:11-19.
2. Gong Y, Slee RB, Fukai N, Baron R, Olsen BR, Warman ML. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 2001; 107:513-523.
3. 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; 346:1513-1521.
4. Van Wesenbeeck L, Cleiren E, Fram J, Beals RK, Benichou O, Scopelliti D, Key L, Renton T, Bartels C, Gong Y, Warman M, Vernejoul MC, Bollerslev J, Van Hul W. Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with an increased bone density. Am J Hum Genet 2003; 72:763-771.
5. Streeten EA, Morton H, McBride DJ. Osteoporosis pseudoglioma syndrome: 3 siblings with a novel LRP5 mutation. J Bone Miner Res 2003; 18(Suppl 2):S35.
6. Wehrli M, Dougan ST, Caldwell K, O'Keefe L, Schwartz S, Vaizel-Ohayon D, Schejter E, Tomlinson A, DiNardo S. Arrow encodes an LDL-receptor-related protein essential for wingless signaling. Nature 2000; 407:527-530.
7. Tamai K, Semenov M, Kato Y, Spokony R, Liu C, Katsuyama Y, F.Hess F, Saint-Jeannet JP, He X. LDL-receptor-related proteins in Wnt signal transduction. Nature 2000; 407:530-535.
8. Johnson ML, Gong G, Kimberling WJ, Recker SM, Kimmel DK, Recker RR. Linkage analysis of a high bone mass trait in humans. Am J Hum Genet 1997; 60:1326-1332.
9. Mao J, Wang J, Liu B, Pan W, Farr G, Flynn C, Yuan H, Takada S, Kimelman D, Li L, Wu D. Low-density lipoprotein receptor-related protein-5 binds to axin and regulates the canonical Wnt signaling pathway. Mol Cell 2001; 7:801-809.
10. Howe LR, Subbaramaia K, Chung WJ, Dannenberg AJ, Brown AM. Transcriptional activation of cyclooxygenase-2 in Wnt-1-transformed mouse mammary epithelial cells. Cancer Res 1999; 59:1572-1577.
11. Mann B, Gelos M, Siedow A, Hanski ML, Gratchev A, Ilyas M, Bodmer WF, Moyer MP, Riecken EO, Buhr HJ, Hanski C. Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. Proc Natl Acad Sci USA 1999; 96:1603-1608.
12. Van Der Heyden MA, Rook MB, Hermans MM, Rijksen G, Boonstra J, Defize LH, Destree OH. Identification of connexin 43 as a functional target for Wnt signalling. J Cell Sci 1998; 111:1741-1749.
13. Babij P, Zhao W, Small C, Kharode Y, Yaworsky P, Bouxsein M, Reddy P, Bodine P, Robinson J, Bhat B, Marzolf J, Moran R, Bex F. High bone mass expressing a mutant LRP5 gene. J Bone Miner Res 2003; 18:960-974.
14. Karsenty G. The genetic transformation of bone biology. Genes Dev 1999; 13:3037-3051.
15. Kato M, Patel MS, Levasseur R, Lobov I, Chang BH-J, Glass DAI, Hartmann C, Li L, Hwang TH, Brayton CF, Lang RA, Karsenty G, Chan L. Cbfa 1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt co-receptor. J Cell Bio 2002; 157:303-314.
16. Frost HM. Bone "mass" and the "mechanostat": a proposal. Anat Record 1987; 219:1-9.
17. Bex F, Green P, Marsolf J, Babij P, Yaworsky P, Kharode Y. The human LRP5 G171V mutation in mice alters the skeletal response to limb unloading but not to ovariectomy. J Bone Miner Res 2003; 18(Suppl A):S60.
18. Duncan RL, Turner CH. Mechanotransduction and the functional response of bone to mechanical strain. Calcif Tissue Int 1995; 57:344-358.
19. Li J, Duncan L, Burr D, Turner C. L-type calcium channels mediate mechanically-induced bone formation *in vivo*. J Bone Miner Res 2002; 17:1795-1800.
20. Alford A, Jacobs C, Donahue H. Ocillating fluid flow regulates gap junction communication in osteocytic MLO-Y4 cells by an ERK1/2 MAP kinase-dependent

mechanism. Bone 2003; 33:64-70.
21. Troussard A, Mawji N, Ong C, Mui A, Arnaud R, Dedhar S. Conditional knock-out of intergrin-linked kinase demonstrates an essential role in protein kinase B/Akt activation. J Biol Chem 2003; 278:22374-22378.
22. Hausler KD, Horwood NJ, Uren A, Ellis J, Lengel C, Martin TJ, Rubin JS, Gillespie MT. Secreted frizzled-related protein (sFRP-1) binds to RANKL to inhibit osteoclast formation. J Bone Miner Res 2001; 16:S153.
23. Robinson J, Chatterjee-Kishore M, Li C, Gironda V, Green P, Bex F. Mechanical loading induces the expression of Wnt pathway genes and activation of the Wnt pathway in osteoblasts enhances loading responses. J Bone Miner Res 2003; 18(Suppl 2):S10.