Biochimica et Biophysica Acta 1853 (2015) 126–135

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Biphasic effects of luteolin on interleukin-1β-induced cyclooxygenase-2 expression in glioblastoma cells

Sylvie Lamy *[,1], Paula Liana Moldovan [1], Aroua Ben Saad, Borhane Annabi

Laboratoire d'Oncologie Moléculaire, Centre de Recherche BioMed, Université du Québec à Montréal, C.P. 8888, Succ. Centre-ville, Montréal, Québec H3C 3P8, Canada

ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 10 October 2014
Accepted 13 October 2014
Available online 18 October 2014

Keywords:
COX-2
Inflammation
Interleukin-1
Luteolin
NF-κB
Glioblastoma

ABSTRACT

Success in developing therapeutic approaches to target brain tumor-associated inflammation in patients has been limited. Given that the inflammatory microenvironment is a hallmark signature of solid tumor development, anti-inflammatory targeting strategies have been envisioned as preventing glioblastoma initiation or progression. Consumption of foods from plant origin is associated with reduced risk of developing cancers, a chemopreventive effect that is, in part, attributed to their high content of phytochemicals with potent anti-inflammatory properties. We explored whether luteolin, a common flavonoid in many types of plants, may inhibit interleukin (IL)-1β function induction of the inflammation biomarker cyclooxygenase (COX)-2. We found that IL-1β triggered COX-2 expression in U-87 glioblastoma cells and synergized with luteolin to potentiate or inhibit that induction in a biphasic manner. Luteolin pretreatment of cells inhibited IL-1β-mediated phosphorylation of inhibitor of κB, nuclear transcription factor-κB (NF-κB) p65, extracellular signal-regulated kinase-1/2, and c-Jun amino-terminal kinase in a concentration-dependent manner. Luteolin also inhibited AKT phosphorylation and survivin expression, while it triggered both caspase-3 cleavage and expression of glucose-regulated protein 78. These effects were all potentiated by IL-1β, in part through increased nuclear translocation of NF-κB p65. Finally, luteolin was able to reduce IL-1 receptor gene expression, and treatment with IL-1 receptor antagonist or gene silencing of IL-1 receptor prevented IL-1β/luteolin-induced COX-2 expression. Our results document a novel adaptive cellular response to luteolin, which triggers anti-survival and anti-inflammatory mechanisms that contribute to the chemopreventive properties of this diet-derived molecule.

1. Introduction

Glioblastoma multiforme is the most common glioma and is a highly malignant primary brain tumor, associated with a poor survival rate [1]. This glioma is known for its invasiveness and high resistance to standard treatments of chemotherapy and radiotherapy [2,3]. The tumor microenvironment regulates glioblastoma development and progression and, among the pool of inflammatory cytokines, elevated levels of interleukin (IL)-1β are believed to contribute to glioblastoma cell proliferation and invasion [4–6]. Thus, blocking the pro-inflammatory activity of IL-1β would be useful in controlling the invasiveness of glioblastoma cells.

Cyclooxygenases (COXs) catalyze the synthesis of prostaglandins from arachidonic acid and include the constitutive isoform COX-1, the inducible isoform COX-2 and a splice variant COX-3 [7]. COX-2, which is regulated by various growth factors and cytokines such as IL-1β [8], is a crucial target for the control of tumors associated with chronic inflammation [9]. Increased COX-2 has been detected in a variety of human malignant tumors including glioblastomas, in which its expression correlated with the histopathological grade of gliomas [10]. High levels of COX-2 have been correlated with higher proliferation rates of tumors and shorter patient survival times [11]. There is also clear evidence of the positive effects of COX-2 inhibitors, including nonsteroidal anti-inflammatory drugs, against a variety of tumors, which exert chemopreventive activities [12].

The nuclear transcription factor-κB (NF-κB) is one of the major transcription factors associated with cancer development [13]. In response to different stimuli, mainly cytokines, glioblastoma cells exhibit elevated levels of NF-κB activity which contribute significantly to tumor progression [14]. Moreover, it was reported that NF-κB

Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; COX-2, cyclooxygenase-2; IκB, inhibitor of κB; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GRP78, glucose-regulated protein 78; IL-1β, interleukin-1β; IL-1Ra, interleukin-1 receptor antagonist; IL-1R1, interleukin-1 receptor type I; JNK, c-Jun amino-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear transcription factor-κB; PMA, phorbol 12-myristate 13-acetate

* Corresponding author at: Laboratoire d'Oncologie Moléculaire, Université du Québec à Montréal, C.P. 8888, Succ. Centre-ville, Montréal, Québec H3C 3P8, Canada. Tel.: +1 514 987 3000x0317; fax: +1 514 987 0246.

