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COMMENTARIES

New Aspects of Wnt Signalling Revealed by Mouse and Human Genetics

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Commentary on:

Glass DA 2nd, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G. Canonical Wnt signalling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell*. 2005 May;8(5):751–64.
Holmen SL, Zylstra CR, Mukherjee A, Sigler RE, Faugere MC, Bouxsein ML, Deng L, Clemens TL, Williams BO. Essential role of β-catenin in postnatal bone acquisition. *J Biol Chem*, 2005 June 3;280(22):21162–68.

There has been much interest in the role of the Wnt signalling pathway in bone formation since the discoveries that gain of function mutation in LRP5 leads to a high bone mass phenotype, and loss of function mutation leads to the osteoporosis-pseudoglioma syndrome (1;2;3). Activation of the canonical Wnt signalling pathway leads to stabilization of β-catenin in the cytoplasm through inhibition of glycogen synthase kinase (GSK)-3β-mediated phosphorylation, resulting in accumulation of cytoplasmic β-catenin, its translocation to the nucleus and transcriptional activation. Such activation in mesenchymal cells results in inhibition of chondrocyte differentiation and promotion of bone formation (4). Research attention has been focussed on the osteoblast lineage, and much evidence has emerged that canonical Wnt activation favours osteoblast differentiation and bone formation (5). Several approaches have been used to establish this role of Wnt signalling. These include development of genetically manipulated mouse models to recapitulate the human mutation syndromes (6;7), and *in vitro* studies showing direct effects of Wnt signalling on osteoblast differentiation and co-operative effects with bone morphogenetic proteins (4;8;9).

Two recent papers make a strong link between Wnt signalling and osteoclast biology. Both studies made use of transgenic mice, in which the osteoblast lineage was targeted to increase Wnt signalling, with the result in each case that the mice showed increased osteoprotegerin (OPG) production and decreased osteoclast formation. Glass *et al*. (10) prepared mice with Cre driven by the α1 (I) collagen promoter, and crossed them with mice with an active mutant β-catenin allele flanked by *loxP* sequences. The resulting mice, with constitutively activated Wnt signalling in osteoblasts, had a severe form of osteopetrosis, including failed tooth eruption, but normal osteoblast numbers. These phenotypes thus appeared to be due to failure of osteoclast formation. Microarray analysis of osteoblasts from these mice identified OPG as a candidate gene that might mediate these effects. OPG production was increased in the β-catenin mutant transgenics, reflecting the ability of the transcriptional activators of Wnt signalling, particularly TCF-1 and perhaps other TCF proteins, to activate the OPG promoter. This revealed yet another interesting link between the osteoblast and the osteoclast lineages, along the lines indicated from the activation of the OPG promoter by Runx2 (11). Although the amounts of OPG produced by the cells from the transgenic mice did not seem to be especially high, the osteoclast phenotype

was beyond doubt. From previous findings one would have expected stimulation of bone formation in these mice. This was not observed, but the authors did not claim to have excluded the possibility of stimulation.

A similar osteopetrotic syndrome due to failed osteoclast development and increased OPG resulted from a different approach used by Holmen *et al*. (12). They also used Cre-lox methods to prepare mice in lacking the adenomatous polyposis coli protein (APC) in osteoblasts. The main function of APC in a complex of proteins with GSK-3β is to contribute to maintaining the normal degradation of β-catenin. Its absence leads to accumulation of β-catenin, resulting in cell-autonomous activation of Wnt signalling in the osteoblast lineage. APC-deficient mice also had severe osteopetrosis, with serum OPG levels three times higher than those in wild type mice. Thus, like the mice described by Glass *et al.* (10), these mice manifest a phenotype of osteoclast deficiency which is not cell autonomous, but show a cell-autonomous requirement for β-catenin signalling in the mature osteoblast (12).

The findings of increased OPG production and decreased osteoclasts in these two studies represent previously unrecognised features of disrupted Wnt signalling in bone. The consequence of genetic manipulation in each case was to lead to osteoblast differentiation and a stage of enhanced OPG production, and hence reduced osteoclast formation and function. A previous attempt to identify low molecular weight activators of the OPG promoter led to compounds that could increase OPG production moderately (13). They were shown to be capable of modifying disease models that required osteoclast inhibition. These two demonstrations (10;12) of the dramatic effect of increased OPG production on osteoclast development might give further impetus to that search. They do not reveal information about effects of Wnt signalling directly within the osteoclast lineage, which remains an open question. A link with osteoclast biology has been shown in studies that revealed the Wnt decoy receptors, secreted frizzled-related proteins (SFRPs), as osteoblast products, and the finding that SFRP-1 and SFRP-3 inhibit osteoclast formation in several *in vitro* systems (14). Consistent with this, mice rendered null for *SFRP-1* were found to have increased osteoclast formation *in vitro*, but also increased bone mass phenotype as they aged, consistent with Wnt signalling enhancing bone mass (15).

Surprised though some might be at the findings of these two mouse genetic studies, it is reassuring to know that there is recent human data that is consistent with the osteoclast findings. The high bone mass phenotype in human subjects with a G171V mutation in *LRP5* is the result of increased osteoblast activity, with normal resorption parameters. On the other hand, patients with autosomal dominant osteopetrosis type I (ADOI) due to T253I mutation in *LRP5,* manifest an osteopetrotic phenotype, with lower numbers of osteoclasts *in vivo* (16;17), but show no increase in bone formation markers (18). In a recent study Henriksen *et al.* (19) prepared osteoclasts from $CD14^+$ monocytes of subjects with ADOI, finding both osteoclast formation and resorption capacity to be normal. This leads them to conclude that the osteoclast phenotype in ADOI is not cell-autonomous, but rather is an indirect outcome of altered control *in vivo* of osteoclast function. They have thus helped to identify different outcomes from two separate mutations in *LRP5.* The G171V mutation leads to increased bone through an osteoblast and bone formation effect, while the T253I mutation does so by reducing osteoclast activity. They note that serum OPG levels have been observed to be 40% higher (non-significant) in T253I mutation patients (20).

The ADOI data provides a new look at Wnt signalling through the osteoblast. The two mouse genetic studies were consistent in showing osteoclast reduction and increased OPG, in each case associated with increased Wnt signalling, either directly by overexpressing β-catenin in osteoblasts, or indirectly by removing the inhibitory APC, with consequent accumulation of β-catenin. The data in patients with ADOI (19)

indicates that, dependent upon the nature and location of mutations in *LRP5,* there can be varying effects on the osteoblast phenotype. The cell signalling and transcription consequences differ in ways that might not be readily revealed by the methods currently in use to overexpress β-catenin. The two mutation sites are in different parts of the YWTD domain of the first propeller structure of LRP5 (21). Although DKK-1 inhibition of LRP binding and activity is associated with the G171V mutation (1), the consequences of the T253I mutation for LRP5/6 binding are not known.

Given the complexity of the Wnt system, with its receptor complex of inhibitory and stimulatory components, its decoy receptors, the multi-protein inhibitory complex acting on GSK-3β, and the necessary phosphorylation steps, it should perhaps be expected that subtly different mechanisms will emerge for the control of activation. Combined input from mouse and human genetics has provided some important new clues.

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