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Research Paper

15-Keto prostaglandin E_2 suppresses STAT3 signaling and inhibits breast cancer cell growth and progression

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ABSTRACT

Overproduction of prostaglandin E_2 (PGE_2) has been linked to enhanced tumor cell proliferation, invasiveness and metastasis as well as resistance to apoptosis. 15-Keto prostaglandin E_2 (15-keto PGE_2), a product formed from 15-hydroxyprostaglandin dehydrogenase-catalyzed oxidation of PGE_2, has recently been shown to have anti-inflammatory and anticarcinogenic activities. In this study, we observed that 15-keto PGE_2 suppressed the phosphorylation, dimerization and nuclear translocation of signal transducer and activator of transcription 3 (STAT3) in human mammary epithelial cells transfected with H-*ras* (MCF10A-*ras*). 15-Keto PGE_2 inhibited the migration and clonogenicity of MCF10A-*ras* cells. In addition, subcutaneous injection of 15-keto PGE_2 attenuated xenograft tumor growth and phosphorylation of STAT3 induced by breast cancer MDA-MB-231 cells. However, a non-electrophilic analogue, 13,14-dihydro-15-keto PGE_2 failed to inhibit STAT3 signaling and was unable to suppress the growth and transformation of MCF10A-*ras* cells. These findings suggest that the α,β-unsaturated carbonyl moiety of 15-keto PGE_2 is essential for its suppression of STAT3 signaling. We observed that the thiol reducing agent, dithiothreitol abrogated 15-keto PGE_2-induced STAT3 inactivation and disrupted the direct interaction between 15-keto PGE_2 and STAT3. Furthermore, a molecular docking analysis suggested that Cys251 and Cys259 residues of STAT3 could be preferential binding sites for this lipid mediator. Mass spectral analysis revealed the covalent modification of recombinant STAT3 by 15-keto PGE_2 at Cys259. Taken together, thiol modification of STAT3 by 15-keto PGE_2 inactivates STAT3 which may account for its suppression of breast cancer cell proliferation and progression.

1. Introduction

An inflammatory lipid mediator prostaglandin E_2 (PGE_2) is formed from arachidonic acid by cyclooxygenase-2 (COX-2) [1]. Overproduction of PGE_2 is constitutively elevated in various human malignancies, such as colon, gastric, lung and breast cancer [2–4]. COX-2-derived PGE_2 plays an important role in inflammation and cancer progression through modulation of several intracellular signaling pathways [5–7].

The intracellular level of PGE_2 is regulated not only by its biosynthesis but also by degradation. The key enzyme involved in catabolism of PGE_2 is 15-hydroxyprostaglandin dehydrogenase (15-PGDH). Expression of 15-PGDH is low in various cancers, including those of colon, stomach, bladder, and breast [8–11]. 15-PGDH knockout mice are susceptible to colon tumor induction [12]. Down regulation of 15-PGDH in breast cancer cells is associated with its gene silencing via hypermethylation of its promoter [11]. Overexpression and activity of 15-PGDH in various cancer cells have been shown to suppress their

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proliferation, invasiveness, metastatic potential, and growth [11,13,14]. Therefore, 15-PGDH is considered as a tumor suppressor [15].

15-Keto PGE_2, an oxidized metabolite of PGE_2 formed by 15-PGDH was initially considered biologically inactive. However, accumulating evidence supports that this prostaglandin modulates the diverse cellular signal transduction pathways. 15-Keto PGE_2 containing the α,β-unsaturated ketone has been known as a ligand of peroxisome proliferator receptor γ (PPARγ) [16]. In line with this notion, 15-keto PGE_2 inhibited the bacterial lipopolysaccharide (LPS)-induced cytokine production in Kupffer cells through activation of PPARγ [17]. Moreover, the mice treated by 15-keto PGE_2 were shown to be resistant to sepsis by LPS [18]. Lu et al. reported that 15-PGDH overexpression resulted in elevated formation of 15-keto PGE_2, whereas downregulation of 15-PGDH elevated the PGE_2 level in hepatocellular carcinoma cells [19]. In addition, 15-keto PGE_2 treatment increased p21 promoter activity via PPARγ activation in hepatocellular carcinoma cells [19]. 15-Keto PGE_2 is metabolized to 13,14-dihydro-15-keto PGE_2 by prostaglandin reductase 2 (PTGR2). Silencing of PTGR2 enhanced 15-keto PGE_2 accumulation and stimulated apoptosis through generation of reactive oxygen species in pancreatic cancer cells [20]. Thus, it is likely that the anti-inflammatory and other cytoprotective activites of 15-PGDH are mediated by its product, 15-keto PGE_2. However, whether 15-keto PGE_2 can inhibit the tumor growth and progression remains largely unknown.

Signal transducer and activator of transcription 3 (STAT3) is a major transcription factor regulating cellular processes involved in proliferation, development, inflammation and cell survival [21]. In response to extracellular stimuli, such as cytokines and growth factors, STAT3 is recruited from the cytosol to their receptors and activated by receptor-associated Janus kinases (JAKs) [22]. Phosphorylation at the tyrosine 705 (Y705) residue facilitates the formation of a STAT3 dimer that translocates to the nucleus. This leads to transcription of target genes responsible for cell cycle progression, such as *Cyclin D1* and *c-Myc*, as well as those involved in cell survival [23]. Aberrant overactivation of STAT3 is linked to tumorigenesis [24,25]. Notably, more than 40% of breast cancers exhibit constitutively activated STAT3 [26]. The increased phosphorylation of $STAT3^{Y705}$ is associated with the metastasis of breast cancer and upregulation of genes involved in cell proliferation in breast cancer tissues [27]. Therefore, targeting the abnormally activated STAT3 signaling has been considered as an important cancer therapeutic strategy [24,28,29].

In this study, we investigated whether 15-keto PGE_2 could inhibit STAT3 signaling and suppress the human breast cancer cell proliferation and tumor growth.

2. Materials and methods

2.1. Cell culture

MCF10A and MCF10A-*ras* cells were cultured in Dulbecco's Modified Eagle's Medium: Nutrient Mixture-F-12 (DMEM/F-12) supplemented with 5% heat-inactivated horse serum from Gibco (Grand Island, NY, USA), 10 μg/ml insulin, 100 ng/ml cholera toxin, 0.5 μg/ml hydrocortisone, 20 ng/ml human epidermal growth factor, 2 mmol/l L-glutamine and 100 units/ml penicillin/streptomycin. MDA-MB-231 and HeLa/P-STAT3-Luc cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) supplied from GenDEPOT (Barker, TX, USA) and 1% antibiotic-antimycotic mixture. PC3 cells were cultivated in RPMI 1640 containing 10% heat-inactivated FBS and 1% antibiotic-antimycotic mixture. These cell lines were grown at 37 °C in humidified atmosphere of 5% CO_2. MCF10A, MDA-MB-231 and PC3 cells were obtained from American Type Culture Collection (ATCC). The MCF10A-*ras* was kindly provided by Prof. Aree Moon of Duksung Women's University, Seoul, South Korea.

2.2. Chemicals and biological reagents

15-Keto PGE_2 (9,15-dioxo-11α-hydroxy-prosta-5Z,13E-dien-1-oic acid) and 13-14-dihydro-15-keto PGE_2 (9,15-dioxo-11α-hydroxy-prosta-5Z-en-1-oic acid) were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Dithiothreitol (DTT) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Primary antibodies for STAT3, P-$STAT3^{Y705}$, JAK2 and P-JAK2 were products of Cell Signaling Technology (Danvers, MA, USA). Antibodies against β-Actin and Lamin B were supplied from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 15-PGDH primary antibody was obtained from Cayman Chemicals. Anti-rabbit and anti-mouse horseradish peroxidase conjugated secondary antibodies were purchased from Zymed Laboratories Inc. (San Francisco, CA, USA). Human recombinant STAT3 protein (catalog number, ab43618) was obtained from Abcam, Cambridge, UK).

