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Ginsenoside Rg3 attenuates cell migration via inhibition of aquaporin 1 expression in PC-3M prostate cancer cells

Xue-Yang Pan, Hao Guo, Jing Han, Feng Hao, Yu An, Yan Xu, Yilixiati Xiaokaiti, Yan Pan, Xue-Jun Li *

State Key Laboratory of Natural & Biomimetic Drugs and Department of Pharmacology, School of Basic Medical Sciences, Peking University, Beijing 100191, China

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ABSTRACT

Ginsenoside Rg3 (Rg3), one of the bioactive extracts found in ginseng root, was reported to have anti-cancer activity in various cancer models. The anti-proliferation effect of Rg3 on prostate cancer cells has been well reported. To test whether Rg3 has an anti-metastatic effect on prostate cancer, we treated a highly metastatic PC-3M prostate cancer cell line with Rg3. We found that Rg3 (10 μM) led to remarkable inhibition of PC-3M cell migration. Simultaneously, exposure to Rg3 suppressed expression of the aquaporin 1 (AQP1) water channel protein, which has previously been reported to be involved in cell migration. Overexpression of AQP1 attenuated Rg3-induced inhibition of cell migration, and introduction of a shRNA targeting AQP1 abrogated the inhibitory effect of Rg3, although the basal level of cell migration was decreased by RNA interference. In mechanism study, estrogen receptor- and glucocorticoid receptor-dependent pathways are proved uninvolved in the AQP1 regulation by Rg3. However, Rg3 treatment triggered the activation of p38 MAPK; and SB202190, a specific inhibitor of p38 MAPK, antagonized the Rg3-induced regulation of AQP1 and cell migration, suggesting a crucial role for p38 in the regulation process. Deletion analysis of the promoter region of AQP1 was also conducted using dual-luciferase assay, which indicated that the −1000 bp to −200 bp promoter region was involved in the AQP1 regulation by Rg3. In all, we conclude that Rg3 effectively suppresses migration of PC-3M cells by down-regulating AQP1 expression through p38 MAPK pathway and some transcription factors acting on the AQP1 promoter.

1. Introduction

Prostate carcinoma is among the most common malignant cancers worldwide (Jemal et al., 2010). Whereas localized prostate carcinoma can be treated by radical prostatectomy or radiation therapy, patients are at increased risk of cancer metastasis (Cooperberg et al., 2009). Hormone manipulation has been developed as an effective resolution for advanced metastatic prostate carcinoma, however, the occurrence of loss or mutation of the androgen receptor in cancer cells results in failure of androgen deprivation therapy (Devlin and Mudryj, 2009). Many other therapeutic agents and protocols have been developed and utilized clinically, however, few of them have been shown to be dramatically effective. Moreover, drug resistance and cytotoxic side effects also impede the use of alternative therapies (Mahon et al., 2011).

Today, natural products have become a valuable resource for the development of new drugs (Cragg et al., 2009). Ginseng, which refers to the root of *Panax ginseng* and its related species, has been used for thousands of years in Asian countries and been reported to exhibit a wide range of medicinal effects. Ginsenoside Rg3, which has been posited to be one of the active ingredients in ginseng, has been shown to have various biological effects including anti-cancer activities (Jia and Zhao, 2009; Jia et al., 2009). Many studies have demonstrated that Rg3 has an anti-proliferation effect in various cancer cell lines including prostate cancer (Chen et al., 2008; Kim et al., 2004). An anti-metastasis activity for Rg3 has also been reported in several models (Iishi et al., 1997; Mochizuki et al., 1995). In this report, we sought to elucidate the effect of Rg3 treatment on a highly metastatic androgen receptor-negative prostate cancer cell line, PC-3M.

Aquaporin (AQP) is a water channel protein family eliciting fundamental functions in water transportation and osmotic homeostasis. The first member to be discovered, Aquaporin 1 (AQP1) is ubiquitously expressed in the human body (Borgnia et al., 1999). In addition to its basic function, AQP1 facilitates cell migration in a variety of cell types (Papadopoulos et al., 2008). Overexpression of AQP1 is common to malignancies from various organs and tissues, and several cancer cell lines expressing high levels of AQP1 exhibit enhanced migration *in vitro* and greater metastatic potential *in vivo* (Verkman et al., 2008). In addition, AQP1 promotes endothelial cell migration and angiogenesis, which is another important factor in tumor progression (Clapp and Martinez de la Escalera, 2006).

* Corresponding author at: Department of Pharmacology, School of Basic Medical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, China. Tel.: +86 10 82802863; fax: +86 10 82802863.
E-mail address: xjli@bjmu.edu.cn (X.-J. Li).

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Cellular AQP1 expression is controlled through a number of mechanisms. Osmotic change is a principal factor in AQP1 regulation and can be elicited through transcription (Jenq et al., 1998, 1999) or proteasome-mediated degradation (Leitch et al., 2001). Additionally, tonicity-independent mechanisms, such as glucocorticoid triggered AQP1 expression, have been described (Moon et al., 1997). Because previous studies have identified Rg3 as having glucocorticoid-like actions in cancer cells (Hien et al., 2010) and glucocorticoid is thought to modulate AQP1 expression, which is important in cell migration, we hypothesized that Rg3 may affect cell migration through AQP1 in PC-3M cells.

We found that Rg3 significantly suppresses cell migration and AQP1 expression in PC-3M cells. Furthermore, we showed that Rg3 treatment activated p38 MAPK, whereby PC-3M cell migration was mediated. We also offer possible mechanisms on the regulatory pattern of AQP1 through deletion analysis of the promoter of AQP1 using luciferase assay.

