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Ginsenoside F1 suppresses astrocytic senescence-associated secretory phenotype

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ABSTRACT

Senescence is one of the hallmarks of aging and identified as a potential therapeutic target in the treatment of aging and aging-related diseases. Senescent cells accumulate with age in a variety of human tissues where they develop a complex senescence-associated secretory phenotype (SASP). SASP in brain could contribute to age-related inflammation and chronic neurodegenerative diseases. We confirmed that senescent astrocytes express a characteristic of SASP *in vitro* by human cytokine antibody array. Ginsenoside F1 suppresses the SASP from astrocytes induced by D-galactose via suppressing p38MAPK-dependent NF-κB activity. A specific inhibitor of p38MAPK, SB203580 significantly decreased the secretion of IL-6 and IL-8, the major components of SASPs. Additionally, treatment of senescent astrocytes with NF-κB inhibitor, BAY 11–7092, also suppressed the secretion of IL-6 and IL-8, suggesting NF-κB was required for SASP. Importantly, conditioned media from senescent astrocytes promoted the migration of glioblastoma cells, such as U373-MG, U251-MG and U87-MG assessed by scratch wound healing. This migration was significantly decreased by F1 treatment in senescent astrocytes. Interestingly, IL-8, the main mediator regulating glioblastoma cell invasion, was suppressed in both transcriptional and protein level. Herein, we propose ginsenoside F1 as a potential therapeutic strategy for reducing the deleterious contribution of senescent astrocytes in aged brain and related diseases.

1. Introduction

Aging is defined as a progressive loss of tissue and organ function over time [1,2]. Although aging itself is not a kind of disease, it is the largest risk factor for host of age-related diseases [3–6]. Nine tentative candidate hallmarks were recently enumerated as potential pharmaceutical targets to improve human health during aging, with minimal side effects [7]. Among these hallmarks is cellular senescence. Cellular senescence is one of the two independent stress-response mechanisms that is able to initially maintain the tissue integrity when organism repair (DNA) falls short [8,9]. Cellular senescence causes a permanent cell growth arrest of proliferative cells and develop senescence-associated secretory phenotype (SASP) that includes cytokines, chemokines, growth factors [10]. This emerging evidence suggest that cellular senescence is a potent anticancer mechanism, but also has been indicating as a driver for aging and aging related diseases [11,12].

Senescent cells are lurking in the heart, liver, kidney and even brain tissues [13,14]. Astrocytes are fundamental for homoeostasis, defense and regeneration of central nervous system. Loss of astroglial function and reactive astroglial cells contribute to aging of brain [15]. Interestingly, astrocytes undergo functional recession with age and partially lose their neuroprotective ability as well as exacerbating neuronal injury in age-related neurodegenerative processes [16,17]. Additionally, astrocytes in the aging brain express characteristics of senescence-associated secretory phenotype, giving rise to the decline in functional capacity of the brain [18]. Important evidence has been demonstrated that senescent astrocytes accumulate in age brain, and further, in brain from patient of Alzheimer's disease [19].

Ginsenosides has long been a popularity of great interest in a wide range of field. Up to date, great challenges by ginsenosides are anti-aging and neuroprotective functions [20,21]. Furthermore, since most of senescent astrocytes express SASP and SASPs reprogram neighboring microenvironment [22]; we examined whether ginsenoside regulates SASP in astrocytes in the present study. We found that ginsenoside F1 significantly inhibited the expression and secretion of IL-6 and IL-8, the major SASP components claimed in aged brain [23]. Accordingly, we propose that ginsenoside F1 maybe one candidate for reducing the contribution of senescence in aging brain and related pathologies.

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2. Materials and methods

2.1. Cells and cell culture

Astrocytic CRT and U373-MG cells were maintained in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum (FBS,G), 100U of penicillin/mL, and 100 μg of streptomycin/mL as previously described [24]. U251-MG-EGFP cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100U of penicillin/mL, and 100 μg of streptomycin/mL. Primary rat and human astrocytes were maintained in 10% FBS-DMEM containing 1% nonessential amino acids (Gibco-BRL, Grand Island, NY, USA). Stable cell line CRT-MG/IL-8p-d2EGFP cells were prepared and maintained as previously described [25].

Hippocampi were aseptically dissected out from embryonic Day 18 Sprague-Dawley rat embryos acquired from OrientBio, Inc. (Seongnam, Korea). After trituration and trypsinization, hippocampal cells were resuspended in plating medium (86.55% of MEM Eagle's with Earle's BSS, 10% re-filtered and heat inactivated FBS, 0.45% of glucose, 100 μM of sodium pyruvate, 200 μM glutamine and 100 mg/L streptomycin, 100 U/mL penicillin). The single-cell suspension was seeded in 100 mm petri-dishes containing poly-L-lysine coated coverslips at a density of 5×10^5/mL. After 4 h, cells were maintained in Neurobasal medium supplemented with 1% B27, 200 μM glutamine, 100U of penicillin/mL, and 100 μg of streptomycin/mL in a humidified atmosphere of 5% CO_2 at 37 °C.

Cerebral cortices were trypsinized and resuspended in plating medium DMEM20S (DMEM, 4 mM L-glutamine, 1 mM sodium pyruvate, 20% FBS, 50 U/mL penicillin and 50 mg/L streptomycin). The cell suspension was seeded in a T75 poly-D-lysine-coated flask (approximately 10 million cells), and fed every 2–3 days with 10 ml DMED20S for 10 days. Then the flask was pre-shaken on the shaker for 1 h at 200 r.p.m. at 37 °C to remove microglial cells. After a shake at 200 r.p.m. overnight, the medium were collected and incubated in untreated petri-dish to remove microglia and astrocytes. Subsequently, cells were pelleted and plated onto poly-D, L-ornithine-coated petri-dishes to achieve a density of 1×10^4 per cm^2 with oligodendrocyte progenitor cell medium (containing 10 ng/mL PDGF-AA and 10 ng/mL bFGF) in a humidified atmosphere of 5% CO_2 at 37 °C.

