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NEWS

Elucidating a Role for Lrp5 in Mechanotransduction

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Approximately five years after the initial studies linking low-density lipoprotein receptor-related protein 5 to human skeletal disease, new research is helping to elucidate the exact contribution of this key cell-surface protein to bone mass regulation. In particular, a recent study published in the August 18th issue of the *Journal of Biological Chemistry* suggests that while knockout mice lacking the protein can sense mechanical load correctly, they are unable to respond properly to it by making new bone.[1] Though the study has not convinced some experts that definitive proof of the concept has been provided, all of the bone specialists who spoke to *BoneKEy* do agree on the importance of the research and the suggestive nature of the findings, which they say represent only the initial stages of an exciting and growing field of research.

The Study

A team of researchers led by Charles Turner, a professor of biomedical engineering at Indiana University-Purdue University Indianapolis, generated genetically engineered knockout mice missing the gene encoding low-density lipoprotein receptor-related protein 5 (Lrp5) and tested their ability to respond to mechanical loading *in vivo*. This protein had previously been identified as a co-receptor for Wnt glycoproteins and thus as an important factor in the Wnt signaling pathway that is increasingly thought to be a key player in bone mass regulation. The investigators found that while wild-type mice produced new bone in response to ulnar loading, both male and female knockouts exhibited significantly decreased levels of new bone formation. Most striking of all were the female knockouts, which had an approximately 99 percent lower relative periosteal bone formation rate than the wild type females. The male knockouts exhibited a lesser, yet still impressive, 88% reduction.

Additional experiments showed that the numbers and activity of early osteoblastic cells in the knockouts were normal. Further *in vitro* studies also suggested that the early stages of the bone cell response to mechanical loading were intact in the knockouts. In these experiments, the investigators cultured primary osteoblasts from knockout and wild type animals and subjected these cells to fluid shear stress, a technique that mimics mechanical loading *in vitro*. After stimulating the cells using this technique, the researchers measured the chemical activity already thought to occur in the early stages of the load-induced response, including the release of ATP and prostaglandin E_2, as well as MAP kinase signaling, and found no deficits in this activity in the knockout mice. They did, however, find a difference in levels of the bone matrix protein osteopontin, whose production is thought to be characteristic of the later stages of the response. Indeed, while wild-type mice showed a significant increase in the amount of osteopontin produced in response to fluid shear stress, the knockouts failed to show a similar rise.

"At a minimum, these experiments show that the standard markers for sensing mechanical load are not affected in Lrp5 deficiency, but the ability of the cells, once reawakened from their quiescent stage, to be nudged toward making bone in response to mechanical load is indeed impaired," says Matthew Warman, a co-author of the current study, senior author of one of the first studies linking inactivating mutations in the protein to bone mass defects in humans, and a Howard Hughes Medical Institute investigator at Children's Hospital, Boston. Observers not directly involved with the current study also tend to agree with this

interpretation that makes a distinction between sensing, and responding to, mechanical loading. "This research really provides strong evidence in support of the idea that Lrp5 has an essential role in the mechanotransduction system, but this role is not in the early stages, in the ability of the cells to detect changes in strain, but in the later stages, in the ability to fully respond to strain," says Russell Turner, a professor in the department of nutrition and exercise sciences at Oregon State University and an expert on bone health. "This is a big first step — though certainly not the last one — in really understanding how the mechanotransduction system works."

While the results suggested that Lrp5 is necessary for a normal bone-forming response to mechanical loading, questions remained whether agents that build bone, such as parathyroid hormone (PTH), also depended upon the presence of the receptor. The researchers concluded that PTH, administered intermittently, acted similarly in both knockout and wild-type animals, since both groups showed similar increases in bone mineral content in the hindlimb, and in volumetric bone mineral density at the distal femur. This particular finding is also viewed by experts as an important new contribution, though some note that the result is consistent with related studies and thus unsurprising. The investigators suggest that patients with osteoporosis-pseudoglioma (OPPG), a low bone mass syndrome characterized by mutations in Lrp5, may benefit from the hormone, since it appears that PTH acts independently of the receptor.

Building a Stronger Case

While the current study provides strong evidence of an association between mutations in Lrp5 and defects in the response to mechanical loading, additional experiments are necessary to strengthen the case that Lrp5 plays a causal role in producing those defects, according to some experts. First, because mice lacking Lrp5 display low bone mass, it is possible that it is this factor that explains the poor response of the knockouts to mechanical loading, notes Roland Baron, a professor of orthopaedics and cell biology at Yale University School of Medicine and also a co-author of one of the earlier papers on the receptor and bone mass. A more convincing experiment better able to pin down a causal role for Lrp5, notes Baron, would be one that compares an Lrp5 knockout mouse to a control animal that also exhibits low bone mass. "It would be very interesting to have a control group that has about the same changes in bone volume and structure as the Lrp5 knockouts. If, with similar changes in structure, the biomechanical properties are different, that would answer the essential question of whether a poor biomechanical response is caused by, and is not just a consequence of, a lack of Lrp5." Other observers counter, however, that the experimenters did take differences in bone mass into account when calculating how much force to apply so that both the knockout and the wild-type animals would experience similar ulnar strain levels.

