NFI-C Regulates Osteoblast Differentiation via Control of Osterix Expression

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ABSTRACT

In bone marrow, bone marrow stromal cells (BMSCs) have the capacity to differentiate into osteoblasts and adipocytes. Age-related osteoporosis is associated with a reciprocal decrease of osteogenesis and an increase of adipogenesis in bone marrow. In this study, we demonstrate that disruption of nuclear factor C (NFI-C) impairs osteoblast differentiation and bone formation, and increases bone marrow adipocytes. Interestingly, NFI-C controls postnatal bone formation but does not influence prenatal transplantation of adipogenic cells from human osteoporotic patients. Notably, bone development. We also found decreased NFI-C expression in an age-related osteoporosis-like phenotype. Finally, NFI-C directly inhibits adipocyte differentiation by suppressing PPARγ expression in Nfic-/- mice showing an age-related osteoporosis-like phenotype. DISCLOSURE OF POTENTIAL CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

INTRODUCTION

Bone marrow stromal cells (BMSCs) have the capacity to differentiate into osteoblasts and adipocytes. Osteogenesis is regulated by several growth and transcription factors, such as transforming growth factor-β (TGF-β), bone morphogenetic proteins (BMPs), Wnt, Hedgehog, Runx2, Osterix, Osx, and β-catenin, whereas adipogenesis is controlled by peroxisome proliferator-activated receptor gamma (PPARγ). With aging, BMSCs become inclined to undergo differentiation into adipocytes, resulting in increased number of adipocytes and a decreased number of osteoblasts in bone marrow. However, the mechanism underlying this differentiation switch remains unknown. The nuclear factor I (NFI) family members of transcription factors are expressed from four highly conserved genes in mammals (named Nfia, Nfib, Nfic, and Nfix). All four NFI genes are expressed in human osteoblasts and osteoblast-like cell lines. In particular, Nfic is highly expressed in normal osteoblasts compared with other NFI family members. In addition, Nfic mice showed defects in alveolar bone formation in molar tooth sockets. However, the exact role of NFI-C in osteoblast differentiation and bone formation remains unclear. In the present study, we investigated the role of NFI-C in osteoblast differentiation and bone formation during osteogenesis.

METHODS

- Animals
- Nfic-/- mice were kindly provided by Dr. Richard M. Gronostajski.
- Micro-C T and Histomorphometric Analyses
- Western Blot Analyses
- RT-PCR and Real-time PCR Analyses
- Histology Analyses
- ChIP Analyses
- Gene-Expression Profiling
- Statistical Analyses

RESULTS

Figure 1. Nfic disruption impairs bone formation during postnatal osteogenesis. (A) Nfic expression was evaluated using real-time PCR analyses in BMSCs derived from aged mice. (B) Western blot analyses. (C) Representative micro-C T images of the mandible and (D) the distal femur. (E) 3D micro-C T images. (F) Micro-C T quantification. (G) T & K staining (a) and (h) versus Kossa staining (b & c). (H) Histomorphometric analyses. (J) Mineral apposition rates (MAR).

Figure 2. Nfic-deficiency increases bone marrow fat as seen in osteoporotic patients. (A) H&E staining. (B) Representative Oil Red O staining images (upper left panel) and quantification of oil red O staining (upper right panel). (C) H&E and (D) IHC staining from bone specimens of an osteoporotic patient. (E) Expression of Nfic-C mRNA was analyzed from gene expression dataset GSE35959 deposited in GEO. (F) Effect of H&E on Nfic expression in hBMCs.

Figure 3. Nfic accelerates osteoblast differentiation and suppresses adipocyte differentiation. (A) ALP staining and IHC staining. (B) Representative Oil Red O staining images (left panel) and quantification of oil red O staining (right panel). (D) P2y expression. (E) P2y promoter activity. (F) Representative micro-C T images and micro-C T quantification of the distal femurs in WT and Nfic mice transplanted with Nfic-expressing BMSCs or mock-infected BMSCs at 10 weeks of age. (G) Histological analyses. (H) H&E staining (left panel) and number of adipocytes (right panel).

Figure 4. Nfic expression in normal tooth. Abnormal alveolar bone formation in Nfic-deficient mice.

Figure 5. Nfic mediates BMP2-Ran2-induced Osterix expression. (A) Immunofluorescence staining of Osx (red). Western blot analyses. (B) Real-time PCR and western blot analyses. (C) Osx promoter activity (D) ChIP analyses. P1 primers: putative Osx-binding motif. P2 primers: negative control locus. (E) Nfic-/- osteoblasts were treated with BMP-2 (300 ng/ml) and/or transfected with the Nfic expression vector. Osx expression was analyzed using real-time PCR. S = * , P = **. (F) Representative micro-C T images and micro-C T quantification of the distal femurs in WT and Nfic mice transplanted with Osx-overexpressing BMSCs or mock-infected BMSCs at 10 weeks of age. Osx (H) Histological analyses. (I) T & K staining (left panel) and number of adipocytes.

Figure 6. Ran2 mediates BMP2-induced Nfic expression. (A) and (B) Nfic promoter activity and mRNA expression. (C) Western blot analyses. (D) Nfic promoter activity. (E) ChIP analyses. P1: representative Ran2-binding motif. P2: negative control locus. (F) Immunofluorescence staining of Nfic (Red) in femurs from E18.5 WT and Nfic-/- mice. Nfic expression was evaluated using real-time PCR and western blot analyses. (G) A model of role of Nfic during osteoblast and adipocyte differentiation.

SUMMARY & CONCLUSION

We found an age-related decrease in Nfic expression in BMSCs. Nfic mice show an age-related osteoporosis-like phenotype with decreased osteoblast differentiation and increased adipocyte differentiation. Nfic overexpression reduced adipocyte differentiation through suppression of PPARγ, but increased osteoblast differentiation in Nfic-/- BMSCs. Transplantation of Nfic-overexpressing BMSCs rescued an osteoporosis-like phenotype in Nfic-/- mice. Nfic directly regulates Osx expression through the BMP-2 signaling pathway during osteoblast differentiation. Ran2 acts upstream of Nfic and regulates Nfic expression through the BMP-2 signaling pathway. These findings suggest that Nfic is an important factor regulating the balance between osteoblast and adipocyte differentiation in BMSCs.

Taken together, our data suggest that Nfic is a new candidate gene that causes osteoporosis. Therefore, regulation of Nfic expression in BMSCs could be a novel therapeutic approach for treating osteoporosis.