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Original Article

Guiqi polysaccharide protects the normal human fetal lung fibroblast WI-38 cells from H_2O_2-induced premature senescence

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Abstract: Objective: This study is to investigate the effects of Guiqi polysaccharide (GQP) on H_2O_2-induced premature senescence in normal human fetal lung fibroblast WI-38 cells. Methods: WI-38 cells were subjected to treatments of GQP, *Angelica sinensis* polysaccharide (ASP), and *Astragalus membranaceus* polysaccharide (AMP), and then treated with H_2O_2 to induce premature senescence. Morphological observation, MTT assay, senescence-associated β-galactosidase activity assessment, telomerase activity determination, cell cycle analysis, and Western blot analysis were performed to evaluate cellular senescence. Results: H_2O_2 treatment induced premature senescence in WI-38 cells, as indicated by the decreased fibroblast proliferation activity and changed cellular morphology. When treated with GQP, ASP, or AMP, the morphological changes in WI-38 cells induced by H_2O_2 could be restored. SA-β-gal activity was elevated in H_2O_2-treated WI-38 cells, which could be decreased by GQP treatment. Moreover, compared with the normal control, H_2O_2 treatment significantly inhibited the telomerase activity of WI-38 cells. However, GQP effectively elevated the telomerase activity of these senescent cells. Furthermore, flow cytometry and cell cycle analysis showed that GQP treatment could abrogate the cell cycle arrest in H_2O_2-treated WI-38 cells, which might contribute to the anti-senescent effects. In addition, GQP significantly affected the p53-p21 and p16-pRb pathways in H_2O_2-treated WI-38 cells. The effectiveness of GQP was superior to AMP or ASP treatment alone. Conclusion: GQP has protective effects in oxidative stress-induced senescence. Our findings suggest the promising role of GQP as an attractive and bio-safe agent with the potential to retard senescence and attenuate senescence-related diseases.

Keywords: Guiqi polysaccharide (GQP), *angelica sinensis* polysaccharide (ASP), *astragalus membranaceus* polysaccharide (AMP), cellular senescence, hydrogen peroxide, WI-38 cells

Introduction

Aging is a multifactorial process involving changes at the cellular, tissue, organ, and whole body levels, which might result in functional decline, disease pathogenesis, and ultimately death. Cellular senescence halts the proliferation of dysfunctional or damaged cells, which plays a critical role in aging [1, 2]. It has been shown that the induction of senescence could prevent cancer through a failsafe mechanism, eliminating cells that are at risk of neoplastic transformation [3, 4]. Normal human fetal lung fibroblast cell line (WI-38), first described by Leonard Hayfliek [5], is one of the classical experimental models for studying cellular aging and senescence. Currently, it is reported that many agents, such as hydrogen peroxide (H_2O_2), radiation, and DNA damaging agent, can induce premature senescence of WI-38 cells, which refers to shortened intrinsic replicate life span in cells under stress conditions [6-12]. In fact, cellular senescence is a complex process that is characterized by physiopathological changes including irreversible proliferation arrest, enlarged and flattened cell morphology, increased senescence-associated β-galactosidase (SA-β-gal) activity, and enhanced senescence-associated heterochromatin foci (SAHF) formation [6, 7, 13].

Plant polysaccharides are often identified as biological response modifiers or immunostimulants [14, 15]. It has been shown that Chinese herbal medicines, *Angelica sinensis* and *Astragalus membranaceus*, can enrich the

blood, and exert anti-tumor and antioxidation effects [16, 17]. Polysaccharide is one of the main active ingredients in *Angelica* and *Astragalus* [16, 18]. Our previous work has shown that a combination of polysaccharides extracted from *Angelica* and *Astragalus* roots, Guiqi polysaccharide (GQP), exhibits a range of antioxidant, anti-aging, and antiviral activities *in vitro* and *in vivo* [19-22]. Particularly, GQP has been found to cause enzymatic changes in d-galactose-induced senescence, which might be beneficial in delaying senescence process [19]. However, the anti-senescence effects of GQP and related mechanisms have not yet been fully investigated.

