Isolation and functional assessment of mouse skeletal stem cell lineage.

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Public Summary:
There are limited methods available to study skeletal stem, progenitor, and progeny cell activity in normal and diseased contexts. Most protocols for skeletal stem cell isolation are based on the extent to which cells adhere to plastic or whether they express a limited repertoire of surface markers. Here, we describe a flow cytometry-based approach that does not require in vitro selection and that uses eight surface markers to distinguish and isolate mouse skeletal stem cells (mSSCs); bone, cartilage, and stromal progenitors (mBCSPs); and five downstream differentiated subtypes, including chondroprogenitors, two types of osteoprogenitors, and two types of hematopoiesis-supportive stroma. We provide instructions for the optimal mechanical and chemical digestion of bone and bone marrow, as well as the subsequent flow-cytometry-activated cell sorting (FACS) gating schemes required to maximally yield viable skeletal-lineage cells. We also describe a methodology for renal subcapsular transplantation and in vitro colony-formation assays on the isolated mSSCs. The isolation of mSSCs can be completed in 9 h, with at least 1 h more required for transplantation. Experience with flow cytometry and mouse surgical procedures is recommended before attempting the protocol. Our system has wide applications and has already been used to study skeletal response to fracture, diabetes, and osteoarthritis, as well as hematopoietic stem cell-niche interactions in the bone marrow.

Scientific Abstract:
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