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COMMENTARIES

Wnt Signaling, LRP5 and Gut Serotonin: Have We Been Targeting the Right Pathway for the Wrong Reasons?

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Commentary on: Yadav VK, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schütz G, Glorieux FH, Chiang CY, Zajac JD, Insogna KL, Mann JJ, Hen R, Ducy P, Karsenty G. Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell*. 2008 Nov 28;135(5):825-37.

Are the OPPG and HBM Phenotypes Due to Wnt Signaling in Bone, to the Control of Peripheral Serotonin Levels by LRP5 in the Gut, or Both?

The recent discoveries that the human low bone mass Osteoporosis Pseudoglioma Syndrome (OPPG) and the High Bone Mass (HBM) phenotypes were due, respectively, to loss- and gain-of-function of the LRP5 receptor (1-3) directed bone research and osteoporosis drug discovery to the Wnt signaling pathway and has resulted in several biologics currently being tested in the clinic for osteo-anabolism. In a stunning and paradigm-shifting manuscript, Dr. Gerard Karsenty and colleagues (4) now report that, contrary to the commonly held belief that the bone mass alterations in OPPG and HBM are due to changes in Wnt signaling in osteoblasts and osteocytes, the skeletal homeostatic function of LRP5 resides in entero-chromaffin cells in the duodenum. In these cells the LRP5 receptor negatively regulates the synthesis and secretion of serotonin in the periphery (*i.e.*, independent of serotonin in the brain). In turn, the decrease in circulating serotonin favors osteoblast proliferation and bone formation, with serotonin negatively affecting osteoblasts through the H1b receptor (4). Several studies in the last few years had shown that bone cells, including osteoclasts, express receptors for serotonin (5-8) and that the effects of serotonin reuptake inhibition lead to a marked decrease in bone formation and bone density in mice and also to increased fracture risk in humans (9-10), although there may be opposite effects on cortical bone, which was not studied here. The truly new findings in the current study from Yadav *et al*. are 1) the identification of the osteoblast serotonin receptor; 2) the strengthened evidence for a negative influence of serotonin on bone formation and more importantly; 3) the finding that LRP5 plays a critical role in the regulation of serotonin synthesis in the gut (Fig. 1).

Yadav *et al*. have reached these quite unexpected conclusions through an impressive series of very creative mouse molecular genetic experiments. The main findings can be summarized as follows: 1) targeted deletion of LRP5 in osteoblasts using the *collagen type 1* promoter-driven Cre failed to reproduce the osteopenia induced by global deletion of the receptor, suggesting that LRP5 might affect bone indirectly; 2) conversely, targeted knock-in of a cDNA encoding the HBM mutation of LRP5 (G171V) using the same promoter failed to induce the same increase in bone mass when solely expressed in osteoblasts compared to when globally expressed, again suggesting that LRP5 could affect bone indirectly; 3) the molecular signature of global LRP5 deletion includes a significant increase in the expression of Tph1, which encodes an enzyme involved in serotonin synthesis outside of the brain (Tph2, not Tph1, is responsible for serotonin synthesis in the brain), and peripheral serotonin levels are high in *LRP5*(-/-) mice and in patients with OPPG; 4) the opposite is true (*i.e.*, serotonin levels are low) in LRP5 G171V

