

In vivo imaging of human Mesenchymal stem cells differentiation in bone defect model.

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To use Mesenchymal stem cells (MSCs) for bone defects/ fractures therapy, noninvasive imaging to evaluate osteogenic differentiation is important. Using dual promoter (MND promoter driving GFP signal as a beacon for cell sorting, and collagen type 1 alpha 1 (col1a1) promoter driving firefly luciferase (luc) for monitoring osteogenic differentiation) reporter gene expressing human mesenchymal stem cells (hMSCs), we evaluated the possibility of imaging osteogenic differentiation in a murine bone defect model. To create the defect, NOD-SCID mice of 20 g underwent an open internal fixation of a custom 10 x 2 x 1.5 mm alloy plate to the femoral shaft, bilaterally, followed by an osteotomy of 1.5 mm of femoral diaphysis; an X-ray scan was performed to confirm defect consistency in surviving mice. The fused reporter gene of luc and HSV-tk driven by the osteogenic-specific promoter col1a1 promoter (2.3 Kb) in a lenti-viral vector, which also has a GFP driven by the constitutively active promoter MND (LVCol2.3-tk/luc-mnd-GFP), was produced in large quantity and concentrated. hMSCs were infected with LVCol2.3-tk/luc-mnd-GFP viral particles with 1 MOI (multiplicity of infection) three times. After 7 days, infected hMSC (hMSC-Col2.3-tk/luc) were sorted using GFP. hMSC-Col2.3-tk/luc subsequently seeded into a 2% alginate hydrogel of less than 10 uL. This hMSC-Col2.3-tk/luc embedded hydrogel construct was directly implanted into the bone defect. At day 1, 3, 5, 7, 9, 11, 14, 18, and 23, hMSC-Col2.3-tk/luc and hydrogel implanted mice were taken Bioluminescence Imaging (BLI). The BLI signals from the site of implant were increased by day 7 after hMSC-Col2.3-tk/luc and hydrogel implantation. And then the BLI signals were decreased by day 14. After day 14, the BLI signals were all gone.

To evaluate the effects of the inducers: Dexamethasone (Dexa), Ascorbic Acid (AA), and beta-glycerophosphate (BGP) of osteogenic differentiation on col1a1 promoter, 10^{-7} M Dexa, 0.05mM AA, and 2mM BGP were treated only each factor and combination of 3 factors to hMSC-Col2.3-tk/luc in vitro. At day 1, 4, 7, 11, 15, 20, 25, 28, and 35, the luciferase activity driven by col1a1 promoter were measured. The luciferase activity by col1a1 of normal media, Dexa, AA or BGP or AA/BGP treated hMSCs was less than Dexa/AA or Dexa/BGP or Dexa/AA/BGP treated one. Till day 25, the luciferase activity by col1a1 of Dexa/AA treated hMSCs was almost same Dexa/AA/BGP treated one. But, at day 35, the luciferase activity by col1a1 of Dexa and AA treated hMSCs was 1.6 fold higher than Dexa/AA/BGP treated one.

This noninvasive imaging system of hMSCs differentiation is useful for understanding hMSCs therapy on bone defects/fractures for the clinical field.