Analyzing The Cellular Contribution Of Periosteum To Fracture Healing Using A Membrane-targeted Tdtomato Transgenic Mouse Model

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Introduction: Although periosteal cells are reported to play an essential role in bone fracture healing, we still lack in vivo evidence to show cellular origin of cells in fracture callus, the differentiation and the fate of those cells.

Methods: To investigate the cell lineage of fracture healing, we generated and analyzed the process of fracture healing in Tamaxifen inducible Prx-CreER-eGFP-Tomato transgenic mice that express, in periosteal cells, CreER and Tomato under the control of a mouse 2.4 kb Prx promoter. A 2.4 kb Prx1 promoter directs the transgene expression in undifferentiated mesenchyme, and the expression is confined to the periosteum and tendons of the limbs at E15.5. Therefore, the 2.4 kb Prx1 promoter would be useful for expressing genes in the periosteum after cartilage formation during fracture healing. In Tamaxifen inducible Prx-CreER-eGFP-Tomato transgenic mice, the periosteal origin of cells could show deep red fluorescence after fracture, but not in the littermates lacking the Cre which served as controls. Cre recombinase was induced by 2 daily i.p. injections of tamoxifen in oil (2mg per mouse), and fracture was performed after the second tamoxifen injection. A unilateral closed tibial fracture was made in Prx mice and control mice at age 12 weeks.

Results: Three days after fracture, cells in the periosteum of Prx mice began to proliferate and migrated into soft callus. Seven and fourteen days after fracture, most of cells in the soft callus of Prx mice were red-fluorescence positive. After one and two months, while Prx-CreER-eGFP-Tomato-expressing cells were still localized within trabecular bone structure.

Discussion: These results suggest that periosteal cells provide mainly cellular origin bone fracture healing. Most of the cells in the soft callus are derived from the mesenchymal progenitors in the periosteum, but not from bone marrow stromal cells. Our experiments showed that the Prx1-CreER-Tomato-expressing cells are osteochondro progenitor cells with both chondrogenic and osteogenic potential. Chondrocytes originated from periosteum can become osteoblasts and osteocytes in endochondral bone formation during fracture healing.

Significance: The Tamaxifen inducible Prx1-CreER-eGFP-Tomato transgenes will offer novel approaches for analyzing lineage commitment and early stages of chondrocyte and osteoblast differentiation during fracture healing.