Disruption of Thrombospondin-2 Accelerates Ischemic Fracture Healing

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Introduction

Thrombospondin-2 is a matricellular protein that is highly upregulated during fracture healing. TSP2 regulates vascularity, vascular reperfusion following ischemia, and mice deficient in TSP2 (TSP2-null) show an alteration in fracture healing which is characterized by enhanced vascularization and a shift to an intramembranous bone healing phenotype. An important, yet largely untapped, therapeutic strategy in fracture repair is to enhance vascularization at the fracture site to counteract post-injury ischemia. This can be achieved by either activating angiogenic pathways or by blocking angiogenesis inhibitors. Several angiogenic growth factors have been evaluated in bone repair models such as Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor-2 (FGF2), Platelet Derived Growth Factor (PDGF) and Thrombin-peptide 508. Exogenous delivery of these compounds has been shown to promote angiogenesis. On the other hand, blocking of angiogenic inhibitors has not been researched as intensely. Therefore, we utilized TSP2-null mice to evaluate whether an absence of TSP2 would result in enhanced ischemic fracture healing by increasing vascular reperfusion and callus vascularization.

Methods

All procedures were approved by the Institutional Animal Care and Use Committee. Male, three month old TSP2-null or wildtype mice (8 or 9 per group) underwent ischemic (femoral artery resection), stabilized tibial fractures and were harvested at 10, 20 or 40 days post-fracture. Tibias were examined using μCT, histology and immunohistochemistry.

Results

Ten days after fracture, TSP2-null mice show 115% more bone volume, 29% greater trabecular thickness, 122% increase in vessel density, and 20% greater cell proliferation in the fracture callus than wildtype (Fig. 1, 2). Twenty days after fracture, TSP2-null mice have 34% greater bone

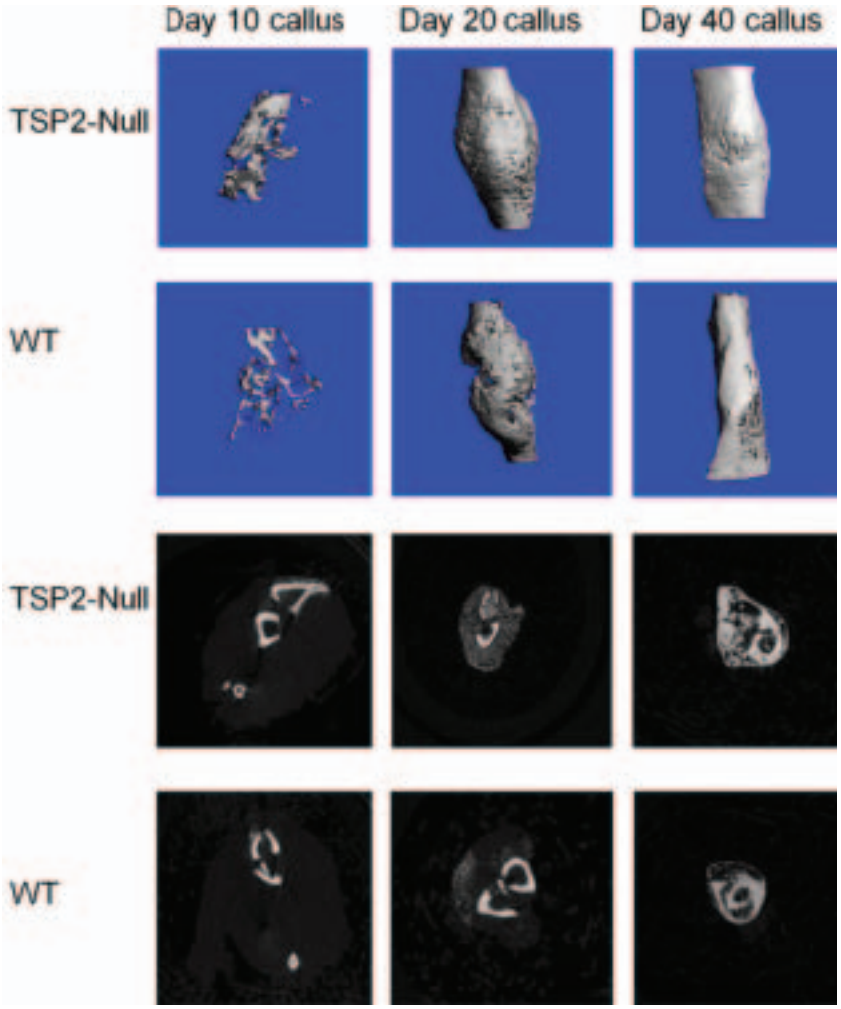

Figure 1. Comparison of μCT between TSP2 null and WT mice at days 10, 20 and 40 post-ischemic fracture.

