SECRETED FRIZZLED RELATED PROTEIN 1 IS A TARGET TO IMPROVE FRACTURE HEALING

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INTRODUCTION
Bone fractures are a major clinical problem among the patient groups with certain diseases that affect the skeleton (osteoporosis, bone-metastasizing cancer and diabetes). The weak bone structure leads not only to high fracture incidence, but also result in delayed repair process. The stages of fracture repair from the initial formation of a hematoma to the induction of bone formation and the bone remodeling to generate lamellar bone have been well described, and have been demonstrated to be regulated by several signaling pathways. Clinical studies as well as transgenic mouse models have shown that the Wnt signaling supports bone formation, and recently, efforts have been made to unravel the role of Wnt signaling during fracture repair and bone remodeling. One approach is to inhibit the negative regulators of Wnt pathway, e.g. Secreted Frizzled Related Protein 1 (sFRP1) which binds extracellular Wnt ligands and prevent their interaction with frizzled receptors in skeletal lineage cells. Our previous studies have shown that physiological activation of Wnt signaling in sFRP1−/− mouse results in improved bone parameters as well as accelerated chondrogenesis, without any deleterious effects. In the present studies, we examined if the appropriate activation of Wnt signaling by sFRP1 ablation can improve fracture repair. We report that fracture healing is accelerated and enhanced (more bone) in sFRP1−/− which gives rise to mechanically stronger bone.

METHODS
Fractures were generated in the mid-shaft of the tibia in WT and homozygous knockout animals. A 30 gauge wire was introduced into the tibial canal by surgery, and a drop weight from a standard height was used to generate a fracture. Healing was monitored at regular intervals by radiographic analyses. Bone samples were collected at 7, 11, 14 and 21 days post fracture for histological and gene expression by qRT-PCR analyses to assess the progression of bone formation and remodeling. The mechanical strength of the healed bone was determined by Torsion test using 55MT MicroTorsion testers (Instron, MA), on days 14 and 28 post-fracture.

RESULTS
By radiographic analysis on day 14, the sFRP1−/− mice demonstrated a higher radio-opacity at the fracture site compared to wild type (Fig. 1), suggesting an early bone union in sFRP1−/− mouse. The histological analyses showed that on day 7, the initial callus appeared similar, but by day 11, reduction in callus growth could be observed in sFRP1−/− mice. Bone remodeling by TRAP staining was evident in the sFRP1−/− as early as day 7, which is not visible in wildtype until day 11. The union of the fracture was achieved in sFRP1−/− mouse by day 21, in contrast to the WT fracture which still exhibited presence of cartilaginous callus along with formation of new bone. Gene expression analyses using RNA from callus tissues showed reduced expression of chondrocyte proliferation markers (Col2a1 and Sox9) in sFRP1−/− mice, consistent with less chondroid tissue in sFRP1−/− compared to wild type mice by histology. Bone remodeling markers, MMP9, TRAP and VEGF were also elevated in sFRP1−/− mice, suggesting active bone remodeling in the absence of sFRP1.

DISCUSSION
A key finding of our studies is that loss of function of one of the secreted frizzled related protein antagonists (sFRP1) has a significant effect in accelerating fracture repair in a normal physiologic manner, as evident by histology and RNA analyses. Reduced chondrocyte proliferation and early bone formation in sFRP1−/− reflect an early commitment of mesenchymal cells towards osteogenesis during fracture repair. Under the influence of induced canonical Wnt signaling, the newly formed bone matures faster in absence of sFRP1, as confirmed by increased expression of bone remodeling markers, and provides enhanced mechanical strength as compared to wild type. Our studies establish a direct improvement of fracture repair by increasing Wnt signaling and also provide a possibility for modulation of the Wnt pathway to achieve better fracture healing in compromised clinical cases.

REFERENCES

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Figure 1. Radiographic analysis of healing tibiae shows improved healing by reduced callus size and union of bone ends in absence of sFRP1. The upper panel shows the tibiae on the fracture day and the lower panel shows callus formation and healing 14 days post fracture.