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Ginsenoside 20(*S*)-Rh2 exerts anti-cancer activity through targeting IL-6-induced JAK2/STAT3 pathway in human colorectal cancer cells

Songhee Han [a,1], Ae Jin Jeong [a,b,1], Heejung Yang [c], Kyo Bin Kang [d], Haeri Lee [a,b], Eun Hee Yi [a,e], Byung-Hak Kim [a,f], Chung-Hyun Cho [a,e,g], Jin Woong Chung [h], Sang Hyun Sung [d,*], Sang-Kyu Ye [a,e,f,i,**]

[a] Department of Pharmacology and Biomedical Sciences, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[b] Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea
[c] College of Pharmacy, Kangwon National University, Chuncheon 24341, Republic of Korea
[d] College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul 08826, Republic of Korea
[e] Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[f] Biomedical Science Project (BK21PLUS), Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[g] Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea
[h] Department of Biological Science, Dong-A University, Busan 47315, Republic of Korea
[i] Neuro-Immune Information Storage Network Research Center, Seoul National University College of Medicine, Seoul 03080, Republic of Korea

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ABSTRACT

Ethnopharmacological relevance: *Panax ginseng* is one of the most well-known medicinal herbs in Korea and China, which has been used for treatment and prevention of cancer, obesity, diabetes, and cardiovascular diseases. Ginsenosides are the major components of *P. ginseng*, having a wide range of pharmacological activities. Among the ginsenosides, protopanaxadiol (PPD)-types reportedly have potent anti-cancer effects. Rh2 is PPD-type ginsenoside, and two stereoisomeric forms of Rh2 as 20(*S*)- and 20(*R*)-Rh2 were selectively isolated recently.

Aim of the study: The biological activities of Rh2 ginsenosides are known to depend on their differences in stereochemistry. Colorectal cancer (CRC) is one of the most lethal neoplasm, and cancer-related death is usually associated with metastasis to other organs. We aimed this study to investigate whether 20(*S*)- and 20(*R*)-Rh2 can suppress tumor invasion in human CRC cells.

Materials and methods: 20(*S*)- and 20(*R*)-Rh2 were isolated from the roots of ginseng. Human CRC cells were incubated with 20(*S*)- or 20(*R*)-Rh2 in the presence or absence of interleukin-6. An MTT assay was used to measure cell viability. Western blot and quantitative real-time PCR analyses were performed to determine levels of expression and phosphorylation. An invasion assay was performed using a Boyden chamber system with the Matrigel-coated membrane to measure cancer cell invasion.

Results: 20(*S*)- and 20(*R*)-Rh2 showed differential cytotoxic activity. Only 20(*S*)-Rh2 decreased cancer cell viability. Additionally, 20(*S*)-Rh2 effectively inhibited IL-6-induced signal transducer and activator of transcription 3 (STAT3) phosphorylation and the expression of matrix metalloproteinases (MMPs), including MMP-1, −2, and −9, resulting in inhibition of cancer cell invasion. Interestingly, these pharmacological activities of 20(*S*)-Rh2 were more potent than those of 20(*R*)-Rh2. Furthermore, combination treatment showed that 20(*S*)-Rh2 enhanced the sensitization of doxorubicin-treated anti-cancer activities in CRC cells.

Conclusion: Our results demonstrated that ginsenoside 20(*S*)-Rh2 has therapeutic potential for the treatment with CRC and may be valuable as a combination partner with more classic chemotherapeutic agents, such as doxorubicin, to treat CRC.

* Corresponding author.
** Corresponding author at: Department of Pharmacology and Biomedical Sciences, Seoul National University College of Medicine, 103 Daehangno, Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea.
E-mail addresses: skysmilez@naver.com (S. Han), lovej89@hotmail.co.kr (A.J. Jeong), heejyang@kangwon.ac.kr (H. Yang), mumyung2@snu.ac.kr (K. Bin Kang), hrlee519@gmail.com (H. Lee), startwo1204@snu.ac.kr (E.H. Yi), protein0826@snu.ac.kr (B.-H. Kim), iamhyun@snu.ac.kr (C.-H. Cho), jwchung@dau.ac.kr (J.W. Chung), shsung@snu.ac.kr (S.H. Sung), sangkyu@snu.ac.kr (S.-K. Ye).
[1] These authors contributed equally to this work.

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1. Introduction

Colorectal cancer (CRC) has a high incidence and mortality rate worldwide. Indeed, the incidence of CRC has increased annually, and almost 50% of patients with CRC eventually die of this cancer (Corvinus et al., 2005). The main reason for cancer-related deaths is not the primary tumor, but distant metastases. CRC is especially prone to metastasis, which is the main cause of death in patients with CRC who die within 5 years after diagnosis (Helling and Martin, 2014; Seo and Kim, 2011). However, there is presently no effective therapy for CRC treatment, even surgical treatment, after cancer metastasis. Because the conventional therapeutic agents for CRC have side effects and limited efficacy, there is a continuing need for the study and development of novel treatments.

