

The Role of the Wnt Pathway in Mediating the Effects of Mechanical Loading

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Introduction

The search for pathways involved in load-induced bone formation was advanced considerably several years ago, when genetic mapping studies among families with osteoporosis pseudoglioma (OPPG) revealed that the low density lipoprotein receptor related protein 5 (LRP5) had important functions in the mammalian skeleton. Mice engineered with a loss-of-function mutation in *Lrp5* exhibit low bone mass, but the weight-bearing portions of the skeleton bear a greater deficit in bone mass than the non weight-bearing portions. Those observations led us to test the hypothesis that *Lrp5* is important in mechanical signaling. When subjected to *in vivo* ulnar loading, we found an 88 to 99% reduction in load-induced bone formation among *Lrp5*^{-/-} mice, compared to wild-type controls.

Shortly after the discovery of families harboring loss-of-function mutations in LRP5, other investigators identified a series of missense mutations in LRP5 that result in abnormally high bone mass (HBM). These mutations result in very high bone mass, reminiscent of osteosclerosis-type disorders. In light of the strong effects of *Lrp5* loss-of-function mutations on mechanotransduction, we investigated whether the *Lrp5* gain-of-function (HBM) alleles might also alter mechano-responsiveness in the bone tissue. Specifically, we asked whether normal expression of one of two different *Lrp5* HBM mutations would enhance load-induced bone gain and/or prevent disuse-induced bone loss.

Materials and Methods

Mice

Lrp5 HBM knock-in mice were engineered by replacing a portion of intron 2 through a portion of intron 4 with targeting constructs that harbored either the G171V (equivalent to residue 170 in the mouse) or the A214V (equivalent to residue 213 in the mouse) within exon 3, using homologous recombination. Both *Lrp5* knock-in mutants (and their WT relatives) were on a mixed genetic background of 129S1/SvIMJ and C57Bl/6J.

Ex vivo strain gauging and in vivo axial loading of the tibia

Four 18 week-old male mice of each *Lrp5* genotype (WT, A214V, G171V) were used for strain measurements under dynamic axial compressive loading. A single element strain gage was applied to the posteromedial surface of the midshaft tibia, which was subsequently mounted between molded knee and ankle fixtures that secured the tibia in the vertical direction. The fixtures were mounted to a Bose 3200ELF mechanical testing system for load application. Strain and force was recorded during a 10-cycle loading bout of comprising increasing force (4.5-8.0 N), which allowed computation of a force:microstrain factor.

For *in vivo* loading, 18-wk old mice were anesthetized under isoflurane and had their right hindlimb (knee to foot) placed in molded loading cups that secured the tibia. A sinusoidal wave form at 2 Hz, 120 cycles, was applied with peak force adjusted for each genotype to achieve 2130 $\mu\epsilon$. Mice were given three bouts of loading with a day of rest between each bout. Intraperitoneal fluorochrome labels were given to facilitate histomorphometric measurements of new bone formation. The right and left tibias were harvested and processed for fluorochrome histomorphometry at 3 different diaphyseal locations (proximal, midshaft, and distal).

Tail suspension and Botox-induced disuse of the lower limb

Sixteen 10 week old female mice from each genotype (WT, A214V, G171V) were used for the tail suspension studies (8 ground control and 8 suspended). All mice were individually housed and a tail harness was used to suspend the experimental mice. Control mice were unencumbered in their cage. Mice were suspended for 24 days prior to sacrifice.

Twenty-four 16 week old male mice from each genotype (WT, A214V, G171V) were used for the unilateral Botox studies. The right hindlimb musculature (quads, hamstrings, calves, tibialis anterior) was injected with 20 μ L of Botox (Allergan, Irvine, CA). The left hindlimb musculature was injected with 20 μ L of saline and served as an internal control. These injections were repeated one week later and the mice were sacrificed 22 days after the first injection.

Mice in the tail suspension and Botox studies were radiographically scanned using pixiMUS II, just prior to intervention, and again at sacrifice. Standard microCT measurements were collected on the dissected and fixed femurs after sacrifice.

Results and Conclusion

Tibial loading:

The tibial loading model significantly increased periosteal bone formation parameters in the loaded limb compared to non-loaded limb at the proximal and midshaft locations, but not at the distal location. The A214V mutant mice had a significantly greater load-induced periosteal bone formation response (MAR, MS/BS, and BFR/BS) compared to WT mice. The G171V mutants, however, were not significantly different from WT mice in their periosteal response to loading. Endocortically, the WT and A214V mutants failed to show a measureable response to loading, but the G171V mutants exhibited significantly increased MAR, MS/BS, and BFR/BS. These data indicate that the G171V mutant mice have a lower strain threshold for initiating endocortical bone formation, compared to WT mice.

Disuse:

In the Botox experiment, we observed a significant decrease BMC in the treated (paralyzed) femur among WT mice (-22%; $p < 0.05$), but both HBM groups were unaffected (3-5% loss, NS). μ CT analysis of the distal femur trabecular bone, however, revealed roughly equivalent bone loss (3-5% decrease in BV/TV, $p < 0.05$), regardless of genotype. In the hindlimb suspension experiment, WT mice lost a significant amount of lower limb BMC (-4.2%; $p < 0.05$), whereas the A214V mice actually gained BMC (+5.0%; $p < 0.05$) and the G171V mice maintained their BMC (-0.4%; NS) throughout the suspension period. Similar to the Botox experiment, μ CT analysis of the distal femur trabecular bone, revealed roughly equivalent bone loss (5-10% decrease in BV/TV, $p < 0.05$), regardless of genotype.

Our data support the hypothesis that the HBM-causing mutations in the Lrp5 receptor (1) confer increased mechanoresponsiveness to the bone tissue in cases of increase mechanical stimulation, and (2) provide protection from disuse-induced bone loss, particularly in the cortical bone compartment.