2.3. Biotinylation of 15-keto PGE_2

To a solution of 15-keto PGE_2 (4 mg) and *N*-(5-Aminopentyl) biotinamide trifluoroacetate salt (6 mg) in dry acetonitrile (1 ml) under Ar atmosphere was added *N*,*N*′-diisopropylcarbodiimide (4 μl) at room temperature. After completion of reaction monitored by thin-layer chromatography (TLC), the resulting mixture was concentrated under reduced pressure and purified by flash column chromatography (ethyl acetate: MeOH = 5 : 1). A white solid (3 mg): ^{1}H NMR (800 MHz, DMSO) δ 7.72 (ddd, J = 14.5, 11.5, 5.4 Hz, 2H), 6.41 (s, 1H), 6.35 (s, 1H), 5.38–5.22 (m, 1H), 5.30 (d, J = 5.7 Hz, 1H), 4.30 (dd, J = 7.4, 5.4 Hz, 1H), 4.13–4.11 (m, 1H), 4.03 (q, J = 7.1 Hz, 1H), 3.51–2.80 (m, 4H), 2.64–2.34 (m, 6H), 2.28–1.89 (m, 13H), 1.63–1.15 (m, 18H), 0.86 (t, J = 7.2 Hz, 3H); 13C NMR (200 MHz, DMSO) δ 214.14, 199.68, 171.75, 162.66, 146.79, 143.09, 131.19, 130.86, 72.25, 70.25, 69.91, 61.01, 60.22, 59.15, 55.77, 55.44, 55.40, 53.27, 52.89, 46.54, 38.30, 38.27, 38.17, 35.18, 34.88, 31.23, 30.83, 28.85, 28.70, 28.20, 28.01, 27.89, 26.32, 25.30, 25.21, 24.71, 23.80, 23.54, 23.40, 22.08, 21.93, 20.74, 13.82; LR-MS (ESI) Calculated for $C_{35}H_{57}N_4O_6S$ (M + H+) 661.4, Found 661.4.

2.4. Western blot analysis

2.4.1. Preparation of cell lysates

After treatment with 15-keto PGE_2 or 13,14-dihydro-15-keto PGE_2, MCF10A-*ras* cells were harvested at indicated time points. The cells were rinsed with cold phosphate-buffered saline (PBS) and then scraped in 1 ml PBS followed by centrifugation at 1700 *g* for 5 min at 4 °C. Whole cell lysates were prepared with 10× Cell Lysis Buffer purchased from Cell Signaling Technology (Danvers, MA, USA) diluted to 1× solution containing 1% of phenylmethylsulfonyl fluoride (PMSF). Cell pellets were resuspended and incubated for 1 h on ice followed by centrifugation at 18,000 *g* for 15 min. Supernatant was collected as whole cell lysates. For obtaining cytosolic and nuclear extracts, buffer A and buffer C were used, respectively. Pellets were resuspended in hypotonic buffer A [0.2% HEPES (pH 7.9), 0.01% $MgCl_2$, 0.07% KCl, 0.007% DTT and 0.003% PMSF] for 15 min on ice, and 0.1% Nonidet P-40 (NP-40) was added for 4 min. The mixture was then centrifuged at 6000 *g* for 5 min at 4 °C. The supernatant contained the cytosolic protein. The pellets were rinsed twice with hypotonic buffer A and resuspended again in hypertonic buffer C [0.4% HEPES (pH 7.9), 0.01% $MgCl_2$, 2.4% NaCl, 0.007% DTT, 0.003% PMSF, 0.005% EDTA and 20% glycerol]. After incubation for 1 h on ice, the mixtures were centrifuged at 18,000 *g* for 15 min at 4 °C. Supernatant was collected as nuclear extract.

2.4.2. Quantification of the protein concentration

The protein concentrations in whole cell lysates were determined by using the BCA protein assay kit supplied from ThermoFisher Scientific (Rockford, IL, USA). The protein concentrations in cytosolic and