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Summary

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Summary - Cell Therapies for Orthopedic Applications

Session Chair: R.J. O'Keefe

Center for Musculoskeletal Research, University of Rochester, School of Medicine and Dentistry, Rochester, NY, USA

In the last several years it has become increasingly apparent that stem cells play an essential role in the maintenance and repair of skeletal tissues. Stem cells are essential for the reparative processes. These cells undergo initial proliferation followed by differentiation where new cells are able to replace and reconstitute lost cells and tissues. Stem cells are also important for maintenance of hard and soft skeletal tissues. With aging, differentiated cells undergo quiescence and death and tissues eventually degenerate. Cells in the stem cell compartment have the potential to replace these cells and slow the degenerative process. The speakers in the session addressed the potential role and use of stem cells for the treatment of orthopedic diseases.

The first speaker was **Dr. Regis O'Keefe** from the University of Rochester, who provided an overview of the session and presented pre-clinical data describing the importance of stem cells in the bone reparative process. Dr. O'Keefe presented data from two models in use in his laboratory, an murine allograft repair model and a fracture healing model in aged mice, both of which illustrate the importance of stem cells and the differentiation potential of stem cells for the reparative process. The allograft model mimicked the scenario in humans whereby healing was limited to the host-graft junction and the allograft segment is not revitalized. In contrast, in autografts, a cuff of bone forms around the graft and the entire segment undergoes remodeling. Surprisingly, Dr. O'Keefe showed that the processing of autograft bone, a procedure that includes ethanol washes and storage at –80 degrees centigrade, also prevents bone healing. To confirm that living stem cells on the autograft stimulate the repair process, Dr. O'Keefe's group transplanted unprocessed autogenous bone (identical strain mouse) from a Rosa 26 mouse, so that histochemical staining could identify the fate of cells transplanted with the autograft. In the new host, the transplanted cells initially proliferated and then differentiated into chondrocytes, osteoblasts, and even endothelial cells. Finally, the laboratory placed C9 cells, which are derived from the C3H 10T½ cell line and express BMP-2, onto the allograft surface and after a one-hour culture, transplanted these cells into the host. The C9 cells stimulated a repair response that was similar to the live autograft. Altogether, the experiments demonstrated that adult stem cell-based and gene-enhanced tissue engineering may offer novel and exciting therapeutic approaches to augment bone allograft healing and repair.

The second speaker was **Dr. T-C. He** from the University of Chicago. Dr. He focused upon the differential ability of the various members of the BMP family to stimulate bone formation. Dr. He used a comprehensive approach whereby he used viral expression vectors to over-express all of the BMP family members from BMP-2 to BMP-15. Both *in vitro* and *in vivo* models were used. Dr. He found that BMPs 2, 7, and 9 were the most stimulatory of osteoblastic differentiation of C2C12 and C3H10T½ cells, while smaller effects were observed with BMP-4 and BMP-7. Dr. He subsequently used two models of ectopic bone formation, both utilizing athymic nude mice. In the first model, C2C12 cells were transduced with the various viral constructs and injected into the thigh musculature. BMPs 2, 6, and 9 induced bone formation radiographically and histologically, with BMP-7 causing slightly smaller effects. In the second model, the bone-inductive potential of each of the BMPs was examined following direct injection of the virus into the thigh musculature. With this method, there was no radiographic evidence of bone formation with any of the BMPs, but histological evidence of cartilage and bone formation were observed with BMPs 2, 6, 7, and 9. These findings demonstrate differences among the various BMPs in osteo-inductive potential and demonstrate improved bone formation when viral gene therapy is combined with administration of a stem cell population. Finally Dr. He used gene array to examine and compare the gene induction patterns of sets of osteo-inductive BMPs (2, 6, and 9), a BMP inhibitor (BMP-3), and a BMP without any known potential to induce bone formation (BMP-12). Dr. He used hierarchical clustering analysis and

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Corresponding author: Regis J. O'Keefe, MD, PhD, Box 666, Department of Orthopaedics, University of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY 14642
E-mail: regis_okeefe@urmc.rochester.edu

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found that osteogenic BMPs had similar patterns of gene expression that were distinct from the other BMPs. While BMP family members have traditionally been grouped based upon structural similarities, the findings suggest that a functional clustering based upon patterns of gene induction may be more appropriate. Furthermore, the findings suggest that BMP-9, which has not previously been extensively studied as a bone-inducing factor, may have an important role in the stimulation of bone formation.

The third speaker was **Gloria Matthews**, from Genzyme. Dr. Matthews reviewed the challenges associated with cartilage repair. Although a number of procedures and different methods are currently used to treat cartilage defects, none of the methods has been completely satisfactory. All methods rely on proliferation and either maintenance of a differentiated chondrocyte phenotype or differentiation of mesenchymal precursors. The simplest method involves arthroscopic cartilage debridement and lavage, but this does not bring new reparative cells or tissues to the defect and the proliferative and repair capacity of the native cartilage is minimal. In contrast, adding to this procedure a method to stimulate subchondral bone marrow, either through micro-fracture or through the drilling of subchondral bone, permits access of bone marrow stem cells to the cartilage defect. Both animal and human studies have established that mesenchymal cells undergo chondrogenesis and produce a hyaline cartilage matrix. However, over time cells dedifferentiate and a less durable and lasting fibro-cartilage matrix is produced. Autologous cartilage transplantation involves the transplantation of articular cartilage capped bone plugs from a donor site to the cartilage defect. However, this procedure has substantial donor site morbidity and is limited to the repair of relatively small defects. Another procedure, autologous cartilage cell transplantation following harvest and cell culture expansion, is an FDA approved cell therapy for cartilage defects. This procedures has several drawbacks as well, including the need for a separate harvesting and an open knee implantation procedure, and the long-term results also suggest that a fibro-cartilage matrix develops with time. In spite of the drawback of the various procedures, essentially all of the methods provide some temporary relief, but the repair is far inferior to native cartilage. Noting the deficiencies of all of the methods, Dr. Matthews described ongoing efforts to create an optimal replacement material, one that would involve a single, arthroscopic procedure using a polymerizable material that could be formed *in situ* with a combination of cells, growth factors, and/or genes.

The final speaker was **Dr. George Muschler** of the Cleveland Clinic Foundation. Dr. Muschler provided a review of his long-standing research program defining the osteoblast precursor population present in the bone marrow compartment. Dr. Muschler showed that small, 2 millimeters per location aspirates of iliac crest bone marrow provides the most efficient harvest of nucleated cells. In culture the nucleated cells form colony-forming units and a subset of colonies stain positively for alkaline phosphatase and have an osteoblast phenotype. Dr. Muschler showed a decline in the number of colony-forming units and osteoblast precursors in the marrow with aging. Since the osteoblast precursors represent only a small portion of the nucleated cells Dr. Muschler went on to describe methods to enrich the osteoblast precursor population. A simple method involves the ability of the osteoblast precursors to adhere to demineralized bone matrix. Dr. Muschler has established methods to pass marrow aspirates through a column of matrix to increase the number of osteoblast precursors. Importantly, this selective enrichment of the bone marrow stem cell population is associated with improved healing potential.

Altogether, the session established the clinical importance of continued translational research to improve musculoskeletal repair. Furthermore the presentations and input from the attendees reached consensus regarding the potential role of stem cells, growth factors, and matrix interactions for optimization of bone and cartilage repair.