Leptin-receptor lineage cells are abundant in fracture nonunion fibrotic tissue and contribute to both osteogenic and fibroblastic processes

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INTRODUCTION: Fracture nonunion is a common complication following a fracture, affecting an estimated 5-10% of individuals who have sustained fractures1. In cases of fracture nonunion, the gap between fractured bone fragments becomes filled with fibrous tissue rather than the expected regrowth of normal bone tissue2. This outcome is problematic because the fibrous tissue lacks the necessary mechanical strength to adequately stabilize the bone fragments3. Consequently, this lack of stability results in persistent pain and the inability of patients to effectively utilize the injured limb2. Currently, there exists a knowledge gap concerning the cellular origins of the fibrous tissue that forms between fractured bone fragments. In our study, we have demonstrated the prevalence of cells expressing the leptin receptor (LEPR+) cells within this fibrous tissue. Notably, our research has also revealed that these LEPR+ cells within the fibrotic tissue express two key markers: ACTA2, commonly associated with myofibroblasts, and osteocalcin (OCN), a well-recognized marker of osteoblasts. This intriguing discovery implies that the LEPR+ cells present in the interfragmentary fibrous tissue may possess a dual role, potentially contributing both to osteogenic (bone-forming) and pro-fibroblastic processes.

METHODS: All experiments were approved by local IACUC. Animally: Lepr−cre (Stock 008320), Rosa26-CAG-loxp-stop-loxp-tdTomato (Stock 007909), Acta2-DsRed (Stock 031160), Ocn-GFP (Stock 017469) were purchased from Jackson Laboratories.

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 were 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 and 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.

Micro-CT: Scans (µCT 45, Scanco Medical, Switzerland) were performed at 6 µm voxel size, 90 kVp, 145 mA, and 0.36 rotation step (180 angular range) per view. Volume of interest was defined as a 4 mm-long cylindrical region whose center was the center of the fracture line. Radiological bone morphological parameters, particularly bone volume fraction (bone volume/total volume; BV/TV), trabecular number (Tb.N.), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) were assessed.

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). Conversely, the micro-CT scans of femurs from the healed fracture model revealed complete bridging between these 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 further confirmed the existence of fibrotic tissue occupying the space between the proximal and distal segments, without any bony bridges (Figure2a-b). Immunofluorescence imaging of non-union femur from LepR-Cre;ZsGreen;Acta2-DsRed showed significantly higher percentage of LEPR+ ACTA2+ cells in the fibrous tissue than the healed fracture counterpart (Figure 3a,c). Immunofluorescence imaging of non-union femur from LepR-Cre;tdTomato;Ocn-GFP showed significantly higher percentage of LEPR+ OCN+ cells in the fibrous tissue than the healed fracture counterpart (Figure 3b,d).

DISCUSSION: Our data suggests that LEPR+ cells are abundantly present in the fibrotic tissue in fracture nonunion. LEPR+ cells in the fibrotic tissue express both ACTA2, a commonly used marker of myofibroblast, and OCN, a commonly used marker of osteoblast. Further studies are currently being performed in to explore the osteoblastic differentiation potential of LEPR+ ACTA2+ OCN+ cells.

SIGNIFICANCE/CLINICAL RELEVANCE: The LEPR+ cells present in the interfragmentary fibrous tissue may possess a dual role, potentially contributing both to osteogenic (bone-forming) and pro-fibroblastic processes.