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Quercetin-3-*O*-glucuronide inhibits noradrenaline-promoted invasion of MDA-MB-231 human breast cancer cells by blocking β_2-adrenergic signaling

Shunsuke Yamazaki [a], Noriyuki Miyoshi [b], Kyuichi Kawabata [c], Michiko Yasuda [b], Kayoko Shimoi [a,b,*]

[a] Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[b] Graduate School of Integrated Pharmaceutical and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[c] Department of Bioscience, Fukui Prefectural University, 4-1-1 Matsuoka Kenjojima, Eiheiji-cho, Yoshida-gun, Fukui 910-1195, Japan

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ABSTRACT

Endogenous catecholamines such as adrenaline (A) and noradrenaline (NA) are released from the adrenal gland and sympathetic nervous system during exposure to stress. The adrenergic system plays a central role in stress signaling, and excessive stress was found to be associated with increased production of reactive oxygen species (ROS). Overproduction of ROS induces oxidative damage in tissues and causes the development of diseases such as cancer. In this study, we investigated the effects of quercetin-3-*O*-glucuronide (Q3G), a circulating metabolite of quercetin, which is a type of natural flavonoid, on the catecholamine-induced β_2-adrenergic receptor (β_2-AR)-mediated response in MDA-MB-231 human breast cancer cells expressing β_2-AR. Treatment with A or NA at concentrations above 1 μM generated significant levels of ROS, and NA treatment induced the gene expression of heme oxygenase-1 (*HMOX1*), and matrix metalloproteinase-2 (*MMP-2*) and -9 (*MMP9*). Inhibitors of p38 MAP kinase (SB203580), cAMP-dependent protein kinase (PKA) (H-89), activator protein-1 (AP-1) transcription factor (SR11302), and NF-κB and AP-1 (Tanshinone IIA) decreased *MMP2* and *MMP9* gene expression. NA also enhanced cAMP induction, RAS activation and phosphorylation of ERK1/2. These results suggested that the cAMP-PKA, MAPK, and ROS-NF-κB pathways are involved in β_2-AR signaling. Treatment with 0.1 μM Q3G suppressed ROS generation, cAMP and RAS activation, phosphorylation of ERK1/2 and the expression of *HMOX1*, *MMP2*, and *MMP9* genes. Furthermore, Q3G (0.1 μM) suppressed invasion of MDA-MB-231 breast cancer cells and MMP-9 induction, and inhibited the binding of [^{3}H]-NA to β_2-AR. These results suggest that Q3G may function to suppress invasion of breast cancer cells by controlling β_2-adrenergic signaling, and may be a dietary chemopreventive factor for stress-related breast cancer.

Introduction

A key component of the stress response involves the activation of the sympathetic nervous system (SNS) and production of mediators such as the noradrenaline (NA)[1] and adrenaline (A) catecholamines. A and NA are both released from the adrenal medulla, and NA is also released from the SNS [1]. Basal circulating levels of NA range from 10 pmol/L to 1 nmol/L, but increase to 100 nmol/L under stress [2]. Similarly, circulating levels of A range from 1 to 10 pmol/L, and increase to 10 nmol/L under stress [2], while the content of NA in organs such as the heart, spleen and some blood vessels was previously reported to be particularly high (5–50 nmol/g of tissue) [3]. Animal research has shown that stress can increase levels of intratumoral NA [4], as well as NA in the ovary and in organs that are typical metastatic sites for ovarian cancer such as the spleen and omentum [5].

Clinical and epidemiological studies previously demonstrated positive associations between stress and cancer progression [6–8]. Epidemiological and animal studies indicated that daily exposure to stress promotes the development of breast cancer [9–11], while oxidative stress that arises after sustained reactive oxygen species (ROS) production was found to be associated with cancer progression [12]. In addition, the overproduction of ROS and reactive nitrogen species can cause oxidative damage to lipids,

* Corresponding author at: Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan. Fax: +81 54 264 5787.
E-mail address: shimoi@u-shizuoka-ken.ac.jp (K. Shimoi).

