

OSTEOBLAST-STIMULATING FACTOR-1 ENHANCES OSTEOGENIC DIFFERENTIATION OF BONE MARROW STROMAL CELLS, BUT IS NOT OSTEOINDUCTIVE

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INTRODUCTION: Osteoblast-stimulating factor-1 (OSF-1), also referred to as pleiotrophin and heparin-binding growth-associated molecule, is a secreted, extracellular matrix-associated, 136-amino acid polypeptide. Although *osf-1* is expressed widely during fetal development, post natal expression is more restricted, the bone and brain being two sites with significantly high concentrations of OSF-1. The protein is synthesized by osteoblasts during early stages of osteogenic differentiation and deposited in the bone matrix ¹. The *osf-1* gene is expressed by primed osteoprogenitors from bone marrow. OSF-1 also functions as a chemoattractant for osteoprogenitors from human bone marrow ². The study examined i) whether OSF-1 influenced osteogenic differentiation of bone marrow-derived cells, ii) whether OSF-1 possessed the osteoinductive potential of Bone Morphogenetic Proteins (BMPs) and iii) the manner in which OSF-1 interacted with BMPs.

METHODS: Bone marrow-derived cells from BDF-1 mice (20-30-week-old) were cultured for 12 days in basal and osteogenic media, to which rhOSF-1 was added in concentrations ranging from ng/ml to pg/ml. Cultures to which 50 ng/ml rhBMP-2 was added served as a positive control. C2C12 cells (murine pluripotent premyoblastic cells) were cultured for 2-6 days with rhOSF-1 and rhBMP-2. At the end of the culture periods, osteogenic differentiation was determined by staining the cultures for alkaline phosphatase (ALP) and assaying for ALP activity, which was expressed per cell (ALP specific activity). Cell proliferation was determined by measuring the DNA content of cultures.

RESULTS: In cultures of mouse bone marrow cells, OSF-1 stimulated specific ALP activity at appreciably low (pg/ml) concentrations, while concentrations in the ng/ml range were ineffective in stimulating ALP. Although stimulation of ALP activity by BMP-2 was greater in comparison to that achieved by OSF-1, the stimulatory effect was observed in presence of ng/ml concentration of BMP-2 compared to the pg/ml concentration of OSF-1. A modest, but significant, mitogenic effect

of OSF-1 was observed at pg/ml concentrations. With respect to pluripotent C2C12 cells, OSF-1 was not osteoinductive as it failed to initiate osteogenic differentiation unlike BMP-2, which initiated osteogenic differentiation evident by the appearance of numerous ALP +ve cells. OSF-1 inhibited BMP-mediated osteoinduction at concentrations as low as 0.05 pg/ml, when present with BMP-2 during the initial osteoinductive phase of culture. However, when OSF-1 was added after initiation of osteogenic differentiation by BMP-2, osteogenic differentiation of BMP-primed cell populations was further enhanced in presence of pg/ml concentrations of OSF-1.

DISCUSSION & CONCLUSIONS: Thus, the effect of OSF-1 on osteogenic differentiation depended on the concentration and the timing at which it was added. The protein was not osteoinductive, it inhibited BMP-mediated osteoinduction during the initial osteoinductive phase, but stimulated osteogenic differentiation of BMP-primed cell populations/ late-stage osteoprogenitors from bone marrow, provided it was present at low concentrations.

REFERENCES: ¹ R.S. Tare, R.O.C. Oreffo, N. M. P. Clarke, H. I. Roach (2002) *Bone* 30 (3): 13S. ² X.B. Yang, H.I. Roach, N.M.P. Clarke, S.M. Howdle, K.M. Shakesheff, R.O.C. Oreffo (2001) *Journal of Bone and Mineral Research* 16 (6): 1179.

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