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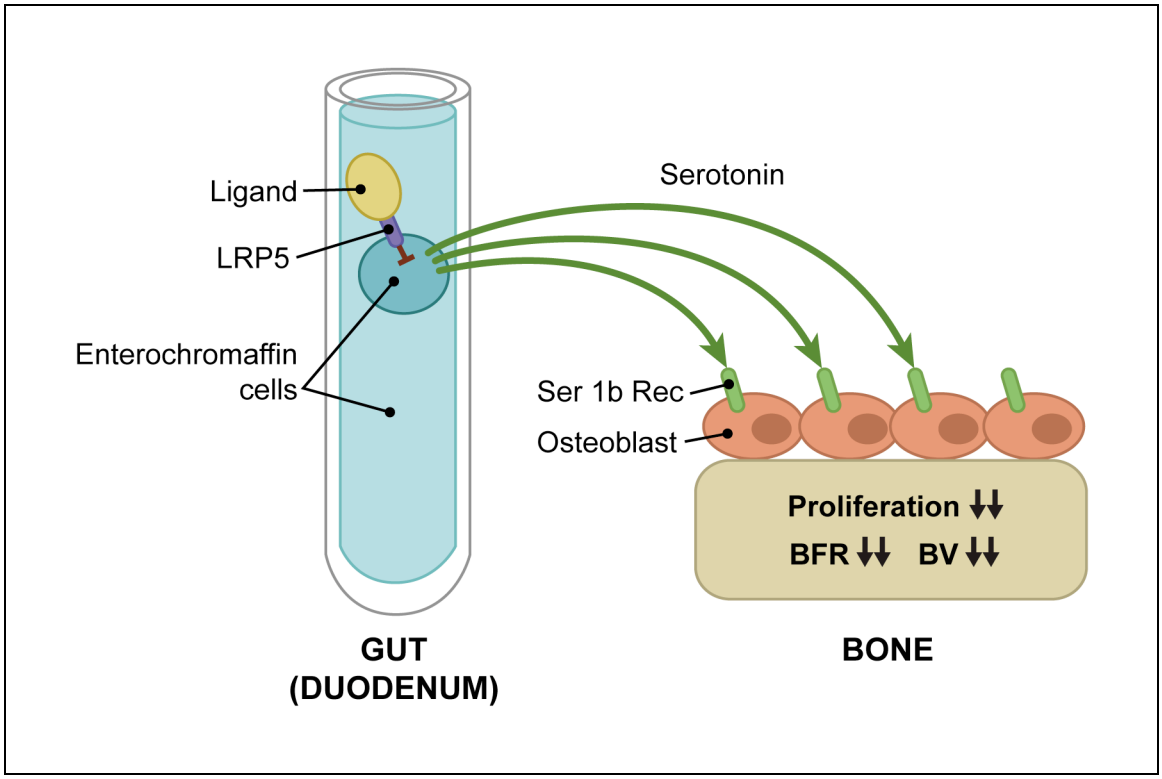

Fig. 1. LRP5 represses peripheral serotonin synthesis and secretion in the gut, favoring bone formation (4). Adapted from Yadav *et al*. *Cell*. 2008 Nov 28;135(5):825-37.

mice and in patients with the HBM phenotype (to be confirmed); 5) the main sources of peripheral serotonin (95%) are enterochromaffin cells in the duodenum and a deletion of LRP5 targeted to the intestine using a Villin promoter to drive Cre expression mimicked the OPPG osteopenic bone phenotype, in sharp contrast to the *Col1*-driven deletion in osteoblasts; 6) the HBM phenotype could be reproduced by knocking-in the G171V LRP5 mutant with the *Villin* promoter, in contrast to the same experiment performed with the *Col1* promoter; 7) dietary and genetic experiments aimed at manipulating circulating levels of serotonin supported the concept that serotonin acts as an inhibitor of osteoblast proliferation and bone formation, and the specific receptor in osteoblasts was identified as Htr1b; 8) the effects of LRP5 on Tph1 in the gut do not appear to be via activation of β-catenin, *i.e*., canonical Wnt signaling; 9) finally, serotonin levels were reported to be elevated four-fold in three OPPG patients and possibly decreased by 50% in HBM patients (Yadav VK, personal communication). Based on these very extensive data obtained through mouse genetic studies, the authors conclude that LRP5 does not have a principal role in osteoblasts and that the OPPG and HBM phenotypes are not due to loss- or gain-of-function in Wnt signaling but rather to the β-catenin- and Wnt-independent effects of LRP5 deletion on gut-derived serotonin.

Less convincing are arguments based on pharmacology or signaling experiments. The authors focus solely on cell proliferation in the osteoblast lineage, which is surely important, but not to the exclusion of differentiation, where Wnt might be critical. In their single experiment studying effects of serotonin on mitotic index in mixed osteoblast cultures, the authors used a single dose of 50μM serotonin to show 50% inhibition of mitotic index. The two-fold increases in mitotic index in osteoblasts grown from Htr1b-deficient and from gut Tph1-deficient mice must be looked at with some reservation as these are heterogeneous primary cultures in which the bones of wild type mice, compared to knockout mice, differed greatly in bone

formation rates and bone mass from the start.

On the other hand, if the findings are indeed confirmed, these studies will open an entirely new field of investigation and novel therapeutic approaches since antagonizing serotonin synthesis in the gut and/or serotonin action on osteoblasts should prove to be highly anabolic. This would have no effect on brain serotonin since serotonin does not cross the blood-brain barrier, although the effects of serotonin levels in the brain on bone formation remain to be elucidated and might be opposite. It will also be of great interest to identify the other pathway participants by which LRP5 regulates serotonin synthesis, since they may serve as targets for the therapeutic regulation of bone formation.

