GSTT1 as a predictive marker and enhancer for osteogenic potential of human adipose-derived stromal/stem cells

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INTRODUCTION: Adipose-derived stromal/stem cells (ASCs) have been extensively studied as cell sources for regenerative medicine for bone due to their excellent proliferative capacity and the ability to obtain a large number of cells with minimal donor morbidity. On the other hand, the differentiation potential of ASCs is generally lower than that of bone marrow-derived stromal/stem cells and varies greatly depending on donors. In this study, we mined a marker that can predict the osteogenic potential of ASC clones and also investigated the usefulness of the molecule as the enhancer of osteogenic differentiation of ASCs as well as its mechanism of action. Through RNA-seq gene analysis, we discovered that GSTT1 (Glutathione S-transferase theta-1) was the most distinguished gene marker between highly osteogenic and poorly osteogenic ASC clones.

METHODS: Total RNA was extracted from each sample using the TRIzol reagent. Its concentration and purity were determined based on A260 and A260/A280, respectively, using a spectrophotometer (Bio-Tek Instruments). RNA sequencing library of each sample was prepared using a TruSeq RNA Library Prep Kit (Illumina, San Diego, California, USA). RNA-seq experiments were performed for hBMSCs cultured under different conditions. RNA-seq analysis was performed using an Illumina HiSeq 2000 system. Buffer R at a final concentration of 1×105 cells/ml. Cells were transfected with either GSTT1 at 100 nM or siControl (scramble control) by electroporation using a 1050 pulse voltage for 30 ms with 2 pulses using a 10 μl pipette tip. Transfected cells were cultured for one day or two days at 37℃ under an atmosphere with 5% CO2.

RESULTS SECTION: The purpose of this study is to discover genetic markers that can promote osteogenic differentiation of adipose stem cells (hADSCs) through mRNA-seq gene analysis. The glutathione S-transferase theta-1 (GSTT1) gene is a member of a protein superfamily that catalyzes the conjugation of reduced glutathione to a variety of hydrophilic and hydrophobic compounds. The selected GSTT1 gene was found to be associated with the enhancement of osteogenic differentiation of adipose stem cells. Knockdown of GSTT1 in high osteogenic ASCs by siGSTT1 treatment reduced mineralized matrix formation. On the other hand, GSTT1 overexpression by GSTT1 transfection or GSTT1 recombinant protein treatment enhanced osteogenic differentiation of low osteogenic ASCs. Metabolomic analysis confirmed significant changes of metabolites related to bone differentiation in ASCs transfected with GSTT1. A high total antioxidant capacity, low levels of cellular reactive oxygen species and increased GSH/GSSG ratios were also detected in GSTT1-transfected ASCs. When the in vivo effect of GSTT1-transfected ASCs on bone regeneration was investigated with segmental long-bone defect model in rats, bone regeneration was significantly better after implantation of GSTT1-transfected ASCs compared to that of control vector-transfected ASCs. In conclusion, GSTT1 can be a useful marker to screen the highly osteogenic ASC clones and also a therapeutic factor to enhance the osteogenic differentiation of poorly osteogenic ASC clones. It also provides a cornerstone for other studies related to the development of gene cell therapeutics with enhanced differentiation potential.

DISCUSSION: In this study, GSTT1 transgenic adipose stem cells could be used in regenerative medicine to improve bone differentiation. In addition, the GSTT1 gene has important significance as a marker for selecting adipose stem cells with potential for bone differentiation in the development of a therapeutic agent for bone regeneration cells.
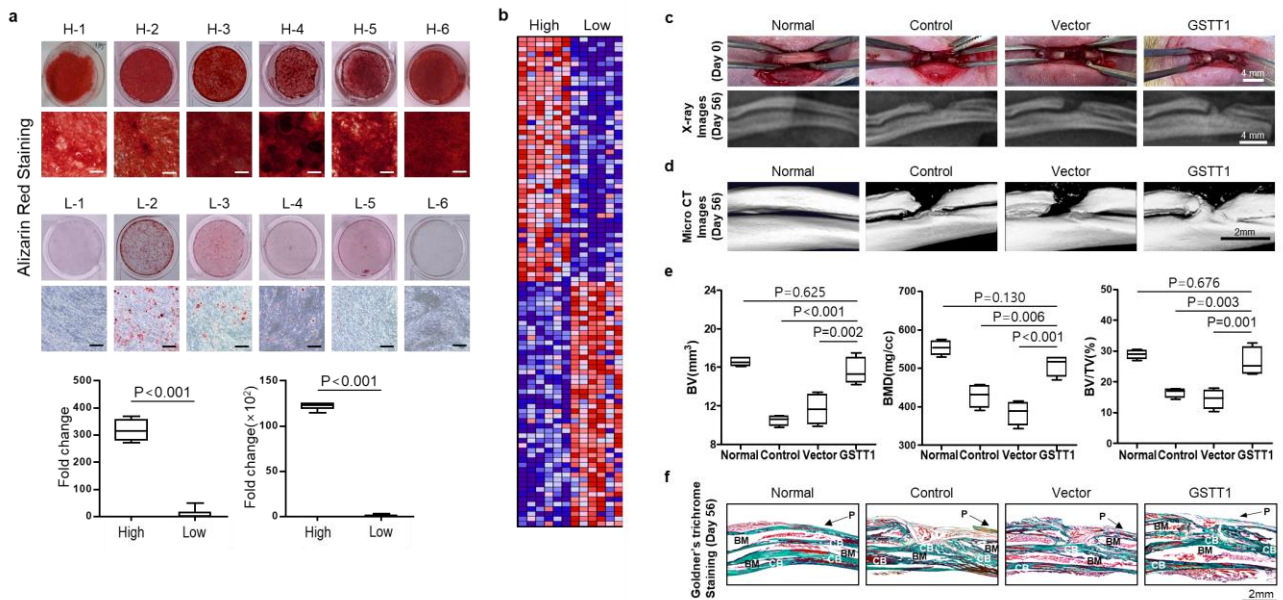
Figure. Identification of novel gene GSTT1 associated with enhanced osteogenic differentiation in hADSCs. These results showed that siGTT1 was successfully transfected and that such transfection inhibited GSTT1 expression in hADSCs, reducing osteogenic differentiation.