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Research Article

Interleukin-4 up-regulates 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in human lung cancer cells

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ABSTRACT

IL-4, an anti-inflammatory cytokine, was found to stimulate 15-PGDH activity in A549 and other lung cancer cells. Increase in 15-PGDH activity was due to increased transcription and decreased protein turnover of 15-PGDH. MMP-9 was shown to result in decreased levels of 15-PGDH in A549 cells. IL-4 induced down-regulation of MMP-9 mRNA and up-regulation of TIMP-1, an inhibitor of MMP-9. These data suggest that IL-4-induced down-regulation of MMP-9 activity may contribute to up-regulation of 15-PGDH in A549 cells. The role of JAK-STAT6 in IL-4-induced 15-PGDH expression was examined by using inhibitors. Inhibitors of JAKs, kaempferol and JAK inhibitor I, attenuated IL-4-stimulated STAT6 phosphorylation and 15-PGDH activity in a comparable concentration-dependent manner. Furthermore, JAK inhibitor I blocked IL-4-induced down-regulation of MMP-9 mRNA and up-regulation of TIMP-1 but not IL-4-stimulated up-regulation of 15-PGDH mRNA. These results indicate that JAK-STAT6 participated in IL-4-induced up-regulation of 15-PGDH through inhibition of MMP-9-mediated degradation. The roles of other signaling kinases in IL-4-induced 15-PGDH expression were also examined by using various inhibitors. Inhibitors of various MAPKs, PI-3K and PKC attenuated significantly IL-4-stimulated 15-PGDH activity indicating that MAPKs, PI-3K/Akt and PKC pathways participated in IL-4-induced up-regulation of 15-PGDH. Our results indicate that IL-4 up-regulates 15-PGDH by increased gene transcription and decreased protein turnover and the up-regulation can be mediated by JAK-STAT6 as well as MAPKs, PI-3K/Akt and PKC pathways.

Introduction

Prostaglandins are biosynthesized from arachidonic acid through consecutive actions of cyclooxygenases (COXs) and various prostaglandin synthases. Two isoforms of COXs have been recognized. COX-1 is expressed constitutively, whereas COX-2 is highly inducible by neoplastic and inflammatory stimuli [1]. One of the ensuing synthases, microsomal prostaglandin E synthase (mPGES), catalyzes the synthesis of proliferative PGE_2 commonly found in epithelial cells [2]. PGE_2 and other prostaglandins are rapidly oxidized by initial oxidation of their 15-hydroxyl group catalyzed by NAD^+-linked 15-hydroxprostaglandin dehydrogenase (15-PGDH) [3]. Interestingly, COX-2 and mPGES were found to over-express in most human tumors and may act as oncogenes

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Abbreviations: IL-4, interleukin-4; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; NSAIDs, non-steroidal anti-inflammatory drugs; COXs, cyclooxygenases; mPGES, microsomal prostaglandin E synthase; MMPs, matrix metalloproteinases; MMP-9, matrix metalloproteinase-9; MMP-2, matrix metalloproteinase-2; TIMP-1, tissue inhibitor of metalloproteinases-1; IRS, insulin receptor substrates; PI-3K, phosphatidylinositol 3-kinase; ERKs, extracellular signal-regulated kinases; IL-4Rα, IL-4 receptor α chain; IL-13Rα1, IL-13 receptor α1 chain

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[4,5], whereas 15-PGDH was recently shown to under-express in several types of human tumors and may function as a tumor suppressor [6–9]. The consequence is that overly synthesized PGE_2 has met with reduced activity of oxidation and inactivation contributing to highly elevated level of PGE_2 and increased tumor growth. Accordingly, up-regulation of 15-PGDH expression which may facilitate catabolism of prostaglandins in tumors may provide a plausible mechanism to induce anti-neoplastic effects. A number of agents have been shown to up-regulate 15-PGDH expression in cancer cell lines. Androgens were first shown to induce 15-PGDH expression in human prostate cancer LNCaP cells [10]. Subsequently, indomethacin [11], dexamethasone [12], TGF-1β (8, 13), histone deacetylase inhibitors [13], calcitriol [14] and diclofenac [15] were later found to stimulate 15-PGDH expression in different human cancer cells. Many of these agents are potential cancer therapeutic agents which may exhibit anti-cancer activity, in part, through induction of 15-PGDH expression.

Interleukin-4 (IL-4) is a cytokine that regulates multiple biological processes, such as proliferation, differentiation and apoptosis in various cell types [16]. IL-4 exerts its function by activating two types of its receptor (IL-4R). The type I receptor is comprised of the JAK1-bound IL-4Rα and JAK3-bound common γ chain (γc). Many nonhematopoietic cells that do not express γc and JAK3 utilize the type II receptor in which IL-4Rα associates with JAK2-bound IL-13Rα1, a low affinity binding receptor for IL-13, or tyrosine kinase (TYK)2-bound IL-13Rα1 [16]. Apparently, IL-13 uses the type II receptor complex for cell signaling. Thus, IL-4 and IL-13 have overlapping biological functions in many cell types that express the type II receptor components. Binding of IL-4 to IL-4Rα induces JAK1-mediated phosphorylation of multiple tyrosine residues in the cytoplasmic region of IL-4Rα. This, in turn, activates two major downstream pathways: the JAK-STAT pathway that induces the expression of IL-4-responsive genes [17] and the insulin receptor substrates (IRS)-phosphatidylinositol 3-kinase (PI-3K) pathway that promotes the growth of target lymphocytes [18]. A potential third pathway that employs MAPK family members as signaling molecules is also recognized [19]. IL-4 is an anti-inflammatory cytokine since it has been shown to suppress pro-inflammatory IL-1β and tumor necrosis factor-α production and activities [20]. IL-4 acting as an anti-inflammatory cytokine is thought to be due to the down-regulation of IL-1β-induced expressions of COX-2 [21,22] and other pro-inflammatory enzymes, such as matrix metalloproteinases (MMPs) [23,24]. IL-4 and IL-13 are also able to induce tumor suppressive 15-lipoxygenase-1 expression in A549 cells [25] and in other mammalian cells [26–28].

Recently, we demonstrated that IL-1β- and TNF-α-induced up-regulation of COX-2 was accompanied by down-regulation of 15-PGDH in human lung cancer A549 cells [29]. We now report that IL-4 induces up-regulation of 15-PGDH expression in A549 cells as well as in other lung cancer cell lines.

Materials and methods

Materials

Culture medium, heat-inactivated fetal bovine serum (FBS) and restriction enzymes were from Invitrogen (Carlsbad, CA). IL-4 and IL-13 were from Peprotech Inc. (Rocky Hill, NJ). JAK inhibitor I was obtained from Calbiochem (San Diego, CA). Kaempferol, other biochemicals and chemicals were all obtained from Sigma-Aldrich (St. Louis, MO). Human pcDNA3-MMP9 expression plasmid was a kind gift from Dr. Rafael Fridman of the Wayne State University. Antibodies against human placental 15-PGDH were generated as described previously [10]. Rabbit anti-STAT6 (Phospho-Tyr^{641}) and Rabbit anti-STAT 6 (Ab-641) were from GenScript (Piscataway, NJ). Mouse monoclonal antibody specific to tissue inhibitors of metalloproteinase-1 (TIMP-1) and mouse monoclonal antibody against MMP-9 were from NeoMarkers (Fremont, CA). Mouse monoclonal antibody specific to pERK and rabbit polyclonal antibody specific to ERK were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The human lung cancer cell lines, A549, H358, H1435 and H460 were supplied by the American Type Culture Collection (Manassas, VA). ECL Western blotting detection system was supplied by the Amersham Pharmacia Biotech (Cardiff, UK).

Cell culture

A549 cells were cultured in RPMI1640 supplement with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 mg/L streptomycin and 100 U/mL of penicillin G at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Cells were plated in 12-well plates and grown for 24 h. Cells were starved for 24 h in a serum-free medium before stimulation. For inhibitor studies, cells were pretreated with the respective inhibitors at working concentrations for 30 min in serum-free medium prior to stimulation.

15-PGDH assay

15-PGDH was routinely assayed by measuring the transfer of tritium from 15(*S*)-[15-^{3}H]PGE_2 to glutamate by coupling 15-PGDH with glutamate dehydrogenase as described previously [30]. Briefly, the reaction mixture contained the following: NH_4Cl, 5 mM; α-keto-glutarate, 1 mM; NAD^+, 1 mM; 15(*S*)-[15-^{3}H]PGE_2, 1 nM, 30,000 c.p.m.; glutamate dehydrogenase, 100 μg; and crude enzyme extract in a final volume of 1 ml of 50 mM Tris–HCl, pH 7.5. The reaction as allowed to continue for 10 min at 37 °C and terminated by the addition of 0.3 ml of 10% aqueous charcoal suspension. The radioactivity in the supernatant after centrifugation (1,000×*g*, 5 min) was determined by liquid scintillation counting. Calculation of the amount of PGE2 oxidized was based on the assumption that no kinetic isotope effect was involved in the oxidation of 15(*S*)-hydroxyl group of 15(*S*)-[15-^{3}H] PGE_2 as a substrate.

Western blotting

Cells were cultured in 12-well plates to achieve approximately 80% confluence and then were starved in RPMI 1640 medium without FBS for 24 h. The culture medium was changed and cells were kept for 1 h before the addition of agonist and various inhibitors. After incubation, cells were harvested and lysed in lysis buffer (1% Nonidet P-40 in 150 mM NaCl, 50 mM Hepes, pH 7.4, 5 mM pyrophosphate, 1 mM sodium orthovanadate, 10 μg/mL aprotinin, 10 μg/mL leupetin, and 1 mM phenylmethyl sulfonyl fluoride) for 1 h on ice. Lysates were cleared by centrifugation at maximum speed on a benchtop centrifuge and then subjected to 12% SDS–PAGE. Proteins were then electrophoretically transferred onto