Is Wnt Signaling, Then, Not Important in Bone?

It would be a great mistake if these provocative findings dampened the current enthusiasm for studying and targeting the Wnt signaling pathway to discover bone anabolics for the treatment of osteoporosis; this would be a counterproductive and erroneous consequence of this groundbreaking paper. While it will certainly force the field to reconsider multiple aspects of bone biology and treatment, it should not change the course of Wnt signaling research in bone and its enormous therapeutic opportunities.

This important point is based on two considerations. First, the evidence is very strong that the human mutations causing the sclerosteosis and van Buchem high bone mass phenotypes are attributable to sclerostin and Wnt signaling in bone (11;12). Many mouse models have also undoubtedly established the fact that Wnt signaling in osteoblasts/osteocytes is strongly anabolic (see (13) for review). Second, the jury is still out as to whether the OPPG and HBM LRP5 mutations indeed do not also contribute directly, *i.e*., within bone, to the bone phenotypes, in addition to the gut-mediated LRP5-dependent changes in peripheral serotonin levels.

As a premise to their studies, Yadav *et al*. mention three observations that they believe challenge the view that OPPG and HBM are Wnt-related diseases: 1) there is no overt skeletal defect in *LRP5*(-/-) embryos; 2) the HBM gain-of-function mutation does not cause bone tumors as Wnt activation does in other organs; 3) (and most importantly) gain- and loss-of-function in β-catenin, the molecular node of canonical Wnt signaling, do not affect bone formation (14;15).

Although possibly correct, these observations are not compelling. Indeed, LRP6, which is closely related to LRP5, appears to be the principal Wnt co-receptor during development. Mice lacking one copy of LRP6 develop normally, but have low bone mass, while embryonic lethality occurs in mice that completely lack LRP6. Importantly, while skeletal patterning is normal in mice lacking LRP5, it becomes abnormal when these mice also lack one copy of LRP6, indicating that both receptors function during development (16), something difficult to attribute to gut serotonin. Furthermore, reductions in bone mass appear additive in mice with mutations in LRP5 and LRP6, indicating that both receptors function postnatally to affect bone mass (16). In contrast to the first point, this could, however, be attributable to effects outside of bone. Second, HBM mutations need not activate Wnt signaling at a level high enough to cause tumors. Increased rates of malignancy have not been reported in mice with mutations in other components of the Wnt signaling cascade that lead to mild activation of Wnt signaling (*e.g*., Dkk1, Kremen or SFRP knockouts). Finally, the use of a *Col1* promoter to activate and inactivate LRP5 function does permit the function of this receptor to be studied in cells committed to becoming osteoblasts, but not in less differentiated cells that have not yet committed to the osteoblast lineage. Last, but not least, and although the data on β-catenin are convincing, events could be downstream of Wnt and LRP5/6 but β-catenin-independent, for instance via cross-talk with other pathways, such as PTH or BMP signaling (17-19) or with non-canonical Wnt signaling, also shown recently to favor bone formation (20-22).

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Is LRP5 a Bona Fide Co-Receptor for Wnts?

In this respect, a most important question is whether or not LRP5, which we know is expressed in osteoblasts and osteocytes, is a bona fide Wnt receptor. As reported in 2001 (1), we had shown that naive cells unresponsive to Wnt could acquire canonical Wnt responsiveness when transfected with LRP5 (see Fig 6A in (1) and Fig. 2 here) and lose their ability to respond to Wnt if transfected with an LRP5 mutant lacking the cytoplasmic C-terminal tail, known to be required for intracellular transduction via Axin recruitment to the plasma membrane. Indeed, Dr. Karsenty's own group reproduced and published similar data in 2002 (see (23), Fig. 6C, "LRP5-dependent activation of gene expression by Wnt proteins") as did several other groups. In the present report however, these data have been ignored on the basis that they represent *in vitro* overexpression results (4), the physiological relevance of which is uncertain. It is, however, a fact that these studies demonstrate that LRP5 is a genuine Wnt receptor, which functions in a manner similar to LRP6, and binds Wnts as well as the Wnt antagonists Dkk1 and sclerostin (24-27). It is also intriguing that the affinity for Dkk1 and sclerostin, two inhibitors of Wnt signaling, are markedly decreased in the G171V HBM mutation (3) and that fat is reduced in the HBM patient's marrow (28), a characteristic feature of decreased Wnt signaling.

