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Wnt Signaling is Essential for Bone Regeneration

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The goal of our study is to determine the role of Wnt signaling in adult bone regeneration. We initiated this experiment by identifying Wnt responsive cells in the intact skeleton of Wnt reporter transgenic BATgal and TOPgal mice. Our data shows hypertrophic chondrocytes and osteocytes responding to Wnt signaling in intact bone. Using *in situ* hybridization, we demonstrated that a number of Wnts and Wnt-related genes were expressed in the periosteum. We further explored whether Wnt signaling is up-regulated in skeletal injury by generating a 1mm mono-cortical tibial defect in Wnt reporter mice. After 24 and 48 hrs, we detected up-regulation of β-galactosidase by RT-PCR demonstrating an increase in Wnt responsiveness. Moreover, histology showed injury sites populated with Wnt responsive cells 72hr after surgery.

Next we modulated Wnt signaling *in vivo* by inhibiting or activating of the Wnt pathway. Perturbation of Wnt signaling was achieved by tail vein injection of Ad-Dkk1, an adenovirus expressing Dkk1 protein. As a negative control, Ad-Fc, an adenovirus expressing the Fc region of a mouse IgG was used. Inhibition of Wnt signaling was confirmed by whole mount X-gal staining of TOPgal tibiae from Ad-Dkk1 infected animals.

To measure the effect of inhibition of Wnt signaling on bone healing, we induced mono-cortical defects after virus infection. Tibiae harvested six days post injury from Ad-Dkk1 or Ad-Fc treated animals underwent histomorphometric analyses using Aniline Blue staining. Histomorphometric analyses showed that Ad-Dkk1 treated tibiae had an 84% reduction in bone formation. Furthermore, *in situ* hybridization for osteogenic markers showed that Ad-Dkk1 treated tibiae did not express *runx2* or *collagen type I* on post-surgical day 4 demonstrating that Dkk1-mediated inhibition of Wnt signaling blocked osteoblast differentiation and bone regeneration.

Another approach used LRP5G171V transgenic mice that carry a gain-of-function mutation in the Wnt co-receptor Lrp5. These mice possess a high bone mass because of constitutively active Wnt signaling. Using the aforementioned injury, on day 6, LRP5G171V mice showed less bone formation than their wild type counterparts. LRP5G171V bone marrow cells and the cells encompassing the injury sites were highly proliferative as compared to these same sites in wild type mice. Our study suggests that Wnt signaling can be a target pathway for therapeutic applications where bone formation is desired.

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