Paper - Basic Science

Paper Session: Fracture Healing:
Cells and Bone

The Effluent of Autologous Bone Graft Preparations Is a Good Source for Skeletal Stem Cells, Which Are Markedly Different From Mesenchymal Stromal Cells

Kimberly Ann Grannis MD*; Mark A Lee MD; Fernando A Fierro PhD; Bryan Le MD; Wei Yao MD*
UC Davis, Sacramento, CA, United States

Purpose: Our objectives were to (1) isolate human skeletal stem cells (SSCs) and identify key differences with mesenchymal stem/stromal cells (MSCs); and (2) identify a good source to isolate SSCs.

Methods: Human SSCs were isolated by flow cytometry-activated cell sorting (FACS), based on the signature CD45-CD51+CD200+. Two sources were investigated: 8t bone marrow aspirates from upper iliac crest, and 8 effluents of procedures using the Reamer Irrigator Aspirator (RIA) system. This latter method is commonly used for the harvest of autologous bone graft, where a large volume of liquid and small particles debris is discarded as waste, which we refer to as RIA-effluent. The osteogenic potential of freshly isolated SSCs was tested in a fracture model in immune-deficient NOD/SCID/IL2Rg-/-(NSG) mice (12,000 cells per mouse, n = 4). Tropism to bone/bone marrow was tested by intravenous injection of fluorescently labeled SSCs (5000 cells per mouse, n = 2) in immune-deficient mice and detection of cells by immunohistochemistry and polymerase chain reaction (PCR). Finally, SSCs were cultured ex vivo for up to 2 weeks and then analyzed using phase contrast microscopy, flow cytometry, and in vitro osteogenic and adipogenic differentiation assays.

Results: We show that higher yields of SSCs can be found in RIA-effluent, as compared to bone marrow aspirates, which we hypothesize relates to an endosteal distribution of the cells. In mice undergoing bone fracture, intramuscular injection of SSCs leads to a significant improvement in bone repair as shown by microCT and calcein staining. Of note, we also show that these cells home preferentially to bone and bone marrow after intravenous injection, suggesting that the cells may be applicable to treat systemic skeletal defects, such as osteoporosis. Both the potent pro-regenerative potential and homing capacity are in sharp contrast to features of MSCs, which are typically applied in much higher numbers and lodge in lungs after systemic administration. However, we also show that after tissue culture expansion, SSCs become undistinguishable from MSCs.

Conclusion: Altogether, our data show that RIA-effluents are a rich source for SSCs, which show unique properties for the development of new cellular properties. A major challenge will be to develop new expansion methods so that the cells can be multiplied, without losing these unique features.