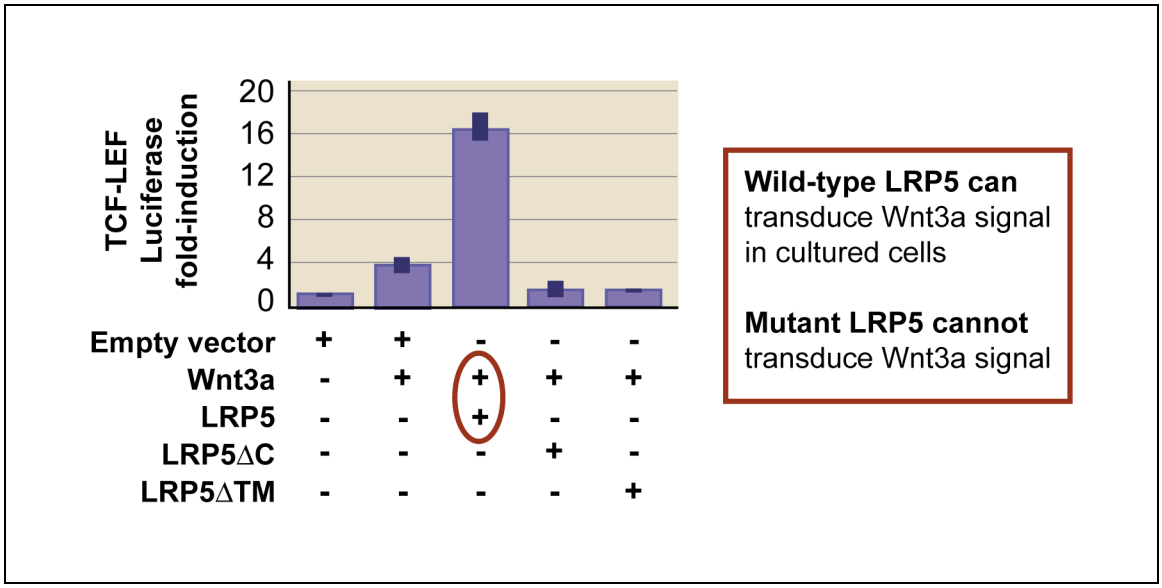

Fig. 2. Studies using cultured cells, as well as pluripotent stem cells, indicate that LRP5 can transduce Wnt signaling (1). Adapted from *Cell*, Volume 107, issue 4, Gong *et al.*, LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development, pp. 513-23, copyright (2001), with permission from Elsevier.

Furthermore, studies from Sawakami *et al*. (29) demonstrate that deletion of LRP5 alters mechanosensing in osteocytes, where loading decreases sclerostin expression (30), something quite difficult to reconcile with a role of circulating serotonin. Finally, O'Brien *et al*. have recently shown that the anabolic response of bone in mice with a constitutively active PTH receptor in osteocytes is blunted by deletion of LRP5 (31). Although the increased circulating levels of serotonin in *LRP5*(-/-) mice could counter the effects on PTH signaling in osteocytes, the fact that PTH decreases sclerostin expression in these cells (32;33) and that LRP5 is indeed a bona fide Wnt receptor that can activate Wnt signaling (see above) seems a much more reasonable explanation. This study would also show, if it was demonstrated that the blunted response is not serotonin-mediated, that LRP5 is essential in this context despite the fact that LRP6 is still expressed in these mice.

Thus, not only is it difficult to reconcile these findings with an exclusive and Wnt-