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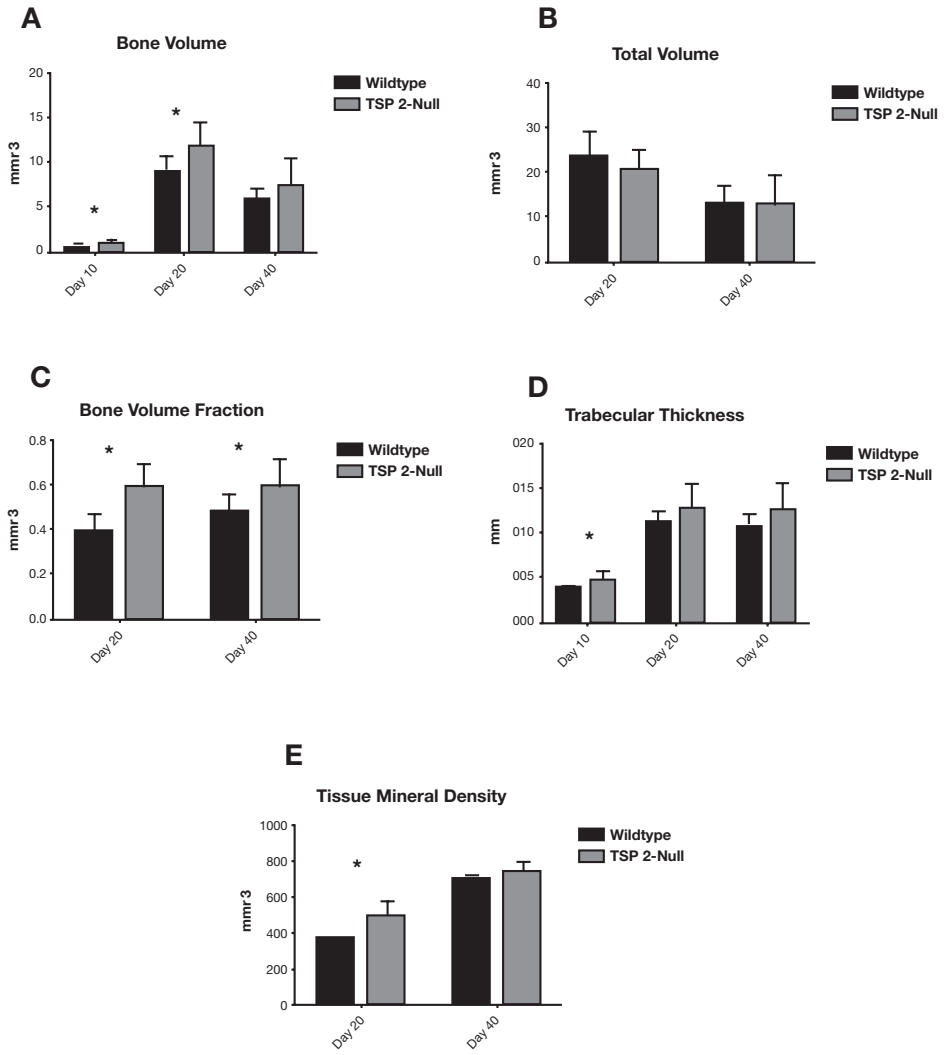

Figure 2. μCT scans show TSP-2 null mice have greater bone volume, bone volume fraction and trabecular thickness after ischemic fracture at days 10 and 20. (A) Quantification of bone volume, (B) Total callus volume, (C) Bone volume relative to total callus volume (Bone Volume Fraction), (D) Trabecular thickness, (E) Tissue mineral density. Values are mean ± SD of TSP2-null and WT mice. * $p < 0.05$.

volume, 37% higher tissue mineral density and 51% increase in bone volume fraction relative to wildtype mice (Fig. 1, 2) By 40 days post fracture the TSP2-null mice still have a 24% increase in bone volume fraction, but all other parameters show no significant difference (Fig. 1,2). The fracture callus in both TSP2-null and wildtype mice shows normal cartilage development, with the TSP2-null mice displaying more hypertrophic cartilage at day 10 than WT (results not shown).

Discussion

The utility of inhibiting TSP2 as a therapeutic agent in compromised fracture healing shows promise. Future directions for this research will include targeting the inhibition of TSP2 specifically during ischemic healing at the time of fracture. It will be important to discern differences in healing between mice that have a complete disruption of the Thbs2 gene (the TSP2-null model used in this study), and those with a disruption of TSP2 at the time of healing. This will allow us to better understand the normal biological significance of TSP2 during bone healing, but also fully establish anti-TSP2 therapy as a viable clinical therapeutic

Significance

We have established that TSP2 is an important negative regulator of ischemic fracture healing. Inhibiting TSP2 could be a novel therapeutic approach to treating ischemic fracture: TSP2-null tibias show more vessels and greatly increased bone volume during the healing process.

Acknowledgements

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