In this study, WI-38 cells were treated with H_2O_2 to establish *in vitro* premature senescence cellular model, and the effects of GQP on cellular senescence and related mechanisms were then investigated. Alterations in cellular morphology, SA-β-gal staining, cell cycle, and molecular expression in H_2O_2-treated WI-38 cells were evaluated and analyzed. This study is the first report concerning the anti-senescence activity of GQP and the related mechanisms, which might support the role of GQP in retarding senescence and attenuating senescence-related diseases.

Materials and methods

Materials

AS and AM were purchased from Minxian Shunfa Medicinal Material Company (Gansu Minxian City, China). Water extraction, ethyl alcohol deposition method and Sevag method [23] were used to obtain Guiqi polysaccharide (GQP), *Angelica sinensis* polysaccharide (ASP), and *Astragalus membranaceus* polysaccharide (AMP) in College of Life Science and Engineering, Lanzhou University of Technology (Lanzhou, Gansu, China). The total carbohydrate content in GQP, ASP and AMP were 87.6%, 64.3% and 75.1%, respectively, as determined by phenol-sulfuric acid method [24]. Before use, GQP, ASP, and AMP were diluted in Dulbecco's modified Eagle's medium (DMEM) and filter-sterilized through a sterile 0.22-μm filter. Dimethyl sulfoxide (DMSO) and MTT were obtained from Sigma, St. Louis, MO, USA. Fetal bovine serum (FBS) and DMEM were purchased from Gibco, Auckland, New Zealand, USA. Cytochemical staining kit of SA-β-gal and BCA protein assay kit were obtained from Beyotime Biotechnology, Haimen, Jiangsu, China. ELISA kit was from R&D Systems, Minneapolis, Minnesota, USA. PVDF membrane was from Bio-Rad, Hercules, CA, USA. Rabbit anti-human anti-p53, anti-p16INK4, and anti-β-actin monoclonal antibodies were purchased from Cell Signaling Technology, Beverly, MA, USA. Cell plates were obtained from Corning, Corning, New York, USA.

Cell culture, drug administration, and H_2O_2 induction

Normal human fetal lung fibroblast WI-38 cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. These cells were cultured in DMEM supplemented with 10% FBS in a 37°C , 5% CO_2 humidified incubator. Cells from 15-25 passages were used in this study to avoid replicative senescence as WI-38 cells have a mean life span of about 45-60 passages.

WI-38 cells were divided into the following groups: (1) the normal control group that was free from intervention; (2) the model group that was treated with H_2O_2, without drug treatments; (3) the GQP group that was pretreated with GQP and then subjected to H_2O_2 induction; (4) the ASP group that was pretreated with ASP and then subjected to H_2O_2 induction; and (5) the AMP group that was pretreated with AMP and then subjected to H_2O_2 induction. For drug administration, the culture medium was removed and 500 μl GQP, ASP, or AMP (diluted in DMEM without FBS) at indicated concentration was added into each well for 2-h incubation. For the concentration dependence assay, the serial concentrations of GQP were set at 0, 0.00064, 0.0032, 0.016, 0.08, 0.4, and 2 mg/ml; for the following experiments, the treatment concentration was set at 0.016 mg/ml. After drug administration, the supernatant was discarded, and H_2O_2 (diluted in DMEM without FBS) was added to incubate the cells for 2 h to induce premature senescence. For the concentration dependence assay, the concentrations of H_2O_2 induction were set at 0, 1, 10, 100, 200, 400, 800, 1600, 3200, and 6400 μmol/L; for the following experiments, the induction concentration was set at 100 μmol/L. After induction, H_2O_2 induction solution was replaced by fresh culture medium, and the following assays were performed.

