BONE REPAIR WITH GENE TRANSFERRED ALLOGENEIC MESENCHYMAL STEM CELLS ON FEMORAL SEGMENTAL DEFECT IN RATS

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Introduction: Bone marrow derived mesenchymal stem cells (MSC) are self-renewing pluripotent progenitor cells, which have been isolated from the whole marrow of chick, mouse, rat, goat and human. The osteogenic potential of marrow-derived MSC has been well defined. In previous studies, our laboratory demonstrated that bone morphogenetic protein-2 gene transfer into MSC enhances the cell differentiation into osteoblast and that implantation of BMP-2 gene transferred autologous MSC effectively induced bone formation and spine fusion in rabbits and pigs.

Although there is great promise for the use of autologous MSC for bone and other musculoskeletal tissue repair, it remains difficult and inconvenient to culture individual MSC for autologous implantation. Furthermore, the effectiveness of MSC culturing in vitro may be limited because the patient’s marrow is damaged or the healthy marrow elements are reduced.

Previous clinical investigative studies have shown that the hemopoietic portion of bone marrow was allogeneically transplantable for disease treatment. Engraftment of allogeneic MSC from allogeneic bone marrow transplantation in children with severe osteogenesis imperfecta enhanced bone formation. Thus, we hypothesize that in association with the short-term administration of immunosuppressants, allogeneic MSC can survive for a period of time in implantation sites of non-ablated recipients. In this scenario after transduction with the BMP-2 gene, these MSC can over-express BMP-2 protein for a period of time in situ, which induces both the implanted allogeneic MSC and the host progenitor cells to express bone matrices and form bone.

In this study we report that BMP-2 gene transferred allogeneic MSC is capable of repairing femoral segmental defects in a rat model, under short-term administration of immunosuppressants. Our results demonstrated that allogeneic MSC is a potential resource for musculoskeletal repair.

Methods: Inbred Fischer 344 (RT1\(^\text{1/2}\)) and Brown Norway (RT1\(^\text{1/2}\)) rats were used for this study. RT1\(^\text{1/2}\) and RT1\(^\text{1/2}\) represent major histocompatibility complexes and differ by strong histocompatibility antigens. A total of 56 rats were used in this study. Fourteen male Fischer 344 rats were used as donors of MSC. Twenty-eight female Brown Norway rats were used as allogeneic recipients. Fourteen female Fischer 344 rats were used as syngeneic recipients.

MSC were isolated from male Fischer rats and transduced with recombinant adenovirus as described previously. A rat femoral segmental defect model with 6 mm right femoral bone defects were used. Recipient rats were divided into 6 groups. Each group received implantation of male Fischer rat MSC transduced with adenovirus carrying human BMP-2 gene (MSC/Adv-BMP2) or male Fischer rat MSC transduced with adenovirus carrying lac Z gene (MSC/Adv-bGal). FK506 (Fujsawa Inc., Deerfield, IL), a powerful immunosuppressant, was administered to rats in two of four allogeneic groups intramuscularly at a dose of 1 mg/kg every day for the first two weeks and every other day for an additional week.

Serial radiographs of rat femurs were examined at two, four, six, and eight weeks post surgery under intramuscularly sedation with ketamine and medetomidine hydrochloride. Radiography of bone formation in the femoral defect site was scored on a 6-point scale. Two randomly selected allograft MSC/Adv-BMP2 rats with FK506 treatment were observed until 24 weeks post surgery at 4 weeks intervals. The cross section area of cortical bone and the bone mineral density (BMD) of cortical bone of united femoral defect specimens were measured by peripheral quantitative computerized tomography (pQCT). The center slice was determined with the center of two outer sides Kirschner wires on the scout view and two other slices were scanned at 1.5 mm intervals from the center slice proximally and distally. The harvested rat femurs were analyzed by Fluorescence in situ Hybridization (FISH) with a rat Y-chromosme probe to determine the fate of implanted male MSC in defect sites. Histological studies were also performed on harvested samples.

Results: The radiographic score is shown in Fig.1. Results showed that a great amount of bone formation was observed in bone defect sites of the syngeneic group of MSC/Adv-BMP2 and the allogeneic group of MSC/Adv-BMP2 with FK506 treatment as early as two weeks post surgery. Bone defects were completely repaired at four weeks post surgery (Fig.2). No bone repair was observed in the MSC/Adv-bGal groups and the allogeneic group of MSC/Adv-BMP2 without FK506 treatment (Fig. 1). PQCT analysis of 8 week samples demonstrated that there was no significant difference on the cortical bone area of newly formed bone between syngeneic and allogeneic transplant; however, the BMD of the syngeneic transplant group of MSC/Adv-BMP2 was significant higher than that of allogeneic transplant group of MSC/Adv-BMP2 with FK506 (100:93, P<0.01). Observation at 24 weeks of the repaired defects of rats in the allogeneic MSC/Adv-BMP2 plus FK506 treatment group indicated that bone remodeling continued with time and no absorption of newly formed bone was observed. FISH analysis found Y-chromosome cells in newly formed bone tissue, which indicated that the implanted allogeneic MSC were involved in bone repair and became a component of the newly formed bone. Histological examination demonstrated similar findings in the syngeneic group of MSC/Adv-BMP2 and the allogeneic group of MSC/Adv-BMP2 with FK506 treatment.

Discussion: Our study demonstrated that allogeneic MSC is a potential source for bone repair. However, an immunosuppressant is necessary in the process to allow the allogeneic MSC to survive in the implant site in the early time period. Our results also demonstrated that allogeneic BMP-2 gene transferred MSC are not only acts as the BMP-2 deliverer in the bone repair process, but also differentiates into osteocytes as a component of newly formed bone. It proves that BMP-2 transferred MSC functions in both paracrine and autocrine effects. Our results further demonstrated that short-term administration of immunosuppressant is sufficient to allow allogeneic BMP-2 gene transferred MSC to induce bone formation/bone repair. Withdrawing the immunosuppressant did not compromise the continuous bone repair process thereafter. No absorption of newly formed bone was observed up to 21 weeks in the absence of the immunosuppressant.

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Fig. 1: Radiographic score of newly formed bone in defect sites.

Fig.2: Serial radiographies after allograft with FK506