Hesperidin Induces Apoptosis by Inhibiting Sp1 and Its Regulatory Protein in MSTO-211H Cells

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Abstract

Hesperidin, a flavanone present in citrus fruits, has been studied as potential therapeutic agents that have anti-tumor activity and apoptotic effects in several cancers, but there is no report about the apoptotic effect of hesperidin in human malignant pleural mesothelioma through the specificity protein 1 (Sp1) protein. We investigated whether hesperidin inhibited cell growth and regulated Sp1 target proteins by suppressing the levels of Sp1 protein in MSTO-211H cells. The ICₕ₀ value of hesperidin was determined to be 152.3 µM in MSTO-211H cells for 48 h. Our results suggested that hesperidin (0-160 µM) decreased cell viability, and induced apoptotic cell death. Hesperidin increased Sub-G₁ population in MSTO-211H cells. Hesperidin significantly suppressed mRNA/protein level of Sp1 and modulated the expression level of the Sp1 regulatory protein such as p27, p21, cyclin D1, Mcl-1, and survivin in mesothelioma cells. Also, hesperidin induced apoptotic signaling including: cleavages of Bid, caspase-3, and PARP, upregulation of Bax, and down-regulation of Bcl-xl in mesothelioma cells. These results show that hesperidin suppressed mesothelioma cell growth through inhibition of Sp1. In this study, we demonstrated that Sp1 acts as a novel molecular target of hesperidin in human malignant pleural mesothelioma.

Key Words: Rotavirus, Hepatitis A virus, Recombinant chimera protein

INTRODUCTION

Malignant pleural mesothelioma (MPM) occurs from the mesothelial cells and is the most common primary tumor of the pleura. MPM is difficult to detect at an early stage and is a highly aggressive cancer (Robinson and Lake, 2005). Approximately 80% of MPM is caused from exposure to asbestos fiber and other factors include simian virus 40, radiation, and erionite (Carbone et al., 2002). Survival time of most patients after their first symptoms is very short (median < 12 months) despite treatments, and conventional treatments such as, chemotherapy and radiotherapy have shown to be quite ineffective (Robinson and Lake, 2005). For more than 20 years, studies for MPM have continued, but no biomarker was used clinically. Diagnostic, therapeutic, and prevention methods for MPM is understood by a variety of molecular pathways. Based on molecular pathways of MPM, diverse clinical trials using targeted agents have been conducted (Zucali et al., 2011). Development of several targeted treatments is necessary for understanding MPM carcinogenesis.

Clinical therapies for cancer treatments are limited to radiation, chemotherapy, immunosuppression and surgery (Neergheen et al., 2009). It is expected that potential chemopreventive and therapeutic activities of polyphenols have an influence on intracellular signaling of cancers (Ramos, 2008). Flavonoids have various biological functions, such as anti-oxidant, anti-tumor, anti-inflammation and anti-allergic effects, at nontoxic concentrations in organisms (Dimmock et al., 1999; Horváthová et al., 2001; Ren et al., 2003). It is generally known that flavonoids have anti-cancer properties. Then, the study for the effects of flavonoids for cancer chemoprevention and chemotherapy is very significant (Ren et al., 2003; Li et al., 2007). Phytochemicals are non-toxic as a natural product in origin and have been known as inhibitors of various transcription factors within in vitro and animal models of cancer (Tsuda et al., 2004; Fresco et al., 2006).

Hesperidin (30,5,9-dihydroxy-40-methoxy-7-orutinosyl flavanone) is a flavanone present in citrus fruits like oranges and lemons (Justesen et al., 1998; Nielsen et al., 2002) (Fig. 1A). Hesperidin was first discovered in 1827, but had been studied as a combination product complex until 1986 (Garg et al., 2001). In nature, hesperidin exist as glycoside form, a beta-7-rutinoside of hesperitin, but dietary hesperidin is hydrolyzed to hesperitin (Preston et al., 1953; Ameer et al., 1996). Hes-
The effect of hesperidin on cell viability in MSTO-211H cells was not understood (Tanaka et al., 1997; Yang et al., 1997; Kong et al., 2010; Sankpal et al., 2011). So highly expressed Sp1 protein upregulate genes concerned in tumor development, growth and metastasis by binding to promoter sequences. Thus, Sp1 protein was expected to be a negative prognostic factor and potential therapeutic target for cancer chemotherapy (Safe and Abdelrahim, 2005). Based on the cancer-related functions of Sp1, it has been expected that Sp1 is a significant target for researches of cancer therapy.

It was not reported whether MPM was influenced by the chemoprevention effects of hesperidin. Also, the association of hesperidin and Sp1 signaling has not been discovered. In the study, we investigated whether hesperidin inhibited cell growth, and regulated Sp1 target proteins, resulting in apoptosis by suppressing the levels of Sp1 protein in MSTO-211H cells.

**MATERIALS AND METHODS**

**Antibodies**

The following antibodies were purchased: anti-p21 (F-5), anti-p27 (C-19), anti-Cyclin D1 (M-20), anti-Sp1 (1C6), anti-caspase-3 (H-277), horseradish-peroxidase-conjugated antibody IgG (all from Santa Cruz Biotechnology, Inc., Santa Cruz, CA). anti-poly ADP-ribose polymerase (PARP) (BD Biosciences, San Diego, California), anti-Mcl-1, anti-survivin, anti-Bid, anti-Bax, anti-Bcl (Cell Signaling, Danvers, Massachusetts), anti-β-actin (AC-74) (Sigma-Aldrich, Inc. St. Louis, Missouri).

**Cell culture**

MSTO-211H cells were purchased from the American Tissue Culture Collection (Manassas, Virginia). The MSTO-211H cells were maintained in Hyclone RPMI-1640 supplemented with 5% fetal bovine serum (FBS) and 100 U/ml each of penicillin and streptomycin (Thermo Scientific, Logan, Utah) at 37°C in a humidified chamber with 5% CO₂ and 95% air, and the medium was changed every 3 days.

**MTS assay**

MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) Assay Kit (Promega, Madison, Wisconsin) was used to measure the viability of MSTO-211H cell, according to the manufacturer’s protocol. The MSTO-211H cells were plated at a density of 3×10⁵ cells/100 μl/well on a 96-well microtiter plate for 24 h. Hesperidin was treated with a final concentration of 0, 40, 80 and 160 μM in cells for 24 h and 48 h, and then a MTS cell proliferation assay reagent was added to the cells and incubated at 37°C in 5% CO₂ for 2 h. The absorbance was measured at 490 nm using GloMax-Multi Microplate Multimode Reader (Promega, Madison, Wisconsin).

Normally, it is known that several transcription factors are upregulated in cancer. Transcription factors may function as targets for the development of new anti-cancer drugs, because these can regulate gene expression by binding to specific DNA sequences within cancer-related gene promoter regions (Safe and Abdelrahim, 2005). The level of Sp1 was elevated in cancer cells compared to normal cells. It was reported that Sp1 was overexpressed in several cancers including prostate cancer, breast cancer, gastric cancer, pancreatic cancer, thyroid cancer, hepatocellular carcinomas, colorectal cancer and lung cancer (Davie et al., 2008; Chuang et al., 2009; Kong et al., 2010; Sankpal et al., 2011). So highly expressed Sp1 protein upregulated genes concerned in tumor development, growth and metastasis by binding to promoter sequences. Thus, Sp1 protein was expected to be a negative prognostic factor and potential therapeutic target for cancer chemotherapy (Safe and Abdelrahim, 2005). Based on the cancer-related functions of Sp1, it has been expected that Sp1 is a significant target for researches of cancer therapy.

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