Available online at www.sciencedirect.com
ScienceDirect
Cellular Signalling 18 (2006) 2131–2142
CELLULAR SIGNALLING
www.elsevier.com/locate/cellsig

Signal pathways involved in the regulation of prostaglandin E synthase-1 in human gingival fibroblasts

Tülay Yucel-Lindberg *,[1], Tove Olsson [1], Tomomi Kawakami

Department of Pediatric Dentistry, Institute of Odontology, Karolinska Institutet, Box 4064, SE-141 04 Huddinge, Sweden

Received 21 February 2006; received in revised form 22 April 2006; accepted 25 April 2006
Available online 7 May 2006

Abstract

Microsomal prostaglandin E synthase-1 (mPGES-1) is the terminal enzyme regulating the synthesis of prostaglandin E_2 (PGE_2) in inflammatory conditions. In this study we investigated the regulation of mPGES-1 in gingival fibroblasts stimulated with the inflammatory mediators interleukin-1 β (IL-1β) and tumour necrosis factor α (TNFα). The results showed that IL-1β and TNFα induce the expression of mPGES-1 without inducing the expression of early growth response factor-1 (Egr-1). Treatment of the cells with the PLA_2 inhibitor 4-bromophenacyl bromide (BPB) decreased the cytokine-induced mPGES-1 expression accompanied by decreased PGE_2 production whereas the addition of arachidonic acid (AA) upregulated mPGES-1 expression and PGE_2 production. The protein kinase C (PKC) activator PMA did not upregulate the expression of mPGES-1 in contrast to COX-2 expression and PGE_2 production. In addition, inhibitors of PKC, tyrosine and p38 MAP kinase markedly decreased the cytokine-induced PGE_2 production but not mPGES-1 expression. Moreover, the prostaglandin metabolites PGE_2 and $PGF_{2\alpha}$ induced mPGES-1 expression as well as upregulated the cytokine-induced mPGES-1 expression indicating positive feedback regulation of mPGES-1 by prostaglandin metabolites. The peroxisome proliferator-activated receptor-γ (PPARγ) ligand, 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2), decreased mPGES-1 expression but not COX-2 expression or PGE_2 production. The results indicate that the inflammatory-induced mPGES-1 expression is regulated by PLA_2 and 15d-PGJ_2 but not by PKC, tyrosine kinase or p38 MAP kinase providing new insights into the regulation of mPGES-1.

Keywords: mPGES-1; PGE_2; PPARγ; $PGF_{2\alpha}$; PKC; p38 MAP kinase; Gingival fibroblasts; Inflammatory mediators

1. Introduction

The inflammatory mediator prostaglandin E_2 (PGE_2) has been implicated in diverse chronic inflammatory diseases including rheumatoid arthritis and periodontitis [1–5]. The biosynthesis of PGE_2 involves three different enzyme systems acting sequentially. The first group of enzymes, phospholipase A_2 (PLA_2) converts membrane lipids to arachidonic acid (AA) [6,7]. The second group of isoenzymes, consisting of the constitutively expressed cyclooxygenase-1 (COX-1), and the inducible cyclooxygenase-2 (COX-2), converts AA to prostaglandin H_2 (PGH_2) [8]. The intermediate prostaglandin PGH_2 is in turn metabolised to diverse prostaglandins including PGE_2, PGF_2, PGD_2, and PGI_2 by multiple enzymes [8,9]. The third group of isoenzymes, prostaglandin E synthase (PGE synthase), which is the terminal enzyme in the synthesis of PGE_2, catalyses the conversion of COX-derived PGH_2 to PGE_2 [10,11]. Recently, three different PGE synthases have been identified and cloned; the inducible, microsomal and glutathione-dependent PGE synthase (mPGES-1), the constitutively and widely expressed cytosolic PGE synthase (cPGES) and the later characterized microsomal and glutathione-independent PGE synthase (mPGES-2) [10,12–14].

The enzyme mPGES-1, which is the focus of this study, is induced by inflammatory stimuli such as LPS, interleukin-1β (IL-1β) and tumour necrosis factor α (TNFα) leading to increased PGE_2 synthesis [15–21]. The induction of mPGES-1, in response to IL-1 β and TNFα, has been reported in various cell types, including cultured gastric fibroblasts, synovial fibroblasts, cardiac fibroblasts, gastric cancer cell lines and gingival fibroblasts [16–21]. Furthermore, studies have suggested that mPGES-1 displays functional coupling with COX-2 to promote delayed PGE_2 synthesis and that mPGES-1 and COX-2 are co-induced by inflammatory stimuli in mouse macrophages and

* Corresponding author. Tel.: +46 8 524 88126; fax: +46 8 7743395.
E-mail address: Tulay.Lindberg@ofa.ki.se (T. Yucel-Lindberg).
[1] These authors contributed equally to this work.

0898-6568/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellsig.2006.04.003

osteoblasts, fibroblasts, chondrocytes and pulmonary A549 cells [22–25]. The role of mPGES-1 in inflammatory-induced PGE_2 has been studied using antisense oligonucleotides blocking mPGES-1 as well as mPGES-1 deficient mice [26–28]. For example in mPGES-1 null mice no augmentation of PGE_2 production was observed in response to LPS indicating that mPGES-1 is essential for LPS-induced PGE_2 production [26,28]. In addition, blocking mPGES-1 expression inhibits osteoclastogenesis and bone resorption in mouse osteoblasts stimulated by the cytokines IL-1β and TNFα [23].

Although the regulation of COX-2 and PGE_2 production has been intensively explored in various types of cells, the signal transduction pathways regulating the expression of mPGES-1 in relation to PGE_2 are not clear. Various signalling pathways, including PKC, p38 and ERK mitogen-activated protein (MAP) kinases, have been reported to be involved in mPGES-1 expression [29,30]. The mPGES-1 promoter contains regulatory elements including activating protein-1, glucocorticoid receptor (GR) and early growth response-1 (Egr-1) binding sites [31,32]. The transcription factors Egr-1 and nuclear factor kappaB (NFκB), have both been suggested to be involved in the regulation of mPGES-1 expression [25,33]. We have previously reported that the inflammatory mediators IL-1β and TNFα induce the expression of mPGES-1 in parallel with PGE_2 production in gingival fibroblasts and that the glucocorticoid dexamethasone inhibits the expression of mPGES-1 [16]. Gingival fibroblasts, the predominant cell type in gingival connective tissue, produce large amounts of PGE_2 and thereby contribute to the increased levels of PGE_2 found in gingival tissue and fluid from patients with periodontitis [4,5,34].

Given the importance of mPGES-1 as a potential therapeutic target for controlling PGE_2 production, it is important to study the regulation of mPGES-1 expression. Here, we investigated the regulation of mPGES-1 with special regards to PLA_2, PKC, tyrosine kinase, p38 MAP kinase, prostaglandin metabolites and the transcription factors Egr-1, GR and NF-κB.

2. Materials and methods

2.1. Fibroblast cultures

Human gingival fibroblasts were established from gingival biopsies obtained from 7 healthy patients, 7 to 12 years of age, with no clinical signs of periodontal disease. The protocol, including the collection of gingival biopsies, was approved by the Ethical Committee at the Huddinge University Hospital. Minced pieces of gingival tissue were explanted to 25 cm^2 Falcon tissue culture flasks containing 5 ml of Dulbecco's Modified Eagle Medium (DMEM; Invitrogen Life Technologies, Scotland, UK) supplemented with 4 mM L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin and 5% fetal calf serum (FCS; Invitrogen). The fibroblasts were obtained by trypsinisation of the primary outgrowth of cells and cultured at 37 °C in a humidified incubator gassed with 5% CO_2 in air and routinely passaged using 0.025% trypsin in phosphate-buffered saline (PBS) containing 0.02% EDTA (Invitrogen). Gingival fibroblasts used for the experiments were cultured in 175 cm^2 tissue culture flasks until reaching confluence and the experiments were performed between the 7th and 14th passage.

The fibroblasts (1.0×10^6) were seeded in Petri dishes (10-cm) in DMEM supplemented with penicillin (50 units/ml), streptomycin (50 μg/ml) and FCS (5%) and cultured at 37 °C for 24 h. The cell layers were rinsed two times with serum-free DMEM followed by the addition of 5.0 ml serum-free medium containing interleukin-1β (IL-1β; R&D Systems, Minneapolis, MN, USA) or tumour necrosis factor α TNFα; R&D Systems) in the presence or absence of substances phorbol-12-myristate-13-acetate (PMA), bisindolylmaleimide (BIS), PD 153035 hydrochloride (PD), SB 203580, arachidonic acid (AA), 4-bromophenacyl bromide (BPB), dexamethasone or the NF-KappaB inhibitor pyrrolidine dithiocarbamate (PDTC) from Sigma-Aldrich (St. Louis, MO, USA) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 15-deoxy-delta (12,14)-prostaglandin J_2 (15d-PGJ_2) from Cayman Chemical (Ann Arbor, MI, USA) at the concentrations indicated in the figure legends. At the end of the incubation period (indicated in the figure legends) the culture medium was collected for PGE_2 determination. The cells were washed twice with ice-cold PBS and were used for RNA or Western blot analysis.

2.2. RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

Gingival fibroblasts were seeded and grown as described above. The cells were treated with different test substances for 6 h and total RNA was isolated

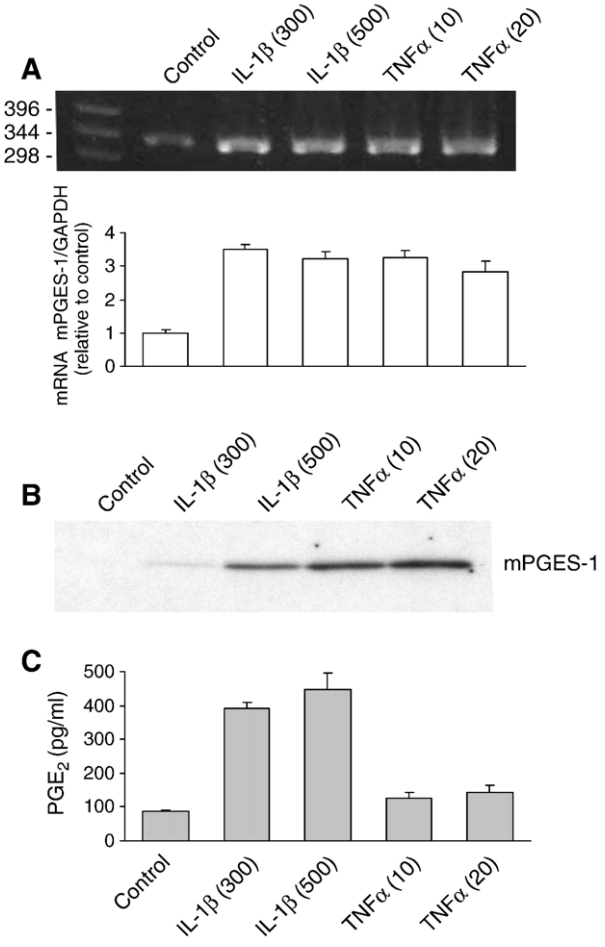

Fig. 1. Effects of IL-1β and TNFα on mRNA and protein expression of mPGES-1 and on PGE_2 production. (A) Gingival fibroblasts were stimulated with IL-1β (300, 500 pg/ml) or TNFα (10, 20 ng/ml) for 6 h and the mRNA expression of mPGES-1 was analysed by RT-PCR as described in Materials and methods. The graph shows semi-quantification of mPGES-1 mRNA expression in relation to GAPDH, expressed as relative to control cells. (B) The cells were stimulated with IL-1β (300, 500 pg/ml) or TNFα (10, 20 ng/ml) for 24 h, lysed and analysed by Western blot using mPGES-1 antibodies as described in Materials and methods. (C) Gingival fibroblasts were stimulated with IL-1β (300, 500 pg/ml) or TNFα (10, 20 ng/ml). After 24 h of incubation the medium was collected and the levels of PGE_2 were determined using RIA. The samples were analysed in triplicates and the results are expressed as mean ± SD. All results are representative of three separate experiments.