Thrombospondin 1 (TSP1) Is Necessary for Fracture Healing Under Normoxic and Ischemic Conditions

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DISCLOSURES: The authors do not have anything to disclose.

INTRODUCTION: Although there has been extensive research into the family of five glycoproteins known as thrombospondins, the role of thrombospondin 1 (TSP1) in fracture healing has been largely neglected1. TSP1 protein is present during the early stages of wound healing, deposited by platelets and expressed by monocytes. TSP1 is implicated in regulation of angiogenesis as well as extracellular matrix organization, fibrosis, and endothelial cell senescence2-6. Our lab has previously demonstrated that an absence of the TSP1 receptor, CD47, results in decreased callus formation and disturbances in mesenchymal progenitor cell (MSC) proliferation7. We hypothesize that TSP1 binding to CD47 during early callus formation is necessary for proper fracture healing and the absence of TSP1 will similarly result in decreased callus size and mesenchymal progenitor cell (MSC) function.

METHODS: With proper animal regulatory approval, wildtype (WT) and TSP1-null mice on a C57Bl/6 background underwent closed-stabilized bilateral femoral fractures or ischemic left tibia fractures, where the femoral artery was ligated. All fractures were generated using a three-point-bend apparatus. Femurs were harvested at 10- and 20-days post-fracture for bilateral femoral fractures and at 15 days post-fracture for ischemic tibia fractures. Limbs were fixed in 4% paraformaldehyde and intramedullary pins were removed. Limbs were scanned every 10 slices and interpolated at 4% paraformaldehyde.

RESULTS: TSP1 depletion results in diminished callus formation by 10 days post-fracture. (Fig A) Through µCT analysis, we observed reductions in bone volume at 10 days post fracture. By 20 days post fracture, we observe significant decreases in bone volume as well as callus volume, bone mineral content and tissue mineral content emphasizing a disruption in osteogenesis in the absence of TSP1 (Fig A). Previous studies as well as our own work have demonstrated that in the absence of TSP1, mesenchymal stem cells (MSCs) are unable to undergo robust osteogenesis. Although there has been extensive research into the family of five glycoproteins known as thrombospondins, the role of thrombospondin 1 (TSP1) in fracture healing has been largely neglected1. TSP1 protein is present during the early stages of wound healing, deposited by platelets and expressed by monocytes. Our findings reveal that TSP1 is necessary for proper fracture healing to occur. Although previous studies have demonstrated a role for TSP1 in bone homeostasis and osteogenesis, this has not been previously linked to impaired fracture callus formation. Our findings support reduced osteogenic potential of MSCs in the absence of TSP1 and further demonstrates a reduced ability to form colonies. TSP1 has been shown to modulate senescence in endothelial cells and this suggests that this role may apply in the context of MSCs as well. Despite TSP1’s anti-angiogenic role, decreased callus formation in the absence of TSP1 suggests a potential compensatory mechanism and could emphasize the importance of TSP1 as a mediator of matrix organization in the context of fracture healing.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings indicate that TSP1 plays an important role in fracture repair. Ongoing studies seek to interrogate the vascularization of the TSP1-null fracture callus and the interplay between TSP1 and its binding partners, TGFβ, CD47 and CD36, to modulate the fracture environment and assist in healing.