Another open question that may merit further scrutiny is whether the defect found in the knockouts is a defect in the later stages of osteoblast maturation, as the authors of the study propose, or whether the defect actually resides somewhere else, perhaps in osteoblast longevity or cell survival. One clue, notes Karl Insogna, director of the Yale Bone Center, and the Yale Core Center for Musculoskeletal Disorders, at Yale University School of Medicine, and also a co-author of one of the first studies linking activating mutations in Lrp5 to high bone mass, is the interesting differences reported in the phenotype of male and female knockout mice. The male knockouts exhibited a more severe phenotype than the female knockouts in terms of bone mass, structure and mechanical properties. The study authors speculate that an interaction between Wnt signaling and estrogen signaling might account for these differences. "The phenotype is more severe in the males, and the authors' suggestion that estrogen may modulate the interaction of Lrp5 in the skeleton is one of the reasons the notion of cell survival might be important, because estrogen clearly has a pro-survival effect," Insogna notes.

A third issue concerns the choice to measure osteopontin and to use changes in the levels of this bone matrix protein as an indicator of a late-stage response to mechanical loading. "The osteopontin knockout mouse doesn't have any particularly dramatic bone phenotype," Insogna notes, and Baron also adds that since Lrp5 knockout mice have low bone mass, it's not surprising that they cannot produce normal quantities of matrix proteins like osteopontin. Consequently, decreases in osteopontin may be a reflection only of low bone mass, and not necessarily of a defect in mechanotransduction caused by Lrp5 deficiency. Baron suggests that the study's main claim would be strengthened with experiments that measure sclerostin, a protein thought to be released by osteocytes in response to mechanical loading. In fact, in an abstract submitted to the annual meeting of the ASBMR, Charles Turner and colleagues have indeed turned their focus to this protein and have linked mechanical loading to changes in sclerostin expression. In doing so, not only has the case for Lrp5 been strengthened, but the prospects for developing a pharmaceutical compound that may help compensate for defective biomechanical responses have also brightened.

An Exercise Pill?

One of the long-term goals of studies on proteins like Lrp5 and their role in mechanotransduction is to use knowledge based from such research to develop an "exercise pill" — that is, a pharmaceutical agent that can mimic the effects of exercise and increase bone formation in patients with osteoporosis and other low bone mass diseases. If Lrp5 is in fact crucial for a normal response to mechanical loading, then researchers now have a starting point for an exercise pill that can act as a surrogate for exercise. "To make a pill, we need a receptor, and now that we have a receptor, we can start working towards drug targets," says Charles Turner, senior author of the study. "We actually now have some biology to work with, while before, though we knew exercise did certain things, it wasn't very clear how one would actually develop any kind of treatment based on that knowledge."

Turner notes that once Lrp5 had been pinpointed as a key receptor in the response to mechanical loading, the next step was to identify the molecule that binds to Lrp5, and to understand how mechanical loading might affect the interaction between this putative binding molecule and the receptor. Though this research is still evolving, Turner and his colleagues think they have identified sclerostin as the Lrp5-binding molecule whose levels are affected by mechanical loading.

Previous research had shown that sclerostin is secreted by osteocytes and, in binding to and antagonizing Lrp5, inhibits bone formation. In their ASBMR Annual Meeting abstract, Turner and colleagues take the story a step further by measuring levels of sclerostin, released from osteocytes, in mice whose ulnar diaphyses were subjected to mechanical loading, and by comparing these levels to those found in control mice whose bones were not subjected to loading. The investigators found that the loaded ulnae exhibited significant decreases in sclerostin, as measured by immunohistochemistry one day after loading, compared to the unloaded bones.

"It looks like one of the key molecules that binds the Lrp5 receptor is sclerostin, and this particular molecule, if you add mechanical loading, is decreased in its secretion from the osteocytes, which permits increased bone formation," Turner says. If further studies can replicate and extend this finding, a drug that mimics exercise could become a realistic possibility, since there is already an antibody drug in development by Amgen that binds to and inhibits sclerostin, and that has been shown to increase bone formation and bone mineral density in ovariectomized rats. (In abstracts submitted to the ASBMR Annual Meeting, this antibody was also shown to improve bone strength in rats with low bone mass, and a humanized anti-sclerostin antibody was shown to increase bone formation, bone mineral density and bone strength in cynomolgus monkeys).