2. Materials and methods

2.1. Drugs and reagents

Ginsenoside Rg3 was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and the purity was at least 95% as determined by HPLC. Rg3 was dissolved in dimethyl sulfoxide (DMSO) in a 100 mM stock solution and stored at −20 °C. Aliquots of stock solution were added directly to the culture media.

The proteasome inhibitor MG132 (z-Leu-Leu-Leu-al, no. C2211) and dexamethasone (no. D1756) were purchased from Sigma-Aldrich. SB202190 (4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)-1H-imidazole), a specific inhibitor of p38 MAP kinase, was purchased from Santa Cruz Biotechnology (no. sc-202334). Estradiol and glucocorticoid receptor antagonizer RU486 (11β-(4-dimethyl-amino)-phenyl-17β-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one) were kindly provided by Prof. Bin He (National Research Institute for Family Planning, Beijing, China).

2.2. Cell culture

The PC-3M prostate cancer cells were obtained from the American-type culture collection (ATCC) and cultured in DMEM medium (GIBCO) supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 U/ml penicillin, and 100 μg/ml streptomycin (Inalco Pharmaceuticals) in a humidified incubator with 5% CO_2 at 37 °C. When treated with Rg3 or other reagents, cells were cultured in low-serum medium (containing 1% FBS) with the indicated concentration of additives.

2.3. Cell proliferation assay

PC-3M cells were plated in 96-well plates at a density of 5×10^3 cells/well and were cultured with DMEM. Twenty-four hours later, cells were treated with a range of concentrations of Rg3 for 24 h. After treatment, cell viability was determined via the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) cell proliferation assay. Briefly, 20 μl of sterile MTT dye (5 mg/ml, Sigma, no. M2128) was added per well and cells were incubated at 37 °C for an additional 4 h. Next, culture media were removed and the crystallized formazan was dissolved in 100 μl of DMSO. The optical density (OD) value of the solute was measured at a wavelength of 570 nm.

2.4. In vitro wound healing assay

Wound healing assays were performed as previously described (Liang et al., 2007) to assess the capacity for cell migration. Briefly, PC-3M cells were plated on a 6-well plate. When cells reached 90–95% confluence, scratches were made with a 200-μl sterile pipette tip. Cells were washed with phosphate buffered saline (PBS) three times and the initial wounds were recorded using an Olympus microscope. Cells were then incubated with medium containing Rg3 for 24 h and the wounds were photographed again. The rate of cell migration was evaluated by the rate of wound closure.

2.5. Transwell migration assay

Cell migration was further investigated using the transwell migration assay. PC-3M cells were suspended in serum-free DMEM (1×10^5 cells) and placed in the upper chamber of a 24-well transwell insert (8 μm pore size) (Corning-Costar). DMEM containing 10% FBS was added to the lower chamber. The plate was incubated at 37 °C for 12 h and the cells were fixed in 4% formaldehyde. The upper chamber was gently wiped with a cotton swab to remove non-migrated cells, and the migrated cells on the lower side of polycarbonate filters were stained with crystal violet and counted under an Olympus microscope (×200 magnification).

2.6. Western blot analysis

After treatment, cells were washed twice with PBS and lysed in RIPA buffer containing 1% Triton X-100, 1% sodium deoxycholate and 0.1% sodium dodecyl sulfate (SDS). Protein concentrations were determined using a BCA protein assay kit (Pierce). Equal amounts of proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore). Anti-AQP1 antibody (no. sc-20810) was purchased from Santa Cruz Biotechnology. Anti-ERK (no. 4695), anti-phospho-ERK (no. 4370), anti-p38 (no. 9212), anti-phospho-p38 (no. 4511), anti-JNK (no. 9258) and anti-phospho-JNK (no. 9255) antibodies were purchased from Cell Signaling Technology. Anti-β-actin antibody (no. A8481) was purchased from Sigma. After incubation with primary antibodies, the membranes were incubated with alkaline phosphatase-conjugated secondary antibodies. Finally, protein bands were detected and visualized by 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt (BCIP, Amresco)/nitro blue tetrazolium (NBT, Amresco), and they were then scanned using an Epson electronic scanner.

2.7. Preparation of total RNA and real-time PCR analysis

Total RNA was isolated from PC-3M cells using TRIzol reagent (Invitrogen, no. 15596-026) according to the manufacturer's directions. Total RNA was reverse transcribed to cDNA using Superscript III First-strand Synthesis Kit (Invitrogen, no. 18080-051) and oligo(dT) primers. For the real-time PCR analysis, a THUNDERBIRD SYBR qPCR Mix (Toyobo, no. QPS-201) was used according to the manufacturer's instructions, and PCR was performed using an Mx3005P QPCR system (Agilent). The selective primers for human AQP1 and GAPDH were as follows: 5′-CCATCCTCTCAGGCATCAC-3′ (forward) and 5′-GGTAGTAGCCAGCACGCATA-3′ (reverse) for human AQP1 (GenBank ID: 297307114); 5′-CAAGGCTGAGAACGGGAAG-3′ (forward) and 5′-GGGCAGAGATGATGACCCTT-3′ (reverse) for human GAPDH (GenBank ID: 83641890). The relative amount of AQP1 mRNA was normalized on the basis of the amount of GAPDH.

2.8. Overexpression of AQP1 in PC-3M cells

A pcDNA3-AQP1 plasmid for overexpression of AQP1 was kindly provided by Prof. Bao-Xue Yang (Peking University Health Science Center, Beijing, China). PC-3M cells were transfected using Lipofectamine LTX and PLUS reagent (Invitrogen, no. 15338-100) and prepared for experiments after 24 h of transfection.