Intramembranous Bone Regeneration is Accelerated in Constitutively Active LRP5 Mutant Mice

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INTRODUCTION: Wnt signaling plays an important role during the development of the skeleton, its homeostasis, disease and repair. How Wnt signaling affects intramembranous bone regeneration in the absence of concomitant endochondral repair, as occurs in distraction osteogenesis or implant fixation, is unclear. Using the bone marrow ablation model in rats, we have previously demonstrated that increased Wnt signaling characterizes the early phase of intramembranous bone regeneration [1]. The current study aims to further determine the effects of Wnt signaling activation during intramembranous bone regeneration in mice and to test the hypothesis that constitutive activation of Wnt signaling accelerates intramembranous bone regeneration.

METHODS: In IACUC approved experiments, we used C57Bl/6 (n = 3-4/group) and constitutively active Wnt signaling by LRP5 mutant (HBM) mice and their wildtype (WT) littermates (n = 3/group). All mice underwent bone marrow ablation surgery of the right femur at 4 weeks old. C57Bl/6 mice were killed at 3, 5, 7, and 14 days after the surgery. HBM and WT mice were killed at 5 days after surgery. At each time point, femurs were harvested, scanned by microCT, and processed for H&E [2]. Region of interest for microCT analysis was the entire marrow volume from 40 to 70% of the total bone length away from femoral condyle. To determine if Wnt signaling is activated, immunohistochemical staining for β-catenin was performed. Statistical analyses were performed using ANOVA for C57Bl/6 mice and student's t-test for HBM mice.

RESULTS: In C57Bl/6 mice, regenerated bone within the ablated marrow compartment peaked at 7 days after the surgery, where the mean BV/TV was 8.1 ± 1.8 % (Figure 1A). Increased immunoreactivity to β-catenin within the ablated marrow compartment preceded an increase in BV/TV, where β-catenin positive cells (DAB positive cells) appeared at day 3. This reconstitution of the empty marrow space by β-catenin positive cells was followed by extracellular matrix (fast green positive) deposition that mineralized and was partially resorbed by day 14. At day 14, β-catenin positive cells (yellow triangles) were limited to bone lining cells. HBM mice had 2-fold more bone at day 5 post surgery than their littermate controls ($p < 0.05$, Figure 1.B).

DISCUSSION: Our studies demonstrate that Wnt signaling enhances intramembranous bone regeneration in a mouse model of marrow ablation. The study adds further support to the concept that modulating Wnt signaling can have significant impacts in clinical scenarios where bone healing occurs via intramembranous bone regeneration, including joint replacement. Pharmacological agents that activate Wnt signaling may allow patients to recover faster from such surgeries.

REFERENCES: [1]Wise, J. K., et al. (2010). PLoS One; [2]Moran, M. M., et al. (2015). J Orthop Res

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Figure 1. (A) MicroCT, histology by H&E, and unphosphorylated β-catenin immunohistochemical staining of femur in C57Bl/6 mice. (B) MicroCT and H&E of femur in WT and HBM mice. (*:p<0.05)