In addition to an anti-sclerostin antibody, the Lrp5 knockout study may also spur the search for other anabolic agents that bypass Lrp5 and that, potentially, might also be effective in improving the bone-forming response to mechanical loading. One such agent is lithium, which was shown by Warman, Baron and others in a 2005 study in the *Proceedings of the National Academy of Sciences* to increase bone mass in mice lacking Lrp5, perhaps by activating the Wnt signaling pathway downstream of the receptor. "It's interesting to ask whether the administration of lithium, or other Wnt agonists that can bypass the Lrp5 receptor, can restore the mechanotransduction response in these mutant Lrp5 mice," Warman says. "There are anecdotal data in humans that people with bipolar disease on lithium have higher bone density than average individuals. The idea that a small molecule, and a relatively safe one, might be efficient at increasing bone strength is very exciting," Warman says.

Finally, while the search for new anabolic agents goes on, the Lrp5 study, with its findings on PTH, will also spur a more detailed examination of this already proven anabolic agent. Researchers are particularly interested in learning whether Lrp5 may be important when lower, more physiological doses of PTH than those tested in the current study are employed. In addition, while Lrp5, at the higher doses used in the study, doesn't appear to be a key player in the response to PTH, uncertainties still remain about the relationship between the workings of PTH and the Wnt signaling pathway. "One of the unanswered questions is whether any aspect of the Wnt signaling cascade is involved in PTH's anabolic effect," notes Insogna. "This is an important question because if bone anabolism can be induced by multiple pathways, perhaps there is some additivity or synergisms that can be gained."

Future Research Directions

While the forecast for drug development is optimistic, numerous questions remain in scientists' basic understanding of the bone cell response to mechanical loading. While studies like the current one have examined individual components of the account, the next challenge is to stitch the individual stories together into a larger narrative, according to Mark Johnson, a professor at the University of Missouri-Kansas City School of Dentistry and senior author of one of the early studies that linked mutations in the gene for Lrp5 to high bone mass in humans. "Where the research really needs to go now is in the direction of integrating all of the individual components into a unifying model that explains the overall process, much in the way we understand, for instance, the glycolytic pathway and all the steps that are involved in converting glucose into ATP energy."

Johnson's laboratory has been particularly interested in this effort. "We are trying to understand the sequence of events and the communication between the cells perceiving the load and the cells ultimately responding, and then trying to figure out how all of these pathways fit together." He believes they have begun to make progress towards this end by uncovering interactions between the prostaglandin and Wnt signaling pathways, and in an abstract submitted to the ASBMR Annual Meeting, revealed experimental evidence of these interactions in osteoblastic and osteocytic cells subjected to fluid flow shear stress.

Based on these initial results, Johnson and colleagues are now testing a two-stage model, in harmony with the findings from the Lrp5 knockout study, that describes the temporal sequence of events that occurs in response to mechanical loading. In the first stage, postulated to occur independently of Lrp5, cells that perceive mechanical load (a process that initial data suggest may first occur in osteocytes), release prostaglandin E_2 (PGE_2), which binds to its receptor and consequently inhibits glycogen synthase kinase 3 beta, an enzyme that regulates levels of intracellular β-catenin. This activity results in activation of the Wnt signaling pathway and activates target genes that Johnson thinks will be important modulators of Lrp5, leading to a second stage characterized by feedback amplification of the Wnt pathway at the level of Lrp5.

"This model would fit well with the results from Turner and colleagues showing no changes in ATP or PGE_2 production in the Lrp5 knockouts," Johnson says. "My explanation would be that the early events occur because those events are Lrp5-independent. But the later events, the critical 'go/no go' signal that leads to bone formation, is the feedback amplification at the level of Lrp5, and when Lrp5 is missing, as in the knockout, this fails to occur."

In addition to this kind of work that aims to integrate the individual parts of the story into a more complete tale, future research may also seek to use some of the experimental methods and approaches pursued in the Lrp5 paper. In particular, the study investigators point to their use of mice that express green fluorescent protein (GFP), which marks bone cells at different stages in the osteoblast lineage, as a promising technique that can help open a window into bone disease. "Many diseases of the bone are diseases of the lineage, where the defect does not lie in making a bone matrix, but rather in generating the cells capable of producing bone matrix," stresses David Rowe, a co-author of the current study who supplied the mice expressing GFP for the study's osteoblast recruitment experiments. "I'm so pleased to see these GFP mice used in a very creative way, and believe this is only the beginning for the use of this reporter in model systems," says Rowe, also a professor of genetics and developmental biology at the University of Connecticut Health Center.

In addition to making the most of new experimental techniques, the current study also points to the fruits of multi-disciplinary research. "The Lrp5 knockout work really demonstrates the melding of scientific expertise in multiple disciplines, where bioengineers, geneticists and mouse modelers all pooled their expertise, resources and ideas," Warman says. "This is the way science should be done."

References

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