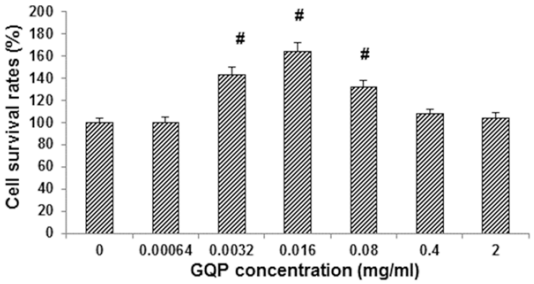

Figure 1. Effects of GQP on proliferation of WI-38 cells. WI-38 cells were treated with 0.00064, 0.0032, 0.016, 0.08, 0.4, and 2 mg/ml GQP, respectively, for 48 h. The cell proliferation was detected by the MTT assay, and the cell survival rate was calculated accordingly. Compared with the normal control group, $^{\#}P < 0.05$.

MTT assay

MTT assay was used to determine the cell viability [25]. WI-38 cells in 100 μl DMEM were seeded into 96-well plates at a density of 8 × 10^3 cells/well. After drug administration and H_2O_2 induction, the medium was replaced by 20 μl MTT (5 mg/ml) for a further incubation for 4 h. Then MTT was removed, and 150 μl DMSO was added to each well. The absorbance (A) at 490 nm was read using a microplate reader. The cell survival rate was calculated using the following equation:

Cell survival rate = $A_{treatment}/A_{control}$ × 100%.

Senescence-associated β-galactosidase (SA-β-gal) activity assessment

SA-β-gal activity was detected using a SA-β-gal staining kit, according to the manufacturer's instructions. Briefly, WI-38 cells were seeded into a 24-well plate. On Day 5 after drug administration and H_2O_2 induction, cells were washed twice with PBS (pH 7.2) and then fixed with 3.7% formaldehyde in PBS for 3-5 min. Next, the cells were incubated in SA-β-gal staining solution (1 mg/ml X-gal, 40 mM citric acid/sodium phosphate pH 6.0, 5 mM potassium ferrocyanide, 150 mM NaCl, and 2 mM $MgCl_2$) at 37°C , without CO_2, for 12-16 h. After rinsed with PBS, cells were observed under microscope, and SA-β-gal positive cells were counted. The experiment was performed in triplicate.

Telomerase activity determination

On day 5 after drug administration and H_2O_2 induction, cells were trypsinized and re-suspended in PBS (pH 7.2) to obtain a concentration of 1 × 10^6 cells/ml. Repeated freeze-thaw cycle method was used to damage cells, and supernatant was collected by centrifugation (5000 × g at 4°C for 20 min). Telomerase activity was detected using a human telomerase (TE) ELISA kit, according to the manufacturer's instructions. Absorbance at 450 nm was read using a microplate reader. The telomerase activity were determined using the following method: the straight line regression equation of the standard curve was calculated with the standard density and the OD value, and then the sample actual density was calculated with the sample OD value and the dilution factor. The experiment was performed in triplicate.

Flow cytometry and cell cycle analysis

On day 5 after drug administration and H_2O_2 induction, cells were trypsinized, and 1 × 10^6 cells were aliquoted in a polypropylene centrifuge tube. The cells were washed with PBS for three times, and re-suspended in 0.1 ml PBS. Then 1 ml 75% ethanol was added, and the cells were stored at 4-8°C overnight. After centrifugation (1500 × g at 4°C for 3 min), cells were collected and incubated in 1 ml DNA staining solution (0.1 mM EDTA in PBS pH 7.4, 0.05 mg/ml RNase A, 50 μg/ml propidium iodide) at 4-8°C for 30 min. Cell cycle was analyzed using a flow cytometer (BD FACSCalibur; Becton Dickinson, NJ, USA), and the results were evaluated using cell quest software.

