Trem2 overexpression in chondrocytes may protect the knee joint from degeneration associated with anterior cruciate ligament injury

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INTRODUCTION: Triggering receptor expressed on myeloid cells 2 (*Trem2*) is thought to modulate inflammatory responses governed by macrophages. Our previous studies[1, 2] indicated that Murphy Roths large (MRL/MpJ) mice, known as super-healers[3] and resistant to osteoarthritis, increase macrophage populations with *Trem2* overexpression during the acute inflammatory phase triggered by anterior cruciate ligament (ACL) rupture, significantly higher than injured C57BL/6 (wildtype mice: *WT*) control. Whether the overexpression in Trem2 was limited to macrophages/inflammatory response or it involved other tissue-based regenerative responses has not been fully investigated. Hence, we developed two new transgenic strains; *LSL-Trem2^{TG};Col2-Cre^{ERT2}*, and *LSL-Trem2^{TG};LysM-Cre*, to conditionally overexpress Trem2 in chondrocytes and macrophages, respectively. We aimed to assess the long-term regenerative response followed by ACL injury in response to elevated levels of Trem2.

METHODS: Male C57B6/J (B6/WT), *LSL-Trem2^{TG} (no Cre control) LSL-Trem2^{TG};Col2-Cre^{ERT2}*, and *LSL-Trem2^{TG};LysM-Cre* mice (10-12 weeks-old at the time of injury, 16-18 weeks old at the time of joint/bone analysis, n= 4-6 per group) were used in this study. *LSL-Trem2^{TG};Col2-Cre^{ERT2}*received tamoxifen to activate Cre, and then after 3 weeks, the ACL rupture was performed. At Day 0, mice were subjected to non-invasive anterior cruciate ligament (ACL) injury by using a single tibial compression overload (10-15M) with a loading rate of 1 mm/s (ElectroForce 3200, TA Instruments). At 4- and 6- weeks post-injury mice were euthanized and whole knees were analyzed with micro-computed tomography (μCT 35, SCANCO Medical AG) to measure epiphyseal trabecular bone microstructure and osteophyte volume. Osteoarthritis progression was evaluated histologically using paraffin embedded knee joints that were sectioned in the sagittal plane and stained with Safranin O and Fast Green. Gene expression was confirmed by immunohistochemical analysis using Trem2 antibody. This study was approved by IACUC.

RESULTS: *LSL-Trem2^{TG};Col2-Cre^{ERT2}* relative to *LSL-Trem2^{TG}*-, *WT*, and *LSL-Trem2^{TG};LysM-Cre* indicated significantly ($p \leq 0.05$) lower osteophyte volume elucidated by micro-CT analysis. Likewise, histological assessment delineated that *LSL-Trem2^{TG};Col2-Cre^{ERT2}* had limited tissue degeneration compared to *LSL-Trem2^{TG}* and *WT*, 6 weeks post injury. These findings were corroborated by the presence and intensity of *Trem2* positive chondrocytes in the articular cartilage (Figure 1).

DISCUSSION: While the short-term response of *Trem2^{high}* macrophages is essential during the acute phase[1, 2], *Trem2^{high}* chondrocytes may confer a long-term protective potential during remodeling phase as seen in *LSL-Trem2^{TG};Col2-Cre^{ERT2}*. Thus, the presence of *Trem2* in the articular cartilage appears to provide protection against the post-injury degenerative events. These effects were evident by limited osteophyte volumes, and diminished tissue degeneration in *Trem2 Col2 Cre+* compared to other groups.

SIGNIFICANCE/CLINICAL RELEVANCE: The current investigation underscores the role of *Trem2* in both macrophages and chondrocytes, and also their roles during acute inflammatory phase and prolonged regenerative phase, respectively. This concept can be implemented as a new therapeutic modality to prevent the development of osteoarthritis post-injury, for example by in-situ injection of sorted *Trem2^{high}* macrophages and *Trem2^{high}* chondrocytes. Also, this concept can be employed for the treatment of *Trem2* deficiency disorders such as dementia and Alzheimer disease.

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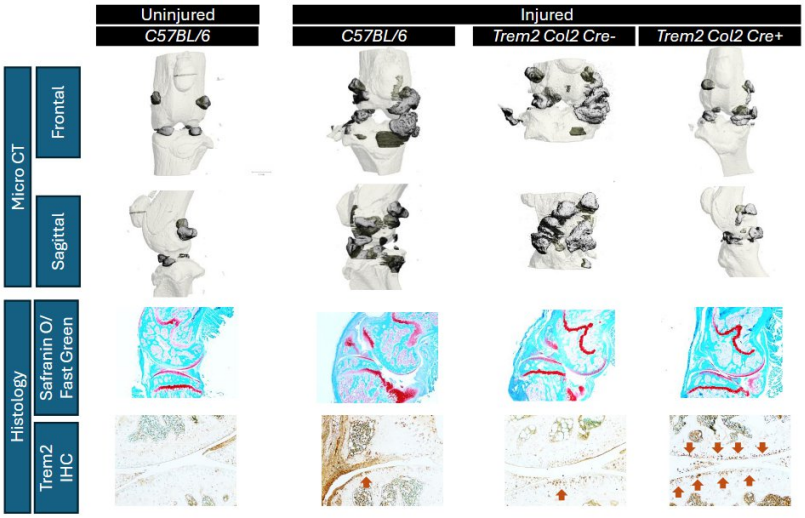

***Figure 1. Trem2* in chondrocytes can prevent osteoarthritis.** MicroCT images indicate that *LSL-Trem2^{TG};Col2-Cre^{ERT2} compared to WT* and *LSL-Trem2^{TG}* -has limited osteophyte volumes; frontal and lateral views. Immunohistochemistry (IHC) indicates that the increased expression of *Trem2* (arrows) in the articular cartilage is associated with less tissue degeneration post injury.