Suppression of PPARγ enhances osteogenic differentiation and bone formation

INTRODUCTION
Peroxisome proliferator-activated receptor gamma (PPARγ) is a transcription factor that plays a key role in adipocyte differentiation, insulin sensitivity, and immune responses. Many studies supposed that PPARγ is a switcher to regulate the fate of stem cell between adipose formulation and bone generation. In literature, human preadipocyte and fetal-femur-derived mesenchymal cells have been reported to inhibit adipocyte differentiation by treated with PPARγ siRNAs. Pluripotent adipose tissue-derived mesenchymal stem cells (ADSCs) provide numbers of advantages over bone marrow-derived mesenchymal stem cells (BMSCs) in bone repairing treatment. However, the role of PPARγ in modulating the balance between adipogenesis and osteogenesis in ADSCs is rare and the pathway has not been fully understood, especially human cells. Therefore, the purposes of this study were (1) to investigate the role of PPARγ in the adipogenic and osteogenic differentiation of human ADSCs (hADSCs); (2) to investigate the bone formation when treated PPARγ siRNAs modified hADSC in SD rat with femur bone defect.

METHODS
Human adipose tissue-derived mesenchymal stem cells (hADSCs) were used in this study. Small interfering RNAs (siRNAs) corresponding to target transcripts of the human PPARγ gene were transfected into hADSCs by using lipofectamine. After transfection, hADSCs were seeding into 6 well plates or 12 well plates for osteogenesis and gene expression test. The osteogenic marker genes such as BMP-2, Runx-2 and ALP were measures by real-time PCR. Mineralization was tested by Alizarin red S staining. For animal study, SD rats were operated a partial defect. After 3 weeks, the rats were scarified to evaluate the bone formation by micro-CT. Significant differences were tested by using ANOVA. The mean of different treatment groups was value < 0.05 was taken as significant.

RESULTS
By interfering transiently with PPARγ mRNA through small interfering RNA (siRNA), a 75% suppression of PPARγ gene expression was observed in hADSCs (Fig. 1). Upon the suppression of PPARγ gene, three of the well-established osteogenic genes, BMP-2, Runx2, and ALP, were up-regulated (Fig. 2). It lead to retardation of adipogenesis and stimulated higher level of mineralization (Fig. 3). In animal study, the bone repair in the group of siRNA-PPARγ hADSCs was better than the group of hADSC alone or control group without cells (Fig. 4).

Fig. 1. Change in PPARγ gene expression with time after PPARγ2-siRNA transfection. After transfected with 50 nM PPARγ2-siRNA for 48 h, the expression of PPARγ was decreased by analyzing real-time PCR (n=3, *p<0.05, **p<0.01 compared to control and mock).

Fig. 2. Change in osteogenic gene expression with time after PPARγ2-siRNA transfection. The osteogenic genes were increased after PPARγ2-siRNA transfection. (n=3, “*” p<0.05, “**” p<0.01, compared to control and mock).

Fig. 3. Effect of PPARγ suppression on the adipogenic and osteogenic differentiation and of hADSCs. The adipogenesis was decreased and osteogenesis was increased after PPARγ2-siRNA transfection. (n=3, “*” p<0.05, “**” p<0.01, compared to control and mock).

Fig. 4 The micro-CT image on rat femur bone defect repairing. The bone repair in the group of siRNA-PPARγ hADSCs was better than the group of hADSC alone or control group without cells

Discussion
Our study clearly indicates that bone formation increase upon the adipogenesis decrease from PPARγ suppression. Moreover, the results generated from this study highlights that the alteration of PPARγ-related pathways might be able to regulate bone homeostasis in human ADSCs. This investigation contributes not only an intensive understand of the path ways might be able to regulate bone homeostasis in human ADSCs. Besides, hADSCs pretreated with PPARγ siRNA would potentially offer better bone repairing function on local application, and cell delivered PPARγ siRNA would be possible to lead less systematic damage. Consequently, regulation of PPARγ-related pathways may shed light of new way for skeletal regeneration by promoting osteogenic differentiation of human ADSCs.