E-mail addresses: lamy.sylvie@uqam.ca (S. Lamy), moldovan.paula_liana@courrier.uqam.ca (P.L. Moldovan), ben_saad.aroua@courrier.uqam.ca (A. Ben Saad), annabi.borhane@uqam.ca (B. Annabi).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbamcr.2014.10.010
0167-4889/

activation is a crucial step in the induction of COX-2 in the brain in response to IL-1β [15]. Binding of IL-1β to its type 1 receptor (IL-1R1) activates a cascade of phosphorylation that results in NF-κB activation [16]. NF-κB is a protein complex consisting of a 65-kDa DNA binding subunit (*Rel* A) and an associated 50-kDa protein which, in the classical NF-κB pathway, is maintained in the cytoplasm in an inactive state by a protein complex called inhibitor of κB (IκB) [17]. Once phosphorylated by the IκB kinases (IKKs), IκB then allows the p65 and p50 subunits of NF-κB to translocate to the nucleus and to bind to the κB recognition sites located in the promoter regions of various NF-κB-regulated genes such as COX-2 [18]. Deregulation of the NF-κB signaling pathway contributes to enhanced glioblastoma cell survival, proliferation, cell cycle progression and chemoresistance; it therefore represents an attractive therapeutic target [19–21].

Numerous studies have linked abundant consumption of foods from plant origins with decreased risk of developing various cancers [22], a chemopreventive effect that is related to the high content of several phytochemicals with potent anticancer and anti-inflammatory properties [23]. Among these, luteolin (3′,4′,5,7-tetrahydroxyflavone), a flavonoid found at high levels in common fruits, vegetables and herbs such as green peppers, olive oil, parsley, celery, thyme, broccoli, cabbages, and chamomile tea [24,25], possesses a variety of neuroprotective [26], anticancer [24] and anti-inflammatory [27] properties. For instance, luteolin was demonstrated to antagonize phorbol 12-myristate 13-acetate (PMA)-induced COX-2 in human brain endothelial cells [28] and to inhibit tumor necrosis factor (TNF)-α-induced COX-2 expression by down-regulating the transactivation of NF-κB and activator protein-1 (AP-1) in JB6 mouse epidermis cells [29]. However, the effect of luteolin on the pro-inflammatory cytokine IL-1β-mediated induction of COX-2 has never been investigated. Here, we report for the first time anti-IL-1β effects of luteolin on the NF-κB-mediated transcriptional regulation of COX-2 expression in U-87 glioblastoma cells.

2. Materials and methods

2.1. Materials

Luteolin (purity ≥ 99%) was purchased from Extrasynthese (Lyon, France). Human recombinant IL-1β and human recombinant IL-1Ra were obtained from R&D Systems (Minneapolis, MN). The MEK inhibitor U0126 and the JNK inhibitor SP60012 were from Calbiochem (La Jolla, CA). Electrophoresis reagents were purchased from Bio-Rad (Mississauga, ON). The anti-ERK (extracellular signal-regulated kinase 1 and 2) (K-23) polyclonal antibody was from Santa Cruz Biotechnologies (Santa Cruz, CA). The monoclonal antibody against GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was from Advanced Immunochemical Inc. (Long Beach, CA). The Alexa Fluor® 488 donkey anti-rabbit IgG antibody was purchased from Invitrogen (Carlsbad, NM). Antibodies for AKT, Bip/GRP78 (glucose-regulated protein 78), caspase-3, cleaved-caspase-3, IκB, lamin A/C, NF-κB p65, SAPK/JNK (c-Jun amino-terminal kinase), survivin, phospho-AKT, phospho-NF-κB p65, phospho-SAPK/JNK polyclonal antibodies and phospho-ERK and phospho-IκB monoclonal antibodies were from Cell Signaling Technology (Beverly, MA). The anti-COX-2 monoclonal antibody was from BD Transduction Laboratories™ (Franklin Lakes, NJ). Anti-mouse and anti-rabbit horseradish peroxidase (HRP)-linked secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA) and enhanced chemiluminescence (ECL) reagents were from Denville Scientific Inc. (Metuchen, NJ). Micro bicinchoninic acid protein assay reagents were from Thermo Scientific (Rockford, IL). All other reagents were from Sigma-Aldrich (Oakville, ON).

