TSG-6 improves healing of critical-sized bone defects in mice

Oliver Küppers1, Mubashir Ahmad1, Jasmin Bülow1, Melanie Haffner-Luntzer1, Karin Scharffetter-Kochanek2, Anita Ignatius1, Verena Fischer1

1Institute of Orthopedic Research and Biomechanics, University Medical Center Ulm, 89081 Ulm, Germany
2Department of Dermatology and Allergic Diseases, Ulm University, 89081 Ulm, Germany

Email of Presenting Author: oliver.kueppers@uni-ulm.de

Disclosures: The authors declare no conflicts of interest.

INTRODUCTION: Critical-sized bone defects are a challenging clinical problem with a high risk of impaired regeneration and non-union development [1]. Therefore, novel therapeutic approaches to enhance bone repair are needed. TNF-stimulated gene 6 protein (TSG-6), a protein with anti-inflammatory and pro-regenerative properties, was shown to be secreted by various cell types including mesenchymal stem cells [2-5] and to improve wound healing [6]. Here, we investigated if TSG-6 can also stimulate bone regeneration.

METHODS: 12-weeks-old male C57BL/6j mice received a critical-sized defect (1.5 mm) in the femur stabilized by an external fixator (linear axial: 18.1 N/mm, torsional stiffness: 1.5 N/mm). A collagen type I gel (5 mg/mL) served as a carrier to transfer 10 or 50 µg recombinant human TSG-6 (rhTSG-6) into the fracture defect, respectively. An unloaded collagen gel and an empty defect served as controls. Bone regeneration was analysed using microcomputed tomography (µCT) and histomorphometry on day 35 post-surgery (n=8/group). The impact of 50 µg rhTSG-6 on innate and adaptive immune cells in the fracture hematoma was investigated on day 1 and 3 using fluorescence-activated cell sorting (FACS, n=4-5/group). In vitro, the role of Tsg-6 on osteoblastic differentiation (OD) was investigated in primary murine mesenchymal stem cells (mMSCs). For small interfering RNA (siRNA) transfection to knock down Tsg-6, mMSCs (25,000 cells/cm²) were reverse transffected with 20 nM siRNA of either negative control (siNT) or siRNA specific for Tsg-6 (n=4/group). The effects of Tsg-6 on OD were analysed by gene expression analysis of the osteogenic markers alkaline phosphatase (Alpl), Runt-related transcription factor-2 (Runx2), Bone γ-carboxyglutamate protein (Bglap) and Sp7 transcription factor (Sp7) and by quantitative measurement of alkaline phosphatase activity. Statistics: Student’s t-test or ANOVA with post hoc Fisher’s LSD test, p<0.05.

RESULTS: The low dosage of 10 µg rhTSG-6 did not improve bone healing on day 35. Notably, the higher dosage of 50 µg rhTSG-6 significantly stimulated bone healing as indicated by an increased percentage of bone bridging of the defect and volume of newly formed bone compared to 10 µg rhTSG-6: 1.17 mm³ vs. 0.57 mm³, p=0.0112) collagen gel (50 µg rhTSG-6 vs collagen gel: 1.17 mm³ vs 0.57 mm³, p=0.0074) and empty group (50 µg rhTSG-6 vs empty: 1.17 mm³ vs 0.24 mm³, p=0.0002) (Figure 1A,B). Histomorphometric analysis of the callus showed a significant increased number of osteoblasts (Figure 1C) and decreased number of osteoclasts (Figure 1D) in the 50 µg rhTSG-6 group compared to all other groups. FACS analysis of the early fracture hematoma revealed no major differences between the groups on d1, but on d3 we observed significantly increased numbers of Tis+cells (CD3+/CD4+) in the 50 µg rhTSG-6 treated group compared to collagen gel and to empty group (Figure 1E). In vitro, the siRNA knockdown of Tsg-6 significantly impaired osteogenic differentiation of mMSCs as indicated by a reduced alkaline phosphate activity on d3 and d6 (Figure 1F) and by a significant downregulation of major osteogenic marker genes such as Alpl, Bglap and Sp7 (Figure 1G).

DISCUSSION: In conclusion, we showed that a dosage of 50 µg rhTSG-6 significantly improved bone regeneration in critical-sized defects in mice by promoting bone formation via increased osteoblastogenesis and reduced osteoclastogenesis. These data were supported by our in vitro findings that revealed TSG-6 as a positive regulator of osteoblast differentiation, thereby confirming previous results [7]. Also, more Tis+cells were present in the fracture hematoma on day 3 in the 50 µg rhTSG-6 treatment group compared to controls. CD4+ - Tis+cells were reported to promote bone formation enhancing osteoblastogenesis [8, 9] and to inhibit RANKL induced osteoclastogenesis [10], which might further explain the improved bone healing observed after TSG-6 treatment.

SIGNIFICANCE/CLINICAL RELEVANCE: We showed that TSG-6 improved the healing of critical-sized bone defects in a pre-clinical model. Our results imply that TSG-6 treatment might offer a new therapeutic option to enhance bone healing in patients with large bone defects. However, further mechanistic investigations are warranted.

Figure 1: TSG-6 improves bone healing by promoting osteoblastogenesis and inhibiting osteoclastogenesis. (A) Representative µCT-images of critical-sized defects on day 35 and (B) quantified bone volume. (C) Number of osteoclasts (NOb/BPm), and (D) number of osteoclasts per bone perimeter (NOb/BPm) in the fracture callus on day 35. (F) Alkaline phosphatase activity and (G) osteogenic marker gene expression of mMSCs on day 3 and 6 of osteogenic differentiation.