# Prevention of Fracture Nonunion Through Eradication of Leptin Receptor-Expressing Cells

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INTRODUCTION:

Fracture nonunion affects 5-10% of individuals who sustained fractures, resulting in persistent pain and the inability of patients to effectively utilize the injured limb. In cases of fracture nonunion, the gap between fractured bone fragments becomes filled with fibrous tissue rather normal bone tissue. Currently there exists a knowledge gap concerning the cellular origins of the fibrous tissue that forms between fractured bone fragments. In this study, we showed that the fibrous tissue in fracture nonunion originated from leptin receptor expressing progenitor cells (LEPR+ cells). Furthermore, eradication of LEPR+ cells resulted in prevention of fracture nonunion.

METHODS:

All experiments were approved by local IACUC.

*Animals:* LepR-cre (Stock 008320), Rosa26-CAG-loxp-stop-loxp-tdTomato (Stock 007909), Rosa26-iDTR (Stock 007900) were purchased from Jackson Laboratories. For LEPR+ cells ablation study, LepR-Cre;Rosa26-iDTR; Rosa26-tdTomato (n=10, 12-16 weeks) and LepR-Cre;Rosa26-tdTomato (n=10; 12-16 weeks) were given daily dose 100 ng of diphtheria toxin through intraperitoneal injection for 10 weeks

*Model of fracture non-union:* Non-union model was adapted from Garcia, P et al 2007. Briefly, the femur was exposed through a lateral approach. Two osteotomies with made with a diamond saw to create a 1.8 mm diaphyseal defect at the mid-shaft of the femur. A needle with both ends flattened was then inserted into the intramedullary canal the fracture gap to stabilize the bone. A clip of 8 mm in length was implanted ventro-dorsally into the femur to provide extra rotational stabilization and to prevent the collapse of the gap between proximal and distal fragments. To induce non-union, the periosteum <2 mm from the gap was stripped by surgical scalpel. Fracture without periosteum removal was used as control.

Radiograph: Radiograph of post-surgical femurs were carried out on anesthetized mice using Faxitrom™ LX-60 System. Fracture healing were scored based on plain radiograph as follows 1 = No bridging bone, 2 = Partial boney bridging, 3 = Complete boney bridging.

Statistical Analysis: Statistical analysis was performed using Student's t-test. $p<0.05$ was considered as significant.

RESULTS: At the 10-week mark post-surgery, we observed a persistent gap in the femurs of mice subjected to a fracture non-union model (Figure 1a). Further examination using micro-CT imaging of the femurs from the non-union model confirmed the lack of bony bridges forming between the proximal and distal fragments (Figure 1b). The nonunion group exhibited a significantly lower BV/TV when compared to the healed fracture group (Figure 1c). Histological analysis revealed that the space between bone fragments were filled with fibrous tissue (Figure 1d) and LEPR+ cells (Figure 1e). Eradication of LEPR+ cells by administration of diphtheria toxin resulted in significantly improved boney bridging between fragments (Figure 2a-b).

DISCUSSION AND CONCLUSION:

To our knowledge, this is the first study that shows prevention of fracture nonunion through inhibition of pro-fibrotic progenitor cell. Inhibition of LEPR+ cells successfully prevent fracture nonunion and resulted in fracture healing.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Result of this study showed the feasibility of inhibition of pro-fibrotic progenitor cell to prevent fracture nonunion.

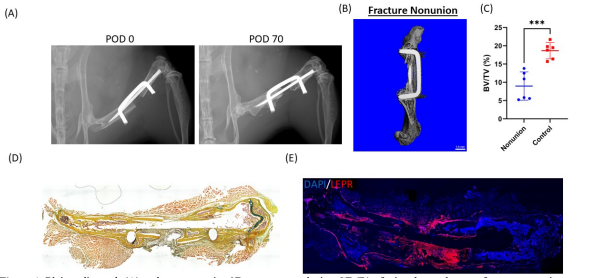


**Figure 1.** Plain radiograph (A) and representative 3D-reconstructed microCT (B) of mice that underwent fracture nonunion model. (C) BV/TV of the peri-fracture area of mice that underwent nonunion vs healed fracture (control) model. Data is represented as mean +/- s.d. ***p<0.001. (D) Representative movat pentachromeimage of surgical femur of mice that underwent fracture nonunion model. (E)Immunofluorescent imaging of mice that underwent nonunion or healed fracture model ten weeks after surgery

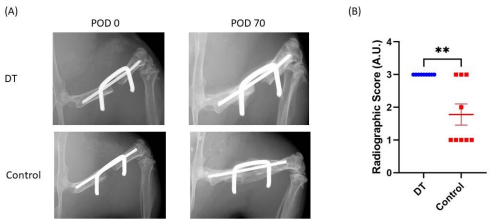


**Figure 2.** Radiograph of femur of mice that underwent LEPR+ cells ablation (DT) versus control. (A) Representative plain radiograph of surgical femur of mice that underwent LEPR+ cells ablation (DT) or control (Control) at immediate postop (POD 0) and at 10 weeks postop (POD 70). (B) Radiographic boney bridging score. 1 = No bridging bone, 2 = Partial boney bridging, 3 = Complete boney bridging. Data is represented as mean +/- s.d. *** p<0.001, ****P<0.0001.