Recent evidence suggests that transcription factor signal transducer and activator of transcription 3 (STAT3) may be a valuable therapeutic target in CRC treatment (Lin et al., 2011; Xiong et al., 2014). Consistently activated STAT3 signaling is observed in patients with numerous types of cancer and in various tumor-derived cell lines, indicating the importance of STAT3 activation in tumorigenesis and cancer progression. In fact, inappropriate activation of STAT3 signaling occurs with surprisingly high frequency in human cancers, including CRC (Corvinus et al., 2005; Kim et al., 2008). STAT3 activation is known as a major factor in colon carcinogenesis and is also involved in the process of metastasis (Ma et al., 2004). Interleukin-6 (IL-6) is a well-known and thoroughly studied cytokine in tumor-associated STAT3 signaling (Musteanu et al., 2010). The proinflammatory cytokine IL-6 acts a major signature cytokine in tumor development and metastasis and is known to be associated with invasive CRC through crosstalk between tumor and immune cells in the tumor microenvironment (Reimers et al., 2013). IL-6 can activate STAT3 signaling, which is associated with tumorigenesis activities including initiation, promotion, invasion, and metastasis (Grivennikov et al., 2009; Waldner et al., 2012), suggesting that IL-6/STAT3 signaling may play an essential role in tumor invasion in CRC (Gordziel et al., 2013).

Ginseng, the roots of *Panax ginseng* C.A. Meyer, is one of the most well-known medicinal herbs in the Far East, particularly in Korea and China. In traditional Korean and Chinese medicine, ginseng has been used for the treatment and prevention of cancer, obesity, diabetes, and cardiovascular diseases. Ginsenosides are the major active components within ginseng extracts and have multiple biological activities without influencing normal cells (Li et al., 2011; Park et al., 2010). The anti-cancer activity of red ginseng extracts is much stronger than that of white ginseng extracts (Lim et al., 2015). Ginsenoside Rh2 is one of the major active components of red ginseng extracts and is known to exert its anticancer effects by inhibiting cell proliferation and inducing apoptosis (Nag et al., 2012; Zhang et al., 2011). Rh2 is classified as 20(*S*) or 20(*R*) according to the orientation of the hydroxyl group at the C-20 position. The recent development of a selective isolation procedure for 20(*S*)- and 20(*R*)-Rh2 enabled in-depth pharmacological studies on the stereoisomers. The two stereoisomeric forms show distinct biological effects according to the orientation of the hydroxyl moiety on C-20 (Kang et al., 2005; Lee et al., 2006).

Many patients with metastatic CRC rely on substitute treatment for conventional chemotherapy using natural products, such as ginseng. In this study, we investigated the pharmacological activities of ginsenosides 20(*S*)- and 20(*R*)-Rh2 on invasion and signaling cascades in CRC cells. 20(*S*)-Rh2 exhibited more potent pharmacological activities in cancer cell invasion and IL-6-mediated STAT3 activation and its signaling cascades than did 20(*R*)-Rh2. Additionally, 20(*S*)-Rh2 effectively inhibited the expression of the STAT3 target genes, the matrix metalloproteinases (MMPs), including MMP-1, −2, and −9. We further observed that the combination of 20(*S*)-Rh2 and the chemotherapeutic agent doxorubicin showed synergistic cytotoxic activity in CRC cells. Our results suggest that ginsenoside 20(*S*)-Rh2 may be a promising therapeutic candidate for combination treatment in metastatic CRC.

2. Materials and methods

2.1. Preparation of ginsenosides 20(S)- and 20(R)-Rh2

Ginsenosides 20(*S*)- and 20(*R*)-Rh2 were prepared in our previous study (Yang et al., 2014, 2012). Briefly, they were isolated by a series of column chromatographic techniques from dry purified extract of *Panax ginseng* prepared by the manufacturing processes, such as column chromatography, and enzyme and acid hydrolysis. The detailed procedure for the manufacturing, isolation, and structural elucidation, and the UHPLC–QTOF/MS chromatogram of the extract are available in the abovementioned references. The purities of 20(*S*)- and 20(*R*)-Rh2 were determined as above 95% by normalization of the peak areas detected by HPLC-UV analysis.

2.2. Reagents

Recombinant human IL-6 was purchased from Peprotech (Rocky Hill, NJ). AG490, a pan-JAK inhibitor, was purchased from Sigma-aldrich (St. Louis, MO, USA). Antibodies specific for phospho-STAT3 (Tyr705, Cat #9145), phospho-STAT3 (Ser727, Cat #9134), STAT3 (Cat #4904), phospho-JAK2 (Tyr1007/1008, Cat #3776), JAK2 (Cat #3230), PARP (Cat #9542), and Caspase-3 (Cat #9662) were obtained from Cell Signaling Technology (Boston, MA, USA).

2.3. Cell lines and culture conditions

Human colorectal cancer cell lines HCT116 and SW620 were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). HCT116 cells were maintained in RPMI 1640 medium (Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS, Life Technologies) and 1% penicillin/streptomycin solution (Life Technologies). SW620 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, HyClone, South Logan, Utah, USA) supplemented with 10% FBS and 1% penicillin/streptomycin solution.

2.4. Cell viability assay

Cells were plated at a density of 40,000 cells into each well of 96-well plates. After an overnight incubation, cells were incubated with either vehicle (0.1% DMSO) or various concentrations of 20(*S*)- or 20(*R*)-Rh2 for 24 h in the presence or absence of doxorubicin (Sigma-aldrich, St. Louis, MO, USA). Cell viability was determined by incubation for 4 h at 37 °C incubator, followed by addition with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (100 μl, 5 mg/ml, Sigma-aldrich). The MTT formazan crystals were dissolved in DMSO and absorbance was measured at 540 nm with an ELISA plate reader (Molecular Devices, CA, USA).

2.5. Western blot analysis

Cells were lysed in a lysis buffer (50 mM Tris–HCl, pH 7.4, 350 mM NaCl, 0.5% NonidetP-40, 10% glycerol, 0.1% SDS, and 1% Triton X-100). The lysates were centrifuged at 13,000 rpm for 20 min at 4 °C and protein amounts were quantified using a Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). Proteins were