MATERIAL AND METHODS

Immunophenotyping
MSCs (1x10^5 cells) were incubated with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (BD Pharmingen, San Diego, CA, USA) against CD11b, CD34, CD45, CD73, CD90, CD105, HLA-DR, and analyzed by flow cytometry (FACScan). For each analysis, an aliquot of cells was also stained with isotype control IgG-conjugated to FITC as a negative control.

In vitro differentiation
In order to induce adipogenic differentiation 2×10^5 in vitro expanded MSC were seeded into a well of a 6-well plate. They were first grown to confluence in MSCGM and the medium was changed every 2–3 days until a confluent cell layer was formed. The cells were stimulated to differentiate into the adipogenic lineage by submitting them to three cycles of alternating culture in Adipogenic Induction Medium (Lonza) and Adipogenic Maintenance Medium (Lonza) according to the manufacturer’s instructions. At the end of these cycles cells were grown for another 7 days in Adipogenic Maintenance Medium. As a control MSC were grown in Adipogenic Maintenance Medium only to exclude spontaneous adipogenic differentiation. To visualize adipocytes, cells were stained with Oil Red O (Sigma), while cell nuclei were counterstained with hematoxylin (Wako). Osteogenic differentiation was induced by exposing 3×10^4 MSC to Osteogenic Induction Medium (Lonza). The medium was changed every 3 to 4 days. As a negative
control, cells were cultured in MSCGM and medium was changed at the same frequency as that for the differentiating MSC. To verify osteogenic differentiation, Von Kossa’s method was used to stain calcium deposits. In order to induce chondrogenic differentiation 2.5×10^5 MSC were washed twice in Incomplete Chondrogenesis Induction Medium (Lonza). Cells were then sedimented by centrifugation at 150 g for 5 minutes and 0.5 mL Complete Chondrogenesis Induction Medium (Lonza) was added to the cell pellet. Complete medium was made by adding 5 μL TGF-β3 (20 μg/ml; R&D systems) to 1 mL incomplete medium. Cells were cultured for 2–3 weeks and the medium was changed every 3 to 4 days. Pellets were harvested and fixed in formalin and embedded in paraffin. Sections of 4 μm were made and stained with Safranin O.

**Adenovirus-mediated gene transduction.**

MSCs and FBs were seeded in culture plates or flasks at a density of 1x10^4 cells/cm^2, and the next day the cells were treated with each adenovirus vector for 1.5 h. The medium containing the vectors was removed and replaced with fresh medium. At the indicated time, fluorescent intensity was measured by a luminometer (Fluoroskan Ascent FL), and luciferase assays were performed using Bright-Glo luciferase assay system (Promega, Madison, WI) according to the manufacturer’s instructions. Luminescent intensity was measured by a luminometer (Fluoroskan Ascent FL).

**Immunohistochemistry**

Colo205/RFP cells (3 x 10^6) were subcutaneously inoculated into 4- to 6-week-old male
Balb/c nu/nu mice. Mice were sacrificed on day 11, serial sections from tumor tissues were processed. Immunohistochemistry was performed with anti-mouse CD34 antibody on tumor section sections to detect tumor blood vessels. Nuclei were stained with Haematoxilin (Wako Pure Chemical Industries, Ltd.). Images were obtained with a fluorescence microscope (BZ-9000).