2.2. Cell culture

A human glioblastoma cell line (U-87 MG) was purchased from the American Tissue Culture Collection (HTB-14™) and maintained in modified Eagle's Minimum Essential Medium (Wisent, 320-036-CL) containing 10% calf serum (HyClone Laboratories, SH30541.03), 1 mM sodium pyruvate (Sigma-Aldrich, P2256), 2 mM L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin (Wisent, 450-202-EL). Cells were cultured at 37 °C under a humidified 95%–5% (v/v) mixture of air and CO_2. Cells were treated with vehicle (0.1% DMSO) or with luteolin and stimulated with IL-1β. All cellular assays were conducted at 85% confluence.

2.3. Western blot analysis

U-87 MG cells were serum-starved in the presence of luteolin, U0126 (10 μM) or SP60012 (10 μM) for 24 h. To study the effects of these molecules on protein expression of COX-2, caspase-3, cleaved caspase-3 or survivin, cells were co-treated with one of these molecules and 50 ng/mL IL-1β or 1 μM PMA for 24 h. To study the phosphorylation status of AKT, IκB, NF-κB p65, ERK and JNK, IL-1β was added to the cells for 5 min after the treatment with luteolin for 24 h. Cells were then washed once with ice-cold phosphate-buffered saline (PBS) containing 1 mM each of NaF and Na_3VO_4 and were incubated in the same buffer solution for 30 min at 4 °C. The cells were solubilized on ice in lysis buffer [150 mM NaCl, 10 mM Tris–HCl, pH 7.4, 1 mM EDTA, 1 mM ethyleneglycol-O, O′-bis(2-aminoethyl)-N,N,N′,N′-tetraacetic acid (EGTA), 0.5% (vol/vol) Nonidet P-40 and 1% (vol/vol) Triton X-100]. To study the translocation of NF-κB p65 into the nucleus, nuclear protein extracts from U-87 MG cells were isolated using the NE-PER Nuclear and cytoplasmic extraction kit (Pierce, Rockford, IL). The resulting lysates or nuclear extracts (25 μg protein) were solubilized in Laemmli sample buffer [125 mM Tris–HCl (pH 6.8), 20% glycerol, 4% SDS, 10% β-mercaptoethanol, and 0.00125% bromophenol blue], boiled for 4 min, and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, proteins were transferred to polyvinylidene difluoride (PVDF) membranes which were then blocked 1 h at 4 °C with 5% nonfat dry milk in Tris-buffered saline/Tween 20 (TBS-T; 147 mM NaCl, 20 mM Tris–HCl, pH 7.5, and 0.1% Tween 20). Membranes were further washed in TBS-T and incubated overnight with the primary antibody in TBS-T containing 3% bovine serum albumin (BSA) and 0.01% sodium azide (NaN_3), followed by a 1 h incubation with HRP-conjugated anti-mouse or anti-rabbit antibodies in TBS-T containing 5% nonfat dry milk. Immunoreactive material was visualized with an ECL detection system. The immunoreactive bands were quantified with ImageJ software (NIH).

2.4. Total RNA isolation, cDNA synthesis and real-time quantitative PCR

Total RNA was extracted from U-87 MG monolayers using TRIzol reagent (Life Technologies, Gaithersburg, MD). For cDNA synthesis, 1 μg of total RNA was reverse-transcribed into cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). cDNA was stored at −80 °C prior to PCR. Gene expression was quantified by real-time quantitative PCR using iQ SYBR Green Supermix (BIO-RAD, Hercules, CA). DNA amplification was carried out using an Icycler iQ5 (BIO-RAD, Hercules, CA) and product detection was performed by measuring the binding of the fluorescent dye SYBR Green I to double-stranded DNA. The following primer sets were provided by QIAGEN (Valencia, CA): COX-2 (QT00040586), IL-1R1 (QT00081263), NF-κB p50 (QT00154091), NF-κB p65 (QT00149415), β-Actin (QT01680476), GAPDH (QT00079247), and PPIA (peptidylpropyl isomerase A; QT01866137). The relative quantities of target gene mRNA against an internal control, β-Actin/GAPDH/PPIA RNA, were measured by following a ΔCt method employing an amplification plot (fluorescence signal vs. cycle number). The difference (ΔCt) between the mean values in the triplicate samples of target gene and those of β-Actin/GAPDH/PPIA RNA was calculated by iQ5 Optical System Software version 2.0 (BIO-RAD, Hercules, CA) and the relative quantified value (RQV) was expressed as $2^{-\Delta Ct}$. Semi-quantitative PCR was performed to examine