Purpose: Our previous work has shown that recombinant human bone morphogenetic protein (rhBMP-7, OP-1) increases cartilage formation in ischemic fractures that have been mechanically stabilized. Since unstable fractures normally heal through endochondral ossification, these findings do not provide evidence that rhBMP-7 directs stem cells to differentiate into chondrocytes. To further explore this possibility, we assessed the ability of rhBMP-7 to induce cartilage formation in stabilized fractures, which normally heal through intramembranous ossification.

Materials and Methods: All procedures were approved by our Institutional Animal Care and Use Committee. Male 129J/B6 mice (3-month-old) were used in this study. Animals were anesthetized with 2% Avertin, a closed fracture was created in the mid-diaphysis of right tibia by three-point bending. All fractures were rigidly stabilized with an external fixator. Thirty microliters of solution containing 10 or 50 µg of rhBMP-7 was injected directly into the fracture site immediately after the creation of fractures. Control animals received 30 µl of 10% lactose only. A set of animals (n=4-5/group) were sacrificed at 3 days after fracture for analysis of tissue vascularization. Tissues between the proximal and distal pins were collected, fixed, and decalcified. Vertical uniform random sections (10µm) were prepared through the whole tissue. Blood vessels were visualized by immunohistochemistry to detect PECAM. The length density and surface density of blood vessels were estimated using stereology. Another set of animals (n=4-5/group) were sacrificed at 10 days after fracture for histomorphometric analysis of fracture healing. Tissues were fixed, decalcified, and paraffin sections were prepared. HBQ staining was performed to visualize bone and cartilage. The volume of callus, bone, cartilage, adipose tissue, and fibrous tissue were estimated using histomorphometry.

Results: Effect of rhBMP-7 on tissue vascularization: Stabilized tibia fractures were treated with 50 µg of rh-BMP-7 or lactose solution and vascularization of the fractured limbs was quantified at 3 days after injury. No significant difference in length density or surface density of blood vessels within the fractured limbs was detected between treated and control fractures (Fig. 1). Effect of rhBMP-7 on bone and cartilage formation in stabilized fractures: At 10 days after injury, control fractures exhibited a small callus and some newly formed bone at the fracture site (Fig. 2A). In controls, cartilage was not detected in 2 of 5 fractures while the other 3 animals had minimal amount of cartilage. In contrast, rh-BMP-7 (10 or 50µg) induced the formation of a large callus and a large amount of bone and cartilage (Fig. 2B). Histomorphometric analyses demonstrated that the volumes of callus, bone, and cartilage in both rhBMP-7 treated groups are all significantly greater than those in PBS-treated controls (Fig. 2C). No significant differences in bone and cartilage were detected between the two rhBMP-7 (10 and 50µg) groups. However, the higher dose of rhBMP-7 induced more robust callus formation that contained more adipose and fibrous tissues (Fig. 2C).

Conclusion and Significance: Data from this current study demonstrate that rhBMP-7 is capable of inducing cartilage and increasing bone formation in a mechanically stable environment, suggesting that BMP-7 can direct stem cells to differentiate into chondrocytes and osteoblasts. In addition, BMP-7 treatment didn’t affect tissue vascularization during early fracture healing.

Acknowledgement: This work is supported by OTA (a research grant to C.L.), NIH (R01 to T.M), and Stryker Biotech (to R.M).

Reference: