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Resveratrol inhibits BK-induced COX-2 transcription by suppressing acetylation of AP-1 and NF-κB in human rheumatoid arthritis synovial fibroblasts

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ABSTRACT

Bradykinin (BK) induces inflammation in rheumatoid arthritis (RA). Resveratrol is a potent activator of Sirt1 which could modulate inflammation through deacetylating histones of transcription factors. Here, we investigated the mechanisms underlying BK-induced COX-2 expression which is modulated by resveratrol/Sirt1 in human rheumatoid arthritis synovial fibroblasts (RASFs). We found that BK-induced COX-2 protein and mRNA expression associated with PGE_2 synthesis, and promoter activity was mediated through B2R receptors, which were attenuated by selective B2R antagonist Hoe140 or transfection with B2R siRNA. BK-induced responses were mediated through PKCμ, MAPKs, AP-1 and NF-κB which were inhibited by their respective inhibitors or siRNAs. Up-regulation of Sirt1 by resveratrol suppressed the BK-induced COX-2/PGE_2 production through inhibiting the interaction of AP-1 and NF-κB with COX-2 promoter in RASFs. BK-induced COX-2/PGE_2 expression is mediated through a B2R-PKCμ-dependent MAPKs, AP-1, and NF-κB cascade. Resveratrol inhibited the phosphorylation and acetylation of p65, c-Jun, and Fos and reduced the binding to the COX-2 promoter, thereby attenuated the COX-2 expression. Therefore, resveratrol may be a promising therapeutic intervention for treatment of inflammatory arthritis.

1. Introduction

The inflammatory milieu of the rheumatoid arthritis (RA) is a major consequence of immune responses, arising from the activation of RA synovial fibroblasts (RASFs) and complex interactions among kinin peptides and cytokines that lead to progressive destruction of joints [1–3]. Bradykinin (BK) has been recognized as a potent mediator of pain and swelling and initiates the release of cytokines from leukocytes in arthritis [4]. Elevated levels of BK have been reported in the patients with inflammatory joint diseases [5,6]. BK may interact with two types of G protein coupled receptors, termed as B1 and B2 [7]. B2 receptors (B2R) are most commonly found in various smooth muscles, whereas B1 receptors (B1R) are found in bone tissues [8]. Most of pathophysiological processes triggered by BK are mediated through activation of B2R which causes hydrolysis of phosphoinositide to generate inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 stimulates an increase in $[Ca^{2+}]_i$ and DAG activates PKC isoforms in different cell types [9–11]. It has been reported that activation of B2R by BK induces COX-2 expression in human gingival fibroblasts and airway smooth muscle cells [12–14]. However, the detailed mechanisms of BK-mediated postreceptor signaling pathways leading to COX-2 expression are not completely understood in human RASFs.

Activation of PKC has been implicated in the development of RA [15]. It has been shown that BK stimulates translocation of PKC isozymes, such as PKC-α, -δ, and -ζ, in several cell systems [10,16], and involved in the regulation of cellular functions. In human RASFs, BK stimulates activation of protein kinase Cδ leading to IL-6 secretion [10]. Activation of PKCε involves in COX-2 expression induced by BK in tracheal smooth muscle cells [12]. BK activates MAPKs such as ERK1/2, p38 MAPK and JNK1/2 which are downstream of PKCs and leading to the expression of various inflammatory genes including COX-2 in different cell types [17,18]. In addition, COX-2 promoter consists of various binding sites for the transcription factors such as NF-κB and AP-1 which

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are activated by MAPKs and leading to the gene expression in various cell types [18,19]. However, the roles of PKC isozyme(s), MAPKs, NF-κB, and AP-1 involved in the BK-induced COX-2 expression in human RASFs remain unclear.

The most potent Sirtuin 1 (Sirt1) activator has been discovered on the basis of a screen for small molecule activators of Sirt1 [20]. Sirt1, a nuclear enzyme of the class III histone deacetylases (HDACs) modulating gene expression, involves in the regulation of pathophysiological processes [21–23]. Several studies have shown that Sirt1 exhibits anti-inflammatory properties in chronic inflammatory diseases [24–26]. However, the effects of Sirt1 on arthritis are controversial. Activation of Sirt1 by resveratrol suppresses the TNF-α-induced inflammation in human osteoarthritic chondrocytes [27] and protects cartilage against osteoarthritis progression in an experimental rabbit inflammatory arthritis model [28]. Whereas overexpression of Sirt1 in RA synovial cells increases production of pro-inflammatory cytokines [29], inhibition of Sirt1 activities in synovial macrophages of RA patients is found to reduce the expression of inflammatory mediators [30]. Therefore, we made an attempt to investigate the role of Sirt1 in BK-induced COX-2/PGE_2 production in human RASFs through overexpression of Sirt1 by resveratrol.

2. Materials and methods

2.1. Reagents and antibodies

Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12 (F-12) medium, fetal bovine serum (FBS), and siRNAs encoded p42 (MAPK1-HSS108536), JNK2 (MAPK9-HSS108551), p38 (MAPK14-HSS102352), c-Jun (JUN-HSS105641), c-Fos (FOS-HSS103799), and p65 (Rela-HSS184266) were purchased from Invitrogen (Carlsbad, CA). Hybond C membrane and enhanced chemiluminescence (ECL) Western blotting detection system were obtained from GE Healthcare Biosciences (Buckinghamshire, UK). Phospho-(Thr^{202}/Tyr^{204})-ERK1/2 (#9101), phospho-(Thr^{180}/Tyr^{182})-p38 MAPK (#9211), phospho-(Thr^{183}/Tyr^{185})-JNK1/2 (#4668), phospho-PKCμ ($Ser^{744/748}$) (#2054), phospho-IκBα (#2859), and phospho-p65 (#3031) antibodies were from Cell Signaling (Danvers, MA). B1R (sc-293196), B2R (sc-136216), COX-2 (sc-19999), p42 (sc-154), JNK2 (sc-827), p38 (sc-33688), phospho-c-Jun (sc-822), c-Jun (sc-1694), IκBα (sc-847), p65 (sc-7151), β-actin (sc-47778), Gsα (sc-823), Lamin A (sc-20680), Ac-Histone H3 (sc-8655-R) and c-Fos (sc-52) antibodies were from Santa Cruz (Santa Cruz, CA). GAPDH antibody was from Biogenesis (Bournemouth, UK). All primary antibodies were diluted at 1:1000 in phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA). Hoe 140, Gö6976, U0126, SB202190, SP600125, Bay11-7082, and tanshinone IIA (TSIIA) were from Biomol (Plymouth Meeting, PA). BI113823 was from Boehringer Ingelheim Pharma KG (Biberach, Germany). Bicinchoninic acid (BCA) protein assay reagent was from Pierce (Rockford, IL). PGE_2 enzyme immunoassay kit was from Cayman Chem. (Ann Arbor, MI). BK, enzymes, siRNAs encoded PKCμ (SASI_Hs01_00239143), B1R (SASI_Hs01_00012974) and B2R (SASI_Hs01_00174697_AS), and other chemicals were from Sigma (St. Louis, MO). SDS-PAGE supplies were from MDBio Inc. (Taipei, Taiwan).

2.2. Isolation and culture of human synovial fibroblasts

This study was approved by the Institutional Review Board, Chang Gung Memorial Hospital. Human RASFs were isolated from synovial tissue obtained from patients with RA who underwent knee or hip surgery. Synovial strips were cut into small pieces and placed in 10-cm dishes. Osteoarthritis synovial fibroblasts (OASFs) were isolated from synovial tissue obtained from patients with OA who underwent knee or hip joint surgery. Synovial fibroblasts were obtained from 26 patients with RA who underwent knee or hip surgery. RASFs were isolated, cultured, and characterized as previously described [30]. Experiments were performed using cells from passages 3 to 6.

2.3. Preparation of cell extracts and Western blot analysis

RASFs were incubated with BK for the indicated time intervals. Samples were analyzed by Western blot, as previously described [31]. The immunoreactive bands were detected by ECL reagents and captured by a UVP BioSpectrum 500 Imaging System (Upland, CA). The image densitometry analysis was quantified by an UN-SCAN-IT gel software (Orem, UT).

2.4. Plasmid construction, transfection, and luciferase reporter gene assays

The upstream region (−483 to +37) of the human COX-2 promoter was cloned into the pGL3-basic vector containing the luciferase reporter system [32]. The point mutations in the NF-κB and AP-1 binding sites of the COX-2 promoter were generated by site-directed mutagenesis that splices by overlap extension. Introduction of two double point-mutation into the AP-1-binding site (AP-1 domain; ACAGTCA to ACAACCA) and NF-κB-binding site (NF-κB domain; GGGACTACCC to GGGAGGACCC) to generate pGL3-COX-2ΔAP-1 and pGL3-COX-2ΔNF-κB, respectively, PCR was amplified from COX-2-luc as previously described [32]. The underlined nucleotides indicate the positions of substituted bases. All plasmids were prepared by using QIAGEN plasmid DNA preparation kits. These constructs were transfected into RASFs by using a Lipofectamine 2000 reagent according to the manufacturer's instructions. After incubation with BK, cells were collected and disrupted by sonication in a lysis buffer (25 mM Tris, pH 7.8, 2 mM EDTA, 1% Triton X-100, and 10% glycerol). After centrifugation, aliquots of the supernatants were used to test the promoter activity by using a luciferase assay system (Promega, Madison, WI). Firefly luciferase activities were standardized for β-galactosidase activity.

2.5. Cell fractions preparation and analysis

RASFs were shifted to serum-free DMEM/F-12 medium for 24 h and incubated with 1 μM BK for the indicated time intervals. The membrane, cytosolic, and nuclear fractions were prepared as previously described [31]. Samples from these fractions were analyzed by Western blot using respective antibodies.

2.6. Transient transfection with siRNAs

RASFs (10^6 cells) were plated in 12-well culture plates for 24 h to about 80% confluence. Cells were washed once with PBS and 0.4 ml of serum-free DMEM/F-12 medium was added to each well. Transient transfection of siRNAs (100 nM) was performed by using Lipofectamine™ RNAiMAX reagent according to the manufacturer's instructions, as previously described [31].

2.7. Reverse transcription–polymerase chain reaction and qRT-PCR analyses

Total RNA was extracted from RASFs as previously described [31]. The cDNA obtained from 1 μg total RNA was used as a template for polymerase chain reaction (PCR) amplification. Oligonucleotide primers were designed on the basis of Genbank entries