Western blot analysis

On day 5 after drug administration and H_2O_2 induction, WI-38 cells were washed with iced PBS and then lysed with cell lysis buffer containing protease inhibitors cocktails for 20 min. Cell extract was collected by centrifugation at 12000 × g for 10 min. Protein concentration was determined using BCA protein assay kit. 50 μg protein was subjected to 12% SDS-PAGE. Proteins were blotted to PVDF membrane by electrophoresis. After blocked with TBS-T (5% nonfat milk in 25 mM Tris-HCl pH 7.4, 3 mM KCl, 140 mM NaCl, and 0.05% Tween), the membrane was subjected to incubation with anti-p53 (1:1000 dilution), anti-$p16^{INK4}$ (1:1000 dilution), and anti-β-actin (1:800 dilution) monoclonal antibodies, respectively, at 4°C overnight. After washing, the immunoblots were then incubated with horseradish peroxidase-

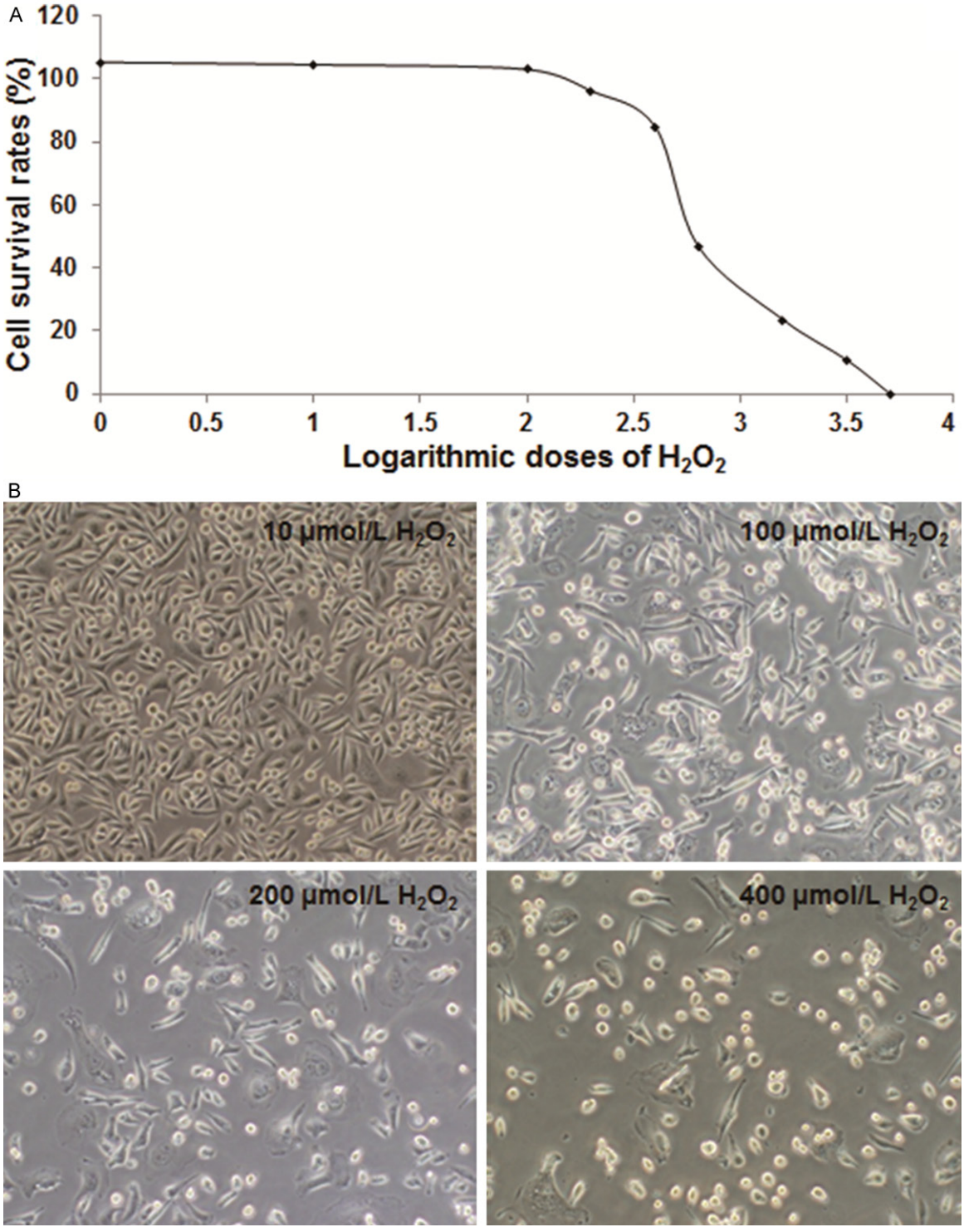

