Delayed cartilage and bone remodeling during fracture healing in matrix metalloproteinase 13 null mutant mice cannot be rescued by bone marrow transplantation

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Introduction: Adult bone does not heal through the production of scar tissue rather through a regenerative process that recapitulates early skeletal development. Matrix metalloproteinase 13 (MMP13, collagenase-3) is an extracellular matrix (ECM) protease whose preferred substrates include the major components of cartilage and bone ECM. MMP13 has been shown to be important for skeletal development via endochondral ossification (1). This observation led us to question the role of MMP13 in skeletal repair. We examined the expression profile of Mmp13 during bone healing and compared bone healing in Mmp13-/- and wild type mice using various repair models in mice.

Materials and Methods: All protocols were approved by the Institutional Animal Care and Use Committee at UCSF. Mmp13-/- mice (12-16 wk old males) and their WT littermates were anesthetized. Closed, standardized non-stabilized fractures were produced as previously described (2). Mechanical testing was performed at days 14, 21 and 28 post-fracture by measuring the moment applied to the callus at the time of failure of the specimen. Bone marrow transplantation from WT, Mmp13-/- or beta-actin green fluorescent protein (GFP) mice into irradiated Mmp13-/- mice from the same genetic background was performed as previously described (3). Monocortical defects (1mm in diameter) were produced on the anterior-proximal tibia (4). Tissues were analyzed by cellular, molecular and histomorphometric analyses on paraffin tissue sections. Histomorphometry data were analyzed using Wilcoxon rank sum tests or ANOVA followed by Bonferroni corrected t-tests.

Results: We previously reported the expression pattern of MMP13 in hypertrophic chondrocytes and osteoblasts in the fracture callus. Our previous work also showed that in the absence of MMP13, there is a delay in the remodeling of cartilage and bone during healing of non-stabilized fractures via endochondral ossification. Here we show that the delayed bone remodeling phenotype is due to an accumulation in the cancellous bone compartment of the fracture callus. Mechanical testing shows that there are no significant differences in callus strength between Mmp13-/- and WT mice at days 14, 21 or 28 post-fracture. PECAM immunostaining and TRAP staining on tissue sections showed no delay in vascular invasion and osteoclast recruitment in the Mmp13-/- callus at day 14. However, the cartilage matrix itself was no invaded in a timely manner. In addition, DIPEN immunostaining showed decreased cleavage of aggrecan by MMPs, indicating that processing of the cartilage matrix is delayed in Mmp13-/- callus.

To understand the cellular basis for the delayed matrix remodeling phenotype, we transplanted WT bone marrow into Mmp13-/- mice. Using this approach, we confirmed that cells in the hematopoietic lineage including osteoclasts were donor-derived, while osteoblasts and chondrocytes in the callus were host-derived (3). Histomorphometric analyses showed no differences in the proportions of cartilage in the callus (CV/TV) at day 14 or the proportion of bone (BV/TV) at day 28 between Mmp13-/- mice that received WT and Mmp13-/--bone marrow (Fig. 1).

To differentiate the consequences of the Mmp13-/-mutation on cartilage and bone during repair, we examined healing via intramembranous ossification in cortical bone defects. By days 21 and 28 there was an increase in the proportion of cancellous bone in the defect in Mmp13-/- compared to WT mice as observed in non-stabilized fractures. However the ratio of compact bone in the defect was not affected (Fig. 2).

Discussion: We demonstrate that MMP13 is required for proper resorption of hypertrophic cartilage and for normal bone remodeling during fracture healing. Transplantation of WT bone marrow, which reconstitutes cells of the hematopoietic lineage, did not rescue the endochondral repair defect, indicating that impaired healing in Mmp13-/- mice is intrinsic to cartilage and bone but does not result from defective osteoclasts. These results are consistent with the expression of Mmp13 which is restricted to hypertrophic chondrocytes and osteoblasts but absent from osteoclasts. Furthermore, Mmp13-/- mice also exhibit a delay in bone remodeling during healing of stabilized fractures and cortical defects via intramembranous ossification, demonstrating that the bone phenotype is independent from the cartilage phenotype. Overall, our findings demonstrate that MMP13 is crucial for normal production and remodeling of cartilage and bone during adult fracture repair. Our data suggest that MMP13 secreted from hypertrophic chondrocytes and osteoblasts produces a pre-processed ECM in cartilage and bone. This pre-processed ECM is then invaded by blood vessels and further modified by osteoclasts secreting MMP9, leading to the production of a processed ECM. This processed ECM then promotes further steps of callus maturation including hypertrophic chondrocyte apoptosis, replacement of cartilage by bone and new bone remodeling.

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