Development of a research platform to improve local bone marrow stromal cell transplantation in the treatment of osteonecrosis

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INTRODUCTION: Ischemic osteonecrosis (ON) is characterized by death of osteocytes and bone marrow cells due to disruption of blood supply to the bone. ON commonly affects the hip, knee, shoulder, elbow, foot, and hand joints. It produces a necrotic bone environment with insufficient recruitment of osteoblasts to repair bone defects which results in irreversible bone destruction. Bone marrow stromal cell (BMSC) therapy is a promising therapy for ON, which can be applied following drilling or core decompression procedures. Yet, the role of BMSC therapy for ON remains controversial, and the optimal method for local delivery of BMSC is unknown. Several key questions related to BMSC transplantation, such as BMSC retention rate, survival, and participation in bone repair, are largely unanswered. The purpose of this study was to develop a rat model of ON for local BMSC transplantation, to optimize the local cell delivery by using gelatin hydrogel, and to determine the early transcriptomic profile of the transplanted BMSCs using a genetic cell labeling method.

METHODS: To prepare for BMSC transplantation, a compound rat of CAG-Tdtomato was generated by crossing Tg (CAG-Ncre) and Rosa26 (CAG-LSL-Tdtomato) rats. BMSCs were isolated from the femur and tibia of the CAG-tdtomato rats (4 weeks old) and cultured in vitro for BMSC selection. Passage 1 (P1) BMSCs were used for transplantation. Flow cytometry was used to determine the purity of the mesenchymal population in the P1 BMSCs. Rat ischemic ON model was created by local vessel disruption of the distal femoral epiphysis using microsurgical technique and characterized by microangiography and histology as previously described [1]. The experiment for quantifying BMSC retention is illustrated in Fig.1. The animals were sacrificed right after the cell transplantation (T0). The distal femoral epiphysis was harvested, and the specimens were processed with tissue clearance protocol for 3D imaging [2] to determine the cell number and distribution. The experiment to investigate the early fate of the transplanted BMSC is illustrated in Fig. 2a. The animals were sacrificed 24 hours after local BMSC transplantation. The specimens were dissected and digested using Collagenase II (0.2%) to release the cells from the bone marrow. The transplanted BMSCs (tdt cells) were sorted for BulkRNASeq. The non-transplanted cells were used as a control. Gene enrichment analyses of GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) were used to reveal the early transcriptomic profile of the transplanted cells.

RESULTS: BMSC preparation: The P1 BMSCs derived from the bone marrow of tdtomato rats showed a red fluorescence by a green laser (Fig.1a, left upper image). Flow cytometry showed that the P1 BMSCs are CD45-, CD73+, and CD90+ (Fig.1a, right upper and lower panel images). When the BMSCs were cultured in an osteogenic medium, mineralization was present in the extracellular matrix. Rat model: Microangiography and H&E images showed a successful induction of ON on Sprague-Dawley rats. Compared to the sham control, ON-induced rats had an absence of vessels (Fig.1b upper panel) and extensive empty lacunae and cavities in the bone marrow in the distal femoral epiphysis 1 week following the ON induction. Local BMSC delivery: For successful cell delivery, stepwise surgical techniques were developed (Fig.1 c-e), including the surgical position, instrument settings (Fig. 1c left panel), drillsings (Fig. 1c middle and right panels), necrotic bone wash procedure (Fig. 1d), and application of cell carrier (gelatin hydrogel, Fig. 1e). Following the development of these techniques, local cell delivery efficiency using PBS vs. gelatin hydrogel was compared. 106 BMSCs were loaded in 25ul PBS or gelatin hydrogel and injected into the distal femoral epiphysis. The 3D reconstructed images showed a lower cell retention in the PBS group compared to the hydrogel group (Fig. 1g). 3D measurement (Fig.1h, Matlab 3D reconstruction images for 3D distribution measurement) showed only 25% of the necrotic epiphysis retained BMSCs in the PBS group. In contrast, in over 70% of the necrotic epiphysis retained BMSCs in the hydrogel group (Fig.2i, p<0.05). 3D cell counting showed that only 1.9 x 106 cells was retained in the PBS group compared to 1.4 x 107 in the hydrogel group (>7 fold increase, Fig.2i(1)). Following successful cell delivery, the injected cells were isolated 24 hours after transplantation via flow cytometry sorting of isolated cells to obtain the RNA. The bulk RNAseq analysis showed a total of 2098 different expression genes (DEGs) between the transplanted BMSC and the non-transplanted BMSC (control group). GO analysis showed that cell necrotic death ranked as #1 and response to oxygen ranked as #10, where the related genes were upregulated in the transplanted BMSCs compared to the non-transplanted BMSCs (Fig.2c). In KEGG analysis, HIF-1 signaling pathway ranked #5, where the related genes were upregulated in the transplanted BMSCs compared to the non-transplanted BMSCs(Fig.2d). Interestingly, the genes related to amino acids/sugar/lipid metabolism processes were upregulated in the transplanted BMSCs compared to the non-transplanted BMSCs (Table).

DISCUSSION: A rat model of ON for investigating cell transplantation was developed by using donor rats with fluorescence reporter, ON induction on wild-type host rats, and application of gelatin hydrogel for BMSC retention following an intraosseous injection. In the rat study, we found significantly improved BMSC retention due to the gelatin hydrogel carrier. Transcriptional analysis of transplanted BMSCs, however, revealed upregulation of cell necrotic death, attributable to the low oxygen environment. Oxygen-releasing materials might be a potential resolution and warrant further investigation to improve BMSC viability in the hypoxic environment.

CLINICAL RELEVANCE: In the United, over 20,000 people develop ON every year. Currently, there is no effective treatment for ON. BMSC transplantation is a promising therapy, but no standardized protocol based on scientific evidence has been established. The current study created a research platform to optimize cell-based therapy for ON.