[1] *Abbreviations used:* NA, noradrenaline; A, adrenaline; Q3G, quercetin-3-*O*-glucuronide; ROS, reactive oxygen species; β_2-AR, β_2-adrenergic receptor; HO-1, heme oxygenase-1; MMP, matrix metalloproteinase; MAPK; mitogen-activated protein kinase.

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proteins, and DNA. A previous study showed that post-weaning social isolation augmented ROS levels in *Ppp1r2*-Cre/fGluN1 knockout mice, which displayed schizophrenia-like behavioral features [13]. Moreover, Nishio et al. demonstrated that oxidative DNA damage is increased in the peripheral blood cells of BALB/c mice exposed to isolation stress [14], and Miyashita et al. reported that urinary excretion of biopyrrins, bilirubin oxidative metabolites, is increased in mice exposed to isolation or confrontation stress [15]. These results together suggest that social stress generates ROS, which increases oxidative damage in mice [14,15]. ROS was also found to participate in the activation of nuclear factor κB (NF-κB), a redox-sensitive transcription factor that is a crucial player in tumorigenesis, and excess ROS produced by cancer cells triggers tumor invasion and angiogenesis *via* NF-κB-mediated matrix metalloproteinase (MMP)-9 induction [16,17]. Moreover, ROS scavenging and/or inhibition of NF-κB activation leads to the suppression of MMP-9 activity and further tumor invasion [18].

Activation of MMPs in tumor invasion and metastasis *via* degradation of the matrix surrounding tumors has been reported [19–21]. The gelatin-degrading MMPs, including MMP-2 and MMP-9, are known to play an important role in cancer cell invasion and metastasis *via* the degradation of type IV collagen, a major component of the basement membrane [22].

β-Adrenergic receptor (β-AR) is composed of three subtypes, β_1, β_2 and β_3. They are G-protein-coupled receptors (GPCRs) that activate adenylate cyclase to elevate intracellular 3′,5′-cyclic adenosine monophosphate (cAMP). β-Adrenergic signaling was previously shown to enhance biological processes involved in cancer progression including angiogenesis, invasion, and metastasis [4,5,23–25]. Moreover, NA and A stimulate the production of pro-invasive molecules such as MMP-2 and -9 from ovarian and other cancer cells, thus increasing the invasive and metastatic potential of these cells [5,24,26–29]. β-Adrenergic signaling also promotes ovarian cancer cell survival by inhibiting anoikis, the normal process of apoptosis that occurs when cells are separated from the extracellular matrix [24]. β_2-AR was found to be present in both IBH and MDA-MB-231 human breast cancer cell lines [30].

We recently reviewed the role of flavonoids in breast cancer prevention [31]. Flavonoids interfere with cancer processes such as proliferation, invasion, and metastasis, and epidemiological and experimental studies performed in animals and cell cultures suggested an inverse association between a higher intake of flavonoids and breast cancer risk. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a typical antioxidative dietary flavonoid that is ubiquitous in many plant foods such as buckwheat, onion, apples, tea, and red wine, mainly in the form of a glycoside [32,33]. As one of the most potent known ROS scavengers of the flavonoid family [34], quercetin ameliorates ROS-mediated cellular damage [35], and likely contributes to protection against various cancers. Recently, quercetin has also been shown to decrease MMP-2 and -9 expression when used at concentrations of 50 and 100 μM, thereby preventing tumor invasion and metastasis *in vitro* [36,37]. However, quercetin is absent or undetectable in the plasma because it is rapidly conjugated with glucuronic acid and/or sulfate after absorption from the small intestine. Major circulating forms of quercetin in human plasma are quercetin-3′-*O*-sulfate, isorhamnetin-3-*O*-glucuronide, and the antioxidative quercetin-3-*O*-glucuronide (Q3G) [38,39], which are present at 0.1–1 μM [40]. In general, biological responses to Q3G are weaker than those to its aglycone, and Kroon et al. [41] suggested that *in vitro* models of dietary polyphenol biological activity should use physiologically relevant flavonoids and their conjugates at appropriate concentrations. We previously reported DNA damage induced by treatment with 4-hydroxyestradiol and NA was suppressed by Q3G (0.1 μM) through the inhibition of NA binding to α_2-AR in MCF-10A human mammary epithelial cells [42].

