

Inhibition of NFAT increases osteoblast differentiation by increasing Fra-2 expression

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Keywords: Osteoblasts, NFAT, AP-1, Fra-2, Calcineurin, Bone

Bone loss and osteoporosis are major public health problems in the elderly. With longer life expectancy in the USA, the number of people that will develop age-related bone loss and osteoporosis is expected to rise to over 61 million by 2020. Osteoblast differentiation is a crucial aspect of bone formation and remodeling, a process that is severely compromised in osteoporosis. Almost all the FDA-approved treatments for building healthier bones, excluding PTH, do not address the decrease in osteoblast differentiation that is seen in osteoporosis and are designed to target osteoclasts and bone resorption. The purpose of this study is to examine the effects of cyclosporine A (CsA) on osteoblast differentiation and elucidate its mechanism of action. CsA, an immunosuppressive agent, is known to cause osteopenia in post-transplantation patients as a result of inhibiting calcineurin and NFAT signaling. The reported effects of CsA on bone are contradictory both in human and animal studies with a common consensus that CsA causes a high turnover bone loss due to an increase in both osteoclast and osteoblast differentiation *in vivo*. Despite this important observation, almost all studies addressing the effects of CsA on bone have focused on the role of Cn/NFAT on osteoclasts.

Methods. The murine clonal osteogenic cell line MC3T3-E1, established from newborn mouse calvaria, was used for this study. Cells were treated with CsA or transfected with NFATc1 siRNA. At the end of the studies, cells were harvested for RNA, cytoplasmic and nuclear protein extractions. Real-time PCR, Western blotting and gel shifts were performed.

Results. MC3T3-E1 osteoblasts express three NFAT isoforms (c1, c2 and c3), and CsA treatment blocks the nuclear

translocation of NFATc1 and c3 and significantly decreases NFAT transactivation. CsA, in a dose-dependent manner, increases alkaline phosphatase (ALP) activity and mineralization. CsA also increases the gene expression of ALP and osteocalcin, markers of osteoblast differentiation. Furthermore, CsA increases osteoblast numbers (N.Ob/BS) by 31% and bone volume (BV/TV) by 18% in an *in vivo* mouse calvariae model (Figure 1).

To elucidate the mechanism by which CsA increases bone formation, we examined the expression of Fra-2, a transcription factor that is known to be associated with osteoblast differentiation. Here we show that CsA increases Fra-2 gene and protein expression as well as AP-1 DNA binding activity. Furthermore, the direct inhibition of NFATc1 by siRNA increases Fra-2 protein expression in osteoblasts.

Conclusion. We demonstrate that NFATc1 negatively regulates osteoblast differentiation by regulating Fra-2 expression. Findings from our studies provide the first documentation of a novel molecular mechanism that describes the role of NFATc1 in osteoblast differentiation. A basis will thereby be provided for the development of a new target for drug design and therapeutic intervention to combat osteoporosis.

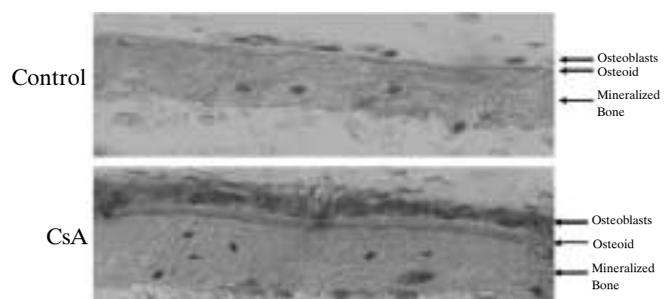


Figure 1. CsA increases osteoblast numbers and bone volume *in vivo*. DMSO (Control) or 1uM CsA dissolved in PBS were injected subcutaneously for 10 days into the calvarial region of 5-day-old mice. At the end of the study, mice were sacrificed, calvariae dissected and modified Goldner's TriChrome was performed.

The UAB Research Foundation (UABRF) is in the process of submitting intellectual property protection for certain novel aspects described in this manuscript.

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Accepted 31 July 2005