THE FATE OF BMP2 TRANSFERRED MESENCHYMAL STEM CELLS IN ALLOGENEIC REPAIR MODEL OF FEMORAL SEGMENTAL DEFECTS IN RATS

Introduction
In a previous study, we reported that BMP-2 engineered allogeneic mesenchymal stem cell (MSC) could repair critical bone defects in rats to the same degree as rats treated with BMP-2 engineered autologous MSC. If the allogeneic group received short-term treatment of the immunosuppressant FK506. We also showed that gene transferred MSC were directly involved in bone repair, in addition to acting as gene deliverers. However, several questions remain to be answered, such as how long these transplanted allogeneic MSCs can survive after discontinuing FK506, whether transplanted allogeneic MSCs migrate from the transplant site. In this study, we investigated the fate of the engineered allogeneic MSC at defect sites after transplantation using fluorescence in situ hybridization (FISH) and possible migration of transplanted allogeneic MSC from transplant defect sites by semi-quantitative polymerase chain reaction (PCR).

Material and Methods
Experimental design. A rat femoral segmental defect model was used in this study. Inbred Fischer 344 (RT1 b) and Brown Norway (RT1 n) rats were used; these strains strongly differ by histocompatibility antigens. Twelve male Fischer 344 rats weighing 90 to 240 g were used as donors of bone marrow derived MSC. 16 female Brown Norway rats weighing 155 to 204g as syngeneic recipients, and 8 female Fischer 344 rats weighing 154 to 197g as syngeneic recipients. Recipient rats were divided into 3 groups; group 1: allogeneic with FK506, group 2: allogeneic without FK506, group 3: syngeneic. MSC from male Fischer rat were all transduced with recombinant adenovirus carrying human BMP-2 gene (MSC/Adv-BMP2). FK506, an immunosuppressant, was administered to rats in group 1 intramuscularly for two weeks at a dose of 1mg/kg, everyday for the first week, and every other day for an additional week. At 2, 4, 6 and 8 weeks after cell implantation, rats were sacrificed and further investigated.

Surgical method. In accordance with protocols approved by the Animal Studies Committee at Washington University, a 6-mm transverse segment of the central diaphysis was created in the rat right femur; a high-density polyethylene fixation plate was attached to the anterolateral aspect of each femur. 8x10^3 cells-collagen mix was implanted in the each defect site of femur.

FISH. Fluorescence in situ Hybridization (FISH) analysis was performed on paraffin-embedded histological sections of the whole operated femur. The rat Y-chromosome probe in plasmid was kindly provided by Dr. Barbara Hoebee (National Institute of Public Health and Environment, the Netherlands). The probe was labeled with digoxigenin by nick translation, then added to the pretreated bone samples. Probe rat were all transduced with recombinant adenovirus carrying human BMP-2 gene (MSC/Adv-BMP2). FK506, an immunosuppressant, was administered to rats in group 1 intramuscularly for two weeks at a dose of 1mg/kg, everyday for the first week, and every other day for an additional week. At 2, 4, 6 and 8 weeks after cell implantation, rats were sacrificed and further investigated.

PCR analysis. Genomic DNA was prepared from liver tissues by standard procedure. The nucleic acid concentration was determined spectrophotometrically. The PCR reaction mixture contained 1µg of genomic DNA, 2.5U SureStart Taq DNA polymerase, 2.5 µl of 100µg/ml primer pair: sense, 5'-GAGAGAGGCACAAGTGTCG-3' and antisense, 5'-GCCTCCTGGAACAGGGCC-3', 0.8 µl of 25 mM dNTP mix, and 10 µl of 10Xreaction buffer in a final volume of 100 µl. After preheat-activation at 95 °C for 10 min, the reaction was performed in a thermal cycler for 30 cycles consisting of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 45 sec, and primer extension at 72 °C for 1.5 min. PCR products were visualized after electrophoresis through a 2 % agarose gel, followed by ethidium bromide staining.

Results
Survival of the transplanted donor cells. The specificity of the rat Y chromosome probe in FISH study was confirmed in control sections of male rat cells as a single red spot over nuclei, and the absence of a color reaction in female cells. The detecting efficiency was found to be 68.4±2.2 % in male positive control samples. In the experimental samples, the signals were observed as that in the cells encapsulated by mineralized matrix, residing within the bone marrow, and around the bone matrix. The number of donor cells within the bone matrix was very high in group 1 until 4 weeks after transplantation, and then decreased. Conversely, the donor cells of group 1 in the bone marrow showed rapid decrease at 2 weeks after transplantation. The cells encapsulated in bone matrix survived for 4 weeks after transplantation in group 1; additionally, the degree of decrease of donor cells resembled to that of group 3. (Fig. 2) The donor cells of group 2 were difficult to detect even at 2 weeks, and there was no new bone formation in vivo.

Discussion
The fate of the MSC has not been clearly reported after implantation into bone repair defect sites. In this study, we used a male MSC implantation into bone defect sites of female recipients system to demonstrate implanted BMP2 gene engineered MSC, either syngeneic or allogeneic with short term treatment with FK506, integrated into the newly formed bone tissue and the periosteum. The results indicated that implanted donor cells differentiated into osteocytes. Moreover, we demonstrated that implanted MSC survived for a period of time at the defect site. We also found that transplanted donor MSC may migrated from the implantation site to other organs in the recipients. These data suggest that maintaining microchimerism for at least 2 weeks might be required to provide the sufficient bone formation in an allogeneic transplant model. The transplanted osteoprogenitor cells might play the primary role in the early phase of bone formation in this model.

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