Role of human nuclear receptor NR2F1 on osteogenic differentiation of human bone marrow mesenchymal stem cell

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Abstract

Bone formation plays an important role in normal human development. This process is regulated by a multitude of factors that ensure proper bone remodeling and growth. Regulation of transcription factors play a central role in determining the differentiation pathway that stromal stem cell or mesenchymal stem cells would commit to in their differentiation. Signaling pathway studies have shown the association of different proteins to osteogenesis through microarray.

Understanding the role of these proteins in osteogenesis would help in implementing scientific and therapeutic utilization of MSCs in bone formation, One of these proteins is an orphan nuclear receptor NR2F1. Human nuclear receptors as part of gene expression pathways are involved variably in adipogenesis and osteogenesis. This study aims to demonstrate the role that this protein plays in the expression of osteogenic transcription factors.

Inhibiting the expression of NR2F1 in MSCs through siRNA transfection of cells grown in osteogenic media have shown decreased expression of osteogenic transcription factors by gene-expression analysis compared with cells grown in osteogenic medium alone.

qRT-PCR results and functional assays shows concordance with the predicted transcription factor association with NR2F1 expression. And outlining that NR2f1 plays an important role in osteogenesis.

Materials and Methods

A clone of a mesenchymal stem cells line, hMSC-TERT, was employed in this study. These cells were cultured in an enhanced DMEM medium .

siRNA-mediated transfection was employed to knockdown NR2F1 and was controlled with a negative control siRNA.

the transfected cells were cultured in osteoblast differentiation media for 7 days.

Cell viability and proliferation was measured consecutively along the the experiment on day 3, 5, and 7 using Alamar Blue assay.

Cytochemical staining techniques were used to measure osteogenesis. Methods used are Alizarin Red S staining, OsteoImage Mineralization assay and ALP quantification assay.

Quantitative Real-Time PCR was used to quantify the expression of target genes involved in osteogenesis by analyzing the cDNA of extracted RNA.

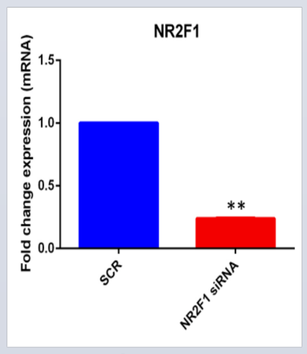

Figure 1

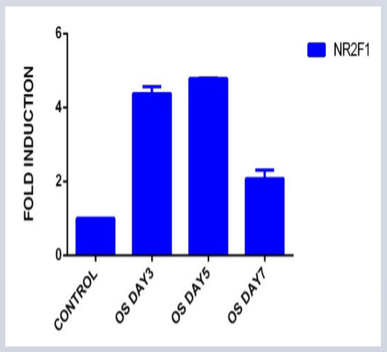

Figure 2

Results

hMSC-TERT cells were knocked down with NR2F1-siRNA. After 3 days of transfection, we validated the NR2F1-siRNA transfection efficiency with qRT-PCR and it exhibited more than 70% down-regulation of the NR2F1 gene. (Figure 1)

NR2F1-siRNA transfected cells showed a slight reduction in proliferation in Alamar Blue proliferation assay compared with control. (Figure 6)

Temporal expression of NR2F1 during osteogenesis shows gradually increase in expression and have a maximum expression at day 5 and then the expression decreases at day 7. (Figure 2)

Alizarin red staining of mineralized nodule formation in NR2F1 transfected cells after 7 days were less compared to control siRNA. (Figure 3) This is further enforced with ALPL quantification and OsteoImage assay. (Figure 4)

In addition, known osteoblast gene expression markers including RUNX2, ALPL, OSC, and BSP were all down-regulated. (Figure 7)

Figure 3

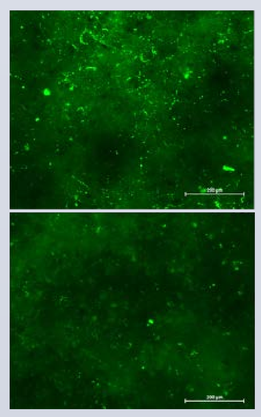

Figure 4

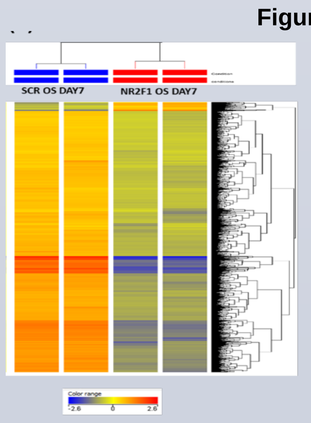

Figure 5

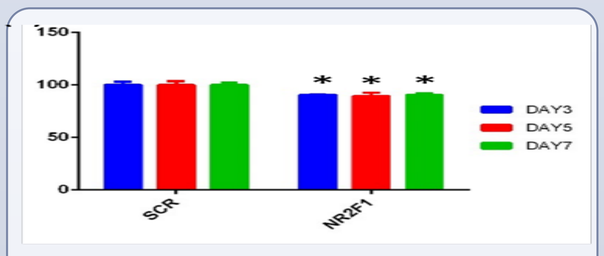

Figure 6

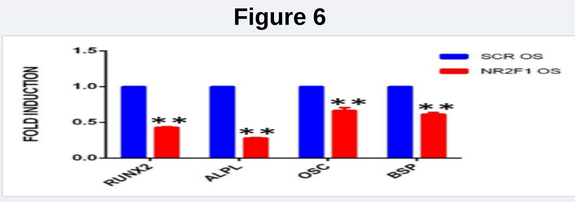

Figure 7

Conclusions

In conclusion, our study reported that NR2F1 mediated the down-regulation of osteoblast differentiation in hMSC-TERT by controlling signaling pathways that promote the expression of osteogenic genes.

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