GATA2 regulates differentiation of bone marrow-derived mesenchymal stem cells

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Supplementary Methods

Antibodies

Allophycocyanin (APC)-labeled human CD14, phycoerythrin (PE)-labeled human CD29, PE-labeled human CD34, fluorescein isothiocyanate (FITC)-labeled mouse/human CD44, FITC-labeled human CD45, FITC-labeled human CD90 and FITC-labeled mouse Sca-1 antibodies were purchased from BD Biosciences. APC-labeled anti-human CD105, PE-labeled mouse CD29, FITC-labeled mouse CD11b, FITC labeled mouse CD34 and PE-labeled mouse CD45 antibodies were purchased from eBioscience.

Cytokine treatment

BM-MSCs were differentiated into adipocyte-lineage cells, with the presence of cytokines as follows: 100 ng/mL each of recombinant human bone morphogenetic protein 4 (rhBMP4), human transforming growth factor-β1 (rhTGFβ1), human interleukin (IL)-1β/IL-IF2z (rhIL-1β), human IL-6 (rhIL-6), human interferon-γ (rhINFγ), human tumor necrosis factor (TNF)-α (rhTNFα), or human IL-17A (rhIL-17A). All cytokines were obtained from R&D Systems.
**Cell cycle fractionation**

In a 6-well plate, $8.0 \times 10^4$ human BM-MSCs were seeded and cultured for 24 hours.

On the next day, control or anti-GATA2 siRNAs were transfected. After 48 hours, the cells were collected and stained in a nucleic acid staining solution (NASS; 0.1 M phosphate-citrate buffer, pH 4.8 with 0.9 % NaCl, 0.5 % bovine serum albumin, 0.02 % saponin) supplemented with 1 μM/mL 7-aminoactinomycin D (7-AAD, Imgenez, SanDiego, CA) for 20 minutes. The cells were washed with PBS and were further stained in the NASS supplemented with 10 ng/mL pyronin Y (Sigma Aldrich, St. Louis, MO) for 5 minutes at 4°C. The cell cycle fractions were identified and quantified using FACSAris II cell sorter and BD FACSDiva software (BD Biosciences, San Jose, CA).

Cells in G0 and G1 phases were determined as 7-AAD\textsuperscript{lo}/pyronin Y\textsuperscript{lo} and 7-AAD\textsuperscript{lo}/pyronin Y\textsuperscript{int/hi}, respectively.
Supplementary Figure Legends

Supplementary Figure 1. Phenotyping of mouse BM-MSCs.

(A) BM-MSCs were induced with iCre to delete the DNA binding domain of GATA2 using the Cre-loxP system. (B) Differentiation of GATA2\textsuperscript{fl} BM-MSCs. Typical adipocytes contained oil drops that were stained with Oil Red O. (C) BM-MSCs from GATA2\textsuperscript{fl} mice expressed cell-surface antigen markers for BM-MSCs. The data was a representative of 2 independent BM-MSC lines.

Supplementary Figure 2. Phenotyping of human BM-MSCs.

(A) Differentiation of human BM-MSCs. Typical adipocytes contained oil drops that were stained with Oil Red O. (B) Human BM-MSCs expressed cell-surface antigens, characteristic of BM-MSCs. The data was a representative of 3 independent BM-MSC lines.

Supplementary Figure 3. Quantitative RT-PCR-based validation analysis for GATA2 knockdown.

The siRNA-mediated GATA2 suppression was confirmed using quantitative RT-PCR.
The expression of GATA2 relative to that of GAPDH was calculated (expressed as mean +/- SD, n = 3). Asterisk, *p* < 0.05.

**Supplementary Figure 4. Increase of G1 phase cells with decreased GATA2 expression in BM-MSCs.**

(A) Representative cell-cycle profile in human BM-MSCs treated with control or anti-GATA-2 siRNA, based on 7-AAD and pyronin Y staining. (D) Percentage of cells in G0 and G1 stages of the cell cycle (expressed as mean +/- SD, n = 3). The average percentages of G0 and G1 phase cells were significantly decreased and increased, respectively, by GATA2 knockdown (*p* < 0.05).

**Supplementary Figure 5. Effects of various cytokines on adipocyte differentiation and GATA2 expression in BM-MSCs.**

Human BM-MSCs were cultured with various cytokines (final concentration, 100 ng/mL) for 16 days, and samples were harvested on days 0, 4, 8, 12, and 16. Oil-drop formation and the expression of GATA2 and aP2 were analyzed using quantitative RT-PCR. (A) rhTNFα, (B) rhINFγ, (C) rhIL-1β, (D) rhIL-6, (E) IL-17A, (F) rhTGFβ1, (G)
rhBMP4. Data was expressed as mean +/- SD (n = 3). Asterisk, $p < 0.05$. 