Figure 2. H_2O_2-induced premature senescence in WI-38 cells. A. WI-38 cells were treated with 100 μl H_2O_2 at the concentrations of 0, 1, 10, 100, 200, 400, 800, 1600, 3200, and 6400 μmol/L, respectively, for 2 h. Then MTT assay was performed to assess the cell survival rate. B. The representative morphology of WI-38 cells treated with 10, 100, 200, and 400 μmol/L H_2O_2 (× 200).

conjugated secondary antibodies at room temperature for 2 h. The enhanced chemiluminescence (ECL) method was used to detect the protein bands.

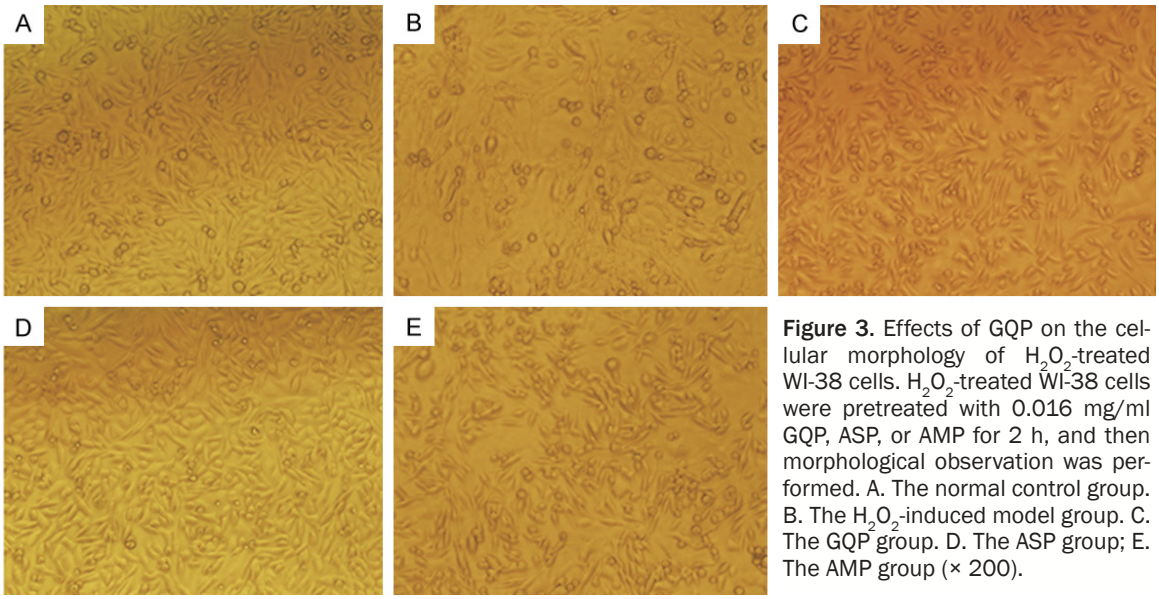

Figure 3. Effects of GQP on the cellular morphology of H_2O_2-treated WI-38 cells. H_2O_2-treated WI-38 cells were pretreated with 0.016 mg/ml GQP, ASP, or AMP for 2 h, and then morphological observation was performed. A. The normal control group. B. The H_2O_2-induced model group. C. The GQP group. D. The ASP group; E. The AMP group (× 200).

Statistical analysis

Data were expressed as mean ± SD. PASW 18.0 software was used for statistical analysis. One-way ANOVA was used for