Therefore, in the present study, we first confirmed β_2-AR expression in MDA-MB-231 cells at both the protein and RNA level by Western blot and RT-PCR analysis, respectively. We also investigated the effects of Q3G at the concentrations detected in human plasma upon catecholamine (NA and A)-induced ROS generation, along with NA-induced β_2-adrenergic signaling including cAMP production, RAS protein activation, phosphorylation of ERK1/2, *MMP2* and *MMP9* gene expression, MMP activity, and NA binding to β_2-AR in MDA-MB-231 breast cancer cells. The role of Q3G, a metabolite of dietary flavonoid, as an anti-metastatic agent in stress-induced cancer progression is discussed.

Material and methods

Chemicals and reagents

NA and A were purchased from Sigma Chemical Co. (St. Louis, MO), and Wako (Osaka, Japan), respectively, and prepared as 10 mM stock solutions in 0.12 M perchloric acid. L-(−)-Isoproterenol hydrochloride (β_2-adrenergic receptor agonist) was from Wako, propranolol hydrochloride (β_2-adrenergic receptor antagonist) was from MP Biomedicals (Tokyo, Japan), and 2′-7′-dichlorodihydrofluorescin diacetate (DCFH-DA) was purchased from Sigma. H-89 Dihydrochloride, an inhibitor of cAMP dependent protein kinase (PKA), came from Cell Signaling Technology (Danvers, MA, USA). SR11302, an inhibitor of AP-1, and Tanshinone IIA, an inhibitor of activation of NF-κB and AP-1 DNA binding, were from Tocris Bioscience (Boston, MA). SB203580, an inhibitor of p38α and p38β MAPK, was purchased from Wako. Quercetin-3-*O*-glucuronide (Q3G) was obtained from Extrasynthese (Genay, France), and dissolved in dimethyl sulfoxide to a concentration of 10 mM and stored at −20 °C until required.

Cell culture

The MDA-MB-231 breast cancer adenocarcinoma cell line was obtained from ATCC and grown in Leibovitz's/L-15 medium (Invitrogen, Carlsbad, CA) supplemented with 50 U/mL penicillin and 50 μg/mL streptomycin (Invitrogen), and 10% (vol/vol) fetal bovine serum (FBS; Invitrogen, Logan, UT), in a CO_2-free, air-only incubator.

RNA extraction

Total RNA was extracted from MDA-MB-231 cells using the Quick Gene RNA cultured cell kit S (Wako) with Quick Gene-Mini 80 (Fuji Film, Tokyo, Japan) according to the manufacturer's instructions. The ratio of RNA sample optical densities measured at 260 and 280 nm was used to evaluate nucleic acid purity, and total RNA concentrations were determined by absorbance at 260 nm. Four hundred nanograms of extracted RNA was transcribed in a final volume of 10 μL using the Prime Script RT reagent kit (RR037A, Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions.

Quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) analysis

A total of 1 μL cDNA solution was added to 19 μL PCR mixture containing 10 μL TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA), 8 μL DNase/RNase-free water, 1 μL house-keeping gene solution (glyceraldehyde-3-phosphate dehydrogenase; *GAPDH*), and 1 μL individual target primer. All primers used in this study were obtained from Applied Biosystems as follows: *ADRB2*, Hs00240532_s1; *MMP2*, Hs01548727_m1; *MMP9*,