Autologous Mesenchymal Stem Cells for Articular and Bone Cell Therapy

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Cartilage lesions are irreversible and reconstructive surgery is currently the only therapeutic option available. Around 20% of people over 65 years old are affected by osteo-arthritis a major functional handicap in the aging population. The resulting cartilage damage has until now been considered irreversible. Joint replacement is currently the only therapeutic option for loss of cartilage substance after trauma and at advanced stages of rheumatoid arthritis or osteo-arthritis. Cell therapy involving transplantation of cells able to regenerate the cartilage matrix specifically in the damaged joint is now an option that warrants serious consideration.

We are trying to combine cell therapy, using mesenchymal stem cells (MSCs) derived from bone marrow and capable of differentiating into the chondrogenic lineage, with transfer of the hBMP-2 gene, a factor that triggers chondrocyte differentiation and secretion of cartilage matrix-specific proteins.

The early limb bud formation stage corresponds to condensation of mesenchymal cells in prechondrogenic regions foreshadowing the future skeleton. This step is controlled by various factors, including the fibroblast growth factor (FGF), the Wingless (Wnt) factor, and members of the Hedgehog gene family. Members of the TGF-b superfamily, such as BMPs and their receptors, are expressed later in developing cartilage elements and enable mesenchymal cell differentiation into cartilage cell lineages. These factors have a pivotal role in triggering cell differentiation, especially into osteocytes and chondrocytes. Studies in rabbits revealed that transplantation of collagen sponges saturated with recombinant hBMP-2 cells in the core of mechanically induced cartilage defects led to regeneration of damaged cartilage within 8 to 24 weeks. These authors did, however, note weak engrafting of neosynthesized tissue to the adjacent cartilage, likely because normal cartilage chondrocytes do not migrate in tissue and are not involved in the repair process. Another study highlighted the role of hBMP-2 in vivo in the synthesis of articular proteoglycans by injection of recombinant protein into the knee joint in mice. Transfer of hBMP-2 genes in a mouse MSC line was compared with administration of recombinant BMP-2 proteins in regenerating bone. Transplantation of mesenchymal cells expressing hBMP-2 genes leads to the production of an osseous callus of good anatomical and biomechanical quality (oriented along the stretch lines), and to quicker repair. The use of engineered mesenchymal cells expressing hBMP-2 induces a paracrine response (recruitment and differentiation of unmodified MSC cells derived from joint bone marrow) and an autocrine response (involvement of autologous MSCs engineered to express BMP-2 in the regeneration process).

Pluripotent MSCs proliferate in culture and differentiate in vitro into chondrocytes, osteocytes, adipocytes or myoblasts according to culture conditions and the effects of specific growth factors. The detection of cell markers specifically expressed by hMSCs (SH2, SH3, Thy1, Stro-1) facilitates their identification in bone marrow cell populations. Moreover, they can be readily cultured and amplified ex vivo since they adhere to plastic. Few studies to date have used this approach for cartilage defect repair. However, in a study conducted by Wakitani's team in New Zealand white rabbits, deep articular cartilage (and sub-chondral bone) lesions were mechanically induced around the femoral condyle in rabbits and then filled with collagen sponges saturated with MSCs. Two weeks after

transplantation, MSCs had differentiated into chondrocytes and regenerated a new cartilage matrix. Twenty-four weeks after transplantation, the distal part of this matrix was gradually replaced by bone until the sub-chondral bone was completely repaired. Note that this cartilage-bone regeneration process works only when the defects reach the sub-chondral bone. This process is likely induced by the secretion of differentiation factors synthesized in bone, e.g. members of the transforming growth factor family (TGF-ß), including bone morphogenetic proteins (BMPs).

Our strategy involves transplanting ex vivo amplified autologous MSCs into the damaged joint and inducing chondrocyte differentiation by hBMP-2 gene transfer. We also study the effects of biodegradable matrices on the induction and maintenance of MSC differentiation into chondrocytes, while monitoring the appearance of differentiation markers in vitro. The last phase involve validating this strategy in several preclinical mouse models. The SCID/hu mouse model will be used for in vivo assessment of the differentiation of human MSCs (hMSCs) transplanted in a matrix and their interaction with subcutaneously grafted synovial cells derived from a human RA patient. This project represents a preclinical phase with a view to enhancing cartilage repair in human patients.