Induction of early growth response-1 gene by interleukin-1β and tumor necrosis factor-α in normal human bone marrow stromal and osteoblastic cells: regulation by a protein kinase C inhibitor

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Abstract

The early growth response-1 (Egr-1) gene has been identified as a nuclear transcriptional factor and implicated in the regulation of growth and differentiation of osteoblastic cells. In the present study, we investigated whether Egr-1 mRNA is expressed and induced by interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in normal human bone marrow stromal (HBMS) and osteoblastic (HOB) cells. Results demonstrate a very low basal expression of Egr-1 mRNA which is induced by IL-1β, and TNF-α in a time- and dose-dependent manner. Egr-1 mRNA induction was detectable within 15 min, reached maximal by 60 min and thereafter declined to basal levels by 120 min. Induction of Egr-1 mRNA by IL-1β and TNF-α was completely inhibited by H-7 suggesting the mediation of protein kinase C. The induction by IL-1β, and TNF-α of Egr-1 mRNA was independent of de novo protein synthesis since this induction was also observed in the presence of protein synthesis inhibitor cycloheximide. Fetal bovine serum and cycloheximide also independently induced the Egr-1 mRNA. Actinomycin D experiments demonstrated that Egr-1 mRNA is degraded very rapidly with a half-life of 30 min. Our results demonstrate the expression of Egr-1 gene and its induction by IL-1β, and TNF-α in normal human bone marrow stromal (osteoprogenitor) and osteoblastic cells in primary cultures. Data also reveal that the expression of Egr-1 gene is inhibited by protein kinase C inhibitor H-7 suggesting that the activation of protein kinase C or other protein kinases resulting in the phosphorylation of specific transcription factor(s) is the first immediate early step in the induction of immediate-early Egr-1 gene by IL-1β, and TNF-α. Results also suggest that Egr-1 is an important mediator of IL-1β and TNF-α action in normal human osteoblastic cells. (Mol Cell Biochem 156: 69–77, 1996)

Key words: human bone marrow stromal cells, osteoblasts, Egr-1, immediate-early gene, interleukin-1β, tumor necrosis factor-α

Introduction

Primary response or immediate-early genes (c-fos, fra-1, Fos-B; c-jun, junB and junD; Egr-1, 2, 3 and 4) are rapidly and transiently induced by mitogens and other stimuli in the absence of de novo protein synthesis and encode transcription factors which are likely mediators coupling early signaling events to long term changes in gene expression (reviewed in ref. [1]. The early growth response-1 (Egr-1) gene, also known as NGFI-A, Zif268, Krox24 and TIS8, was originally

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identified as a rapid early response gene to variety of growth factors and serum proteins [1-3]. Egr-1 gene encodes a 533 amino acids, 57 kDa phosphoprotein containing three putative DNA-binding zinc finger motifs of the Cys2-His2 subclass which bind to a specific DNA sequence CGCCCCCGC in a zinc dependent manner [1-3].

Interestingly, Egr-1 gene is expressed predominantly in bone and cartilage during mouse fetal development and the kinetics of its expression is very similar to another nuclear transcriptional factor c-fos [4]. Retinoic acid increases the expression of Egr-1 mRNA in primary cultures of fetal rat calvarial and SV-40 immortalized rat preosteoblastic (RCT-1) cells [5]. The promoters of several cytokines including insulin-like growth factor II (IGF-II) [6, 7], Egr-1 [8], platelet-derived growth factor-A (PDGF-A) chain [9], bone morphogenetic protein-2 (BMP-2) and BMP-4 [10] have Egr-1 binding motif suggesting that Egr-1 could regulate the expression of various growth factors/cytokines which also play key role in controlling bone metabolism. Cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are known to regulate the functions of both osteoblasts and osteoclasts [11-17], and have been shown to induce the expression of Egr-1 in human fibroblasts [18]. These observations suggest that Egr-1 may regulate normal skeleton development and bone metabolism and also point to more broader role of Egr-1 in signal transduction in diverse biological processes including bone remodeling.

Bone marrow stromal cells have long been considered as the source of osteoprogenitor cells [19, 20] and the renewal of the osteoblast (bone-forming cells) population at the bone surface occurs via differentiation of osteoprogenitor cells along the osteoblastic lineage [21, 22]. Results from our laboratory have also shown that glucocorticoids induce differentiation of human bone marrow stromal (HBMS) cells into cells which display an osteoblastic phenotype i.e. increased alkaline phosphatase activity, cAMP production in response to PTH, osteocalcin production in response to 1,25(OH)2D3, and bone matrix mineralization [23]. Since the expression and regulation of Egr-1 gene in normal human osteoblastic cells are not known, we examined the expression of Egr-1 mRNA and its induction by IL-1β and TNF-α in normal human bone marrow (osteoprogenitor) stromal (HBMS) and osteoblastic (HOB) cells in primary cultures. In this study, we demonstrate that IL-1β and TNF-α rapidly and transiently induce the expression of Egr-1 mRNA in HBMS and HOB cells and the induction is mediated through protein kinase signaling pathway(s).

Materials and methods

Materials

H-7 [1-(5-isouquinolinesulfonyl)-2-methylpiperazine, HC1], Denhardt's reagent, salmon sperm DNA, Dulbecco's phosphate-buffered saline (PBS), crude bacterial collagenase, trypsin-EDTA, Histopaque-1077, fetal bovine serum (FBS) and Dulbecco's modified Eagle medium (MEM): Ham's F-12 medium (1:1) were obtained from Sigma Chemical Company (St. Louis, MO.). Proteinase K was purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Human recombinant IL-1β and TNF-α were purchased from R&D Systems (Minneapolis, MN). Concentrated stock solution of IL-1β was made in PBS and required amounts of IL-1β were added directly to the medium.

Human bone marrow stromal cell culture

Human bone marrow stromal cells were isolated as described previously [23]. Briefly, bone marrow was harvested by gently flushing the marrow compartment with DMEM/F-12 medium containing heparin (10 units/ml) and DNase (1 μg/ml). Bone marrow cells were pelleted by centrifugation, re-suspended in 20 ml of α-MEM containing 10% FBS and subjected to Histopaque-1077 (Sigma) gradient. Marrow cells at the interface were harvested, washed three times with the medium and seeded in T-175 culture flasks at a density of 4 x 105 cells/cm2.

Human bone cell culture

Human bone cell culture

Human ribs obtained from surgery patients were transported to the laboratory in tissue culture flasks containing DMEM/F-12 medium and were processed immediately or after storage overnight in the refrigerator. The bone marrow stromal cells were isolated as described previously [23]. Briefly, bone marrow was harvested by gently flushing the marrow compartment with DMEM/F-12 medium containing heparin (10 units/ml) and DNase (1 μg/ml). Bone marrow cells were pelleted by centrifugation, re-suspended in 20 ml of α-MEM containing 10% FBS and subjected to Histopaque-1077 (Sigma) gradient. Marrow cells at the interface were harvested, washed three times with the medium and seeded in T-175 culture flasks at a density of 4 x 105 cells/cm2.