Homozygous Deletion of the Sost Gene Results in Faster Union and Increased Hard Callus Formation

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Introduction
Sclerostin, transcribed by the Sost gene, is a negative regulator of bone formation. Sclerostin expression is specific to osteocytes and its secretion blocks the canonical Wnt/β-catenin pathway through binding to LRP5, inhibiting osteoblast differentiation. This is such that the SOST KO (Sost−/−) mouse demonstrates an extremely high bone mass. Fracture repair is dependent on bone formation and could therefore be enhanced in the absence of sclerostin. We therefore sought to examine bone repair in the SOST KO mouse.

Hypothesis
Mice homozygous for Sost deletion will demonstrate enhanced repair compared to wild type mice with larger, stronger calluses.

Methods
Female homozygous-null mice (Sost−/−), with a well-documented high bone mass phenotype1, were compared to wildtype (WT, Sost+/+) littermates after distal tibial closed fracture. Fractures were stabilized by external fixation with flexible stability such that endochondral repair occurred. Time points examined were 1 week (initial mesenchymal and cartilage tissue formation), 2 weeks (cartilage callus ossification) and 4 weeks (union and hard callus remodeling). Outcome measures were X-ray, MicroCT for hard callus bone content and architecture, QCT, and histology to assess cartilage callus formation and removal. Sample sizes ranged from N=6 to N=10 for each group and statistical significance was determined using non-parametric Mann-Whitney Tests.

Results
Observed fracture union from x-rays and histological sections suggested a trend for enhanced union rate in Sost−/− compared to WT. At 1 week all calluses contained condensed mesenchymal tissue with signs of cartilage matrix production (stained in blue figure 1) at the fracture site. There was no sign of morphological cartilage callus formation in all samples except one Sost−/− fracture callus. At 2 weeks there was a 94% decrease in avascular/cartilage content in the Sost−/− calluses compared to WT (p<0.05), consistent with a trend to increase in rate of union in Sost−/− mice. By 4 weeks all fractures had united in both genotypes such that no cartilage callus remained (Figure 1).

Discussion
At the early stage of repair Sost−/− mice showed no alteration in initial cellular recruitment and cartilage tissue differentiation compared to WT (Figure1). Mineralized callus area was increased at 1 week in Sost−/− mice compared to WT suggesting hard callus formation at the periosteal surface was enhanced at this very early time point. By 2 weeks Sost−/− calluses showed enhanced cartilage callus removal and earlier hard callus union, along with increased bone mineral content, density, volume and moment of inertia. During hard callus remodeling at 4 weeks, Sost−/− mice continued to have increased callus content but reduced callus area, resulting in a smaller, denser hard callus. This suggests that the absence of sclerostin resulted in enhanced endochondral ossification to union resulting in a smaller, denser hard callus.

Conclusion
This study is the first to present detailed examination of fracture repair in the complete absence of sclerostin. As expected, hard callus formation was enhanced in Sost−/− mice. Interestingly this was a result of both increased periosteal intramembranous callus formation and advanced endochondral repair. In addition we noted formation of a smaller, denser callus after union in Sost−/− mice. Further analyses of samples at 10 days post fracture will more closely assess the advanced endochondral repair noted. In addition, analysis of mechanical properties at 4 weeks will determine if the united smaller, denser Sost−/− fracture calluses are stronger than WT.

Significance: The results of this work suggest a role for sclerostin regulation of bone formation in fracture repair, and thus propose the potential application of sclerostin inhibitors in orthopedies as well as osteoporosis.

References

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