2.2. Reagents

Ginsenosides Rg1, Re, F1, Rh1, Rg2, PPT, Rb1, Rd, Gyp75, F2, Rg3, Rh2, CK and PPD, with a purity of more than 98%, were prepared with High Performance Liquid Chromatography (HPLC). Each ginsenoside was dissolved in dimethyl sulfoxide (DMSO) as 10 mg/mL solution. Corticosterone, BAY11-7082, SB203580 and D-galactose were purchased from Sigma (St. Louis, MO).

2.3. Senescence induction and assessment

Astrocytic CRT cells were induced to senescence by exposure to 20 g/L D-galactose supplemented in complete culture medium. Briefly, proliferating cells were treated with above indicated concentration of D-galactose for consecutive 14 days. Later, cells were scored for senescence markers, including SA-β-gal activity and the presence of persistent DNA-damage foci. Senescence-associated β-galactosidase (SA-β-gal) staining was performed using a SA-β-gal kit (#9860, Cell Signaling Technology, Inc. MA) in accordance with the manufacturer's protocol. The cells were fixed for 10–15 min at room temperature, then rinsed twice with PBS and stained with staining solution at a final pH of 6.0 for at least overnight. The SA-β-gal positive cells develop blue color and were counted under a phase-contrast microscope. DNA-damage foci were assessed by immunostaining for 53BP1. For DNA-damage foci and SA-β-gal positivity, random fields were shown. Fluorescent images were quantified using CellProfiler, an open source software program (http://www.cellprofiler.org). SA-β-gal staining was quantified by light microscopy and a researcher that was blinded to the treatments. Specific inhibitor of p38MAPK, SB203580 and NF-κB inhibitor, BAY 11–7092 were added at 12th day in case of SASP assessment.

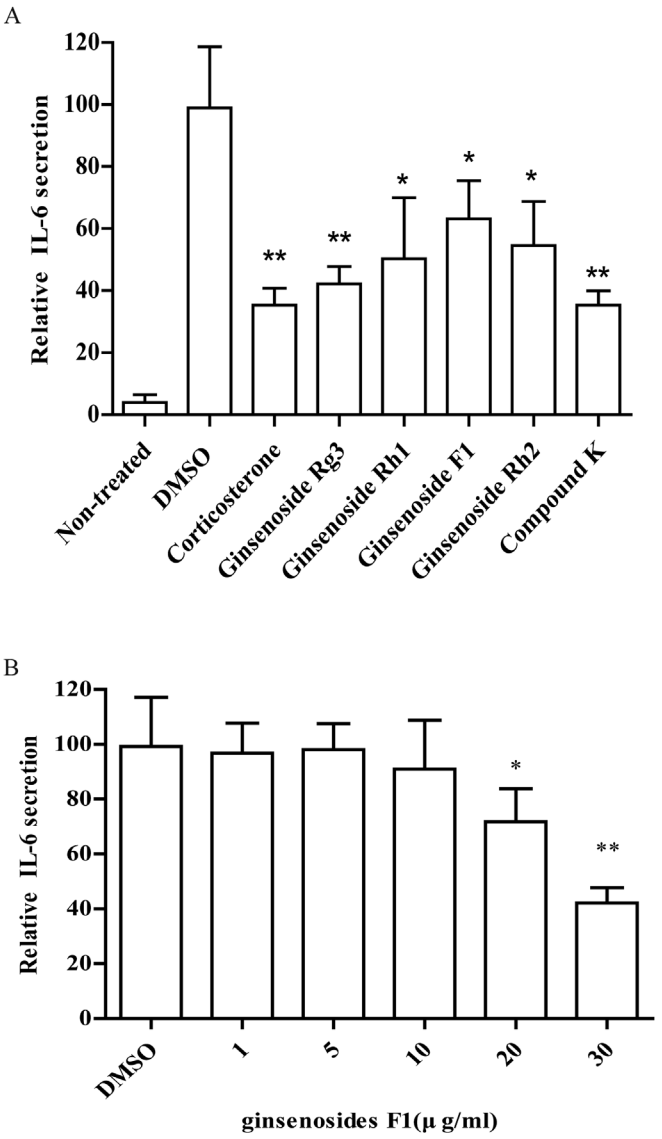

Fig. 1. Selected ginsenosides suppresses IL-6 secretion. A. Senescent astrocytic CRT cells were prepared with 20 g/L D-galactose for 14 days. Ginsenoside F1, Rh1, Rg3, Rh2 and CK were used with 20 μg/mL to treat senescent astrocytic CRT cells at the 12th day, after which conditioned medium (CM) were collected and analyzed with Ray Biotech IL-6 ELISA kit. Cells treated with DMSO (vehicle) as negative control and 100 nM Corticosterone as positive control. B. Senescent astrocytic cells were treated with a serial of ginsenoside F1. CM were collected and analyzed for IL-6 secretion. * indicates $p < 0.05$ versus DMSO, ** indicates $p < 0.01$ versus DMSO.

2.4. Cell proliferation assay

Cell viability was evaluated by the WST-1 assay, which is based on the enzymatic cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenase present in viable cells. In brief, after 24 h treatment, 20 μl of WST-1 was added to each well and the plates were incubated at 37 °C for 2 h. The plates were then centrifuged and 100 μl of the medium was withdrawn to be determined by measuring the absorbance value at a wavelength of 450 nm using microplate reader.