Introduction: During the early stages of bone healing, skeletal stem cells are recruited to the injury site and differentiate into chondrocytes and osteoblasts in response to various signals in the injured environment. There are several potential sources of adult skeletal stem cells within the local environment of bone and at a distance from the bone, including bone marrow, periosteum, soft tissues, blood and blood vessels. Although cells derived from periosteum and bone marrow can differentiate into chondrocytes or osteoblasts in vitro and accelerate healing in vivo, the contributions of these stem cells to bone healing have not been established in vivo. Here we have developed a new method to trace cells derived from the periosteum, bone marrow and particularly the endosteum during healing via intramembranous and endochondral ossification in mice.

Materials and Methods: All procedures followed protocols approved by the Institutional Animal Care and Use Committee. Following euthanasia, cortical bone grafts were isolated from the tibia of Rosa26 donor mice that express the LacZ transgene ubiquitously. To follow cells derived from the periosteum, the bone marrow and the endosteum were removed and the periosteum left intact. The opposite was done to follow cells derived from the bone marrow or endosteum only. For positive controls, the tissue was left intact. For negative controls, all tissues were removed from the cortical bone grafts. Under anesthesia, bone grafts were transplanted into cortical defects created with a drill on the anterior-proximal surface of the tibia in wild type host mice. The orientation of the graft was similar to the endogenous bone or switched. Bone grafts were either allowed to heal without additional injury or a non-stable tibial fracture was created adjacent to the graft. Tissues were collected at day 10 (n=5 per group), processed and cryo-embedded. Cryosections were stained with X-gal to identify cells derived from the graft (blue staining).

Results: Bone grafts healed via intramembranous ossification. To track periosteum- and bone marrow/endosteum-derived cells during bone graft healing, we analyzed the distribution of labeled cells (Fig. 1). We observed that the periosteum gave rise to osteoblasts and osteocytes at the periosteal surface and that the endosteum and bone marrow contributed to osteoblasts and osteocytes within the marrow cavity. In negative controls no graft-derived cells were detected. In positive controls, we observed graft-derived cells both at the periosteal and endosteal surfaces. Therefore, both periosteum and endosteum contributed to bone formation and bridging of the graft and host cortical bone (Fig. 1).

Non-stabilized fractures created adjacent to the graft healed via endochondral ossification. Lineage analysis was performed to determine the contributions of periosteum and bone marrow/endosteum to cartilage and bone formation in the fracture callus. Cells derived from the periosteum gave rise to osteoblasts and chondrocytes in the fracture callus while cells derived from the endosteum gave rise to osteoblasts only (Fig. 1). When bone marrow was left intact, we mainly detected osteoblasts derived from the graft and rarely chondrocytes. Negative and positive controls were performed as described above to confirm the validity of this method. When the periosteum was placed in the environment of the endosteum, periosteum-derived cells could still differentiate into osteoblasts and chondrocytes. When endosteum and bone marrow were placed in the environment of the periosteum, cells could not only give rise to osteoblasts but also chondrocytes, although not to the same extent as the endogenous periosteum.

Discussion: To distinguish the cellular contributions of periosteum and endosteum/bone marrow to bone healing, we developed an in vivo lineage analysis approach using genetically labeled bone grafts. Our findings are the first to directly demonstrate the contributions of these tissues to cartilage and bone formation during bone repair in vivo. During healing via intramembranous ossification, both periosteum and bone marrow/endosteum can give rise to osteoblasts and chondrocytes. In contrast, during healing via endochondral ossification only the periosteum can give rise to chondrocytes while both periosteum and bone marrow/endosteum can give rise to osteoblasts. These data suggest that these tissues contain distinct pools of stem cells. In addition, we showed that the lack of chondrogenic potential of the endosteum is not due to the presence of chondrogenesis inhibitors within the marrow cavity since periosteum was still able to undergo chondrogenesis when placed in the environment of the bone marrow/endosteum. Indeed, placing the bone marrow/endosteum in the environment of the periosteum increased their chondrogenic potential. Overall these results show important differences in the differentiation potentials of periosteum and bone marrow/endosteum in vivo.

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