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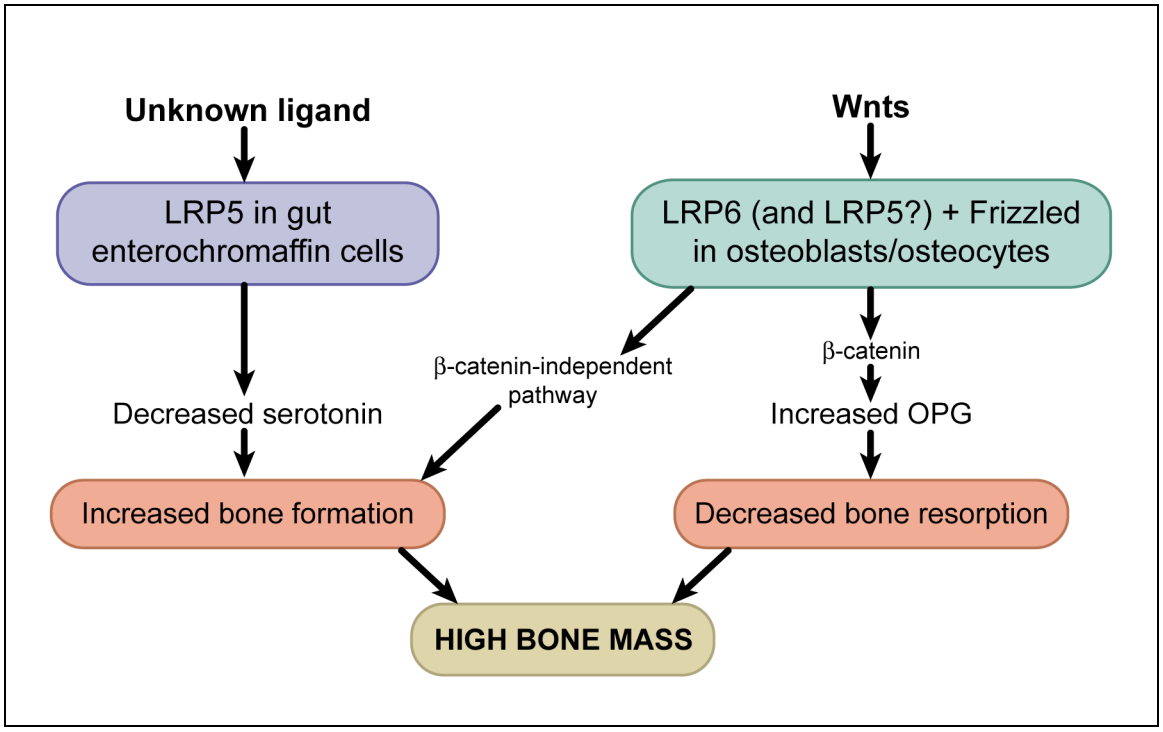

Fig. 3. Two, not just one, LRP-dependent anabolic pathways.

independent role of LRP5 outside of the bone environment (namely in the gut) but it is also difficult to imagine, with LRP5 being expressed in osteoblasts/osteocytes and Wnts being present in the bone environment, that the binding of one to the other does not affect Wnt signaling in these cells. Yadav *et al*. would suggest that this occurs mostly via LRP6, with the role of LRP5 only a redundant one and contributing minimally. But minimally is not the same as not at all, and future studies will have to address this point. In this context, it is also important to point out that Yadav *et al*. added a Flag epitope to the C-terminus of LRP5 in their knock-in studies. Since Wnt signal transduction relies on ligand-dependent modifications of the C-terminus, the investigators may have inadvertently damaged the receptor's ability to respond to Wnt signaling, similar to what has been reported when a myc epitope was added to the C-terminus (26). However, the fact that this Flagged receptor worked in the gut to induce an increase in serotonin and the bone phenotype remains a quite compelling piece of data.

What Explains the Negative Findings of Yadav *et al*.?

First, the effects of serotonin may dominate and the contribution of LRP5 could indeed be too small to be seen at the ages and skeletal sites studied in the paper. Second, the use of the *Col1-Cre* to induce the deletion of LRP5 and to knock-in the HBM LRP5 mutant in osteoblasts may drive expression after the time at which LRP5-dependent Wnt signaling influences osteoblast precursors, LRP5 and/or Wnt signaling being essential only at early stages of precursor commitment (before *Col1* is turned on). Yadav *et al*. do not discuss this possibility and have not tested other Cre drivers in the bone system. Much work will be required to clarify this point. Thus, and although the data presented in this excellent manuscript are very compelling regarding an LRP5-dependent regulation of gut-derived serotonin and bone formation, the jury is still out as to whether LRP5 contributes at least in part to the bone phenotypes in OPPG and HBM patients.

In conclusion (Fig. 3), this outstanding paper will force the bone field to rethink the whole story of LRP5 and to incorporate the role of

peripheral serotonin in bone homeostasis, and in particular to determine if its deleterious effects also apply to cortical bone, a question not addressed in the Yadav *et al*. (4) study. But, even if independent of LRP5 in bone, Wnt signaling in the bone environment is a dominant anabolic pathway that remains critical for the establishment and maintenance of bone mass in mice and in humans, and will probably remain for the foreseeable future the main focus of new anabolic therapeutic approaches to increase bone formation and bone mass in low bone mass patients, as well-illustrated, for instance, by the impressive results obtained in humans with antibodies to sclerostin.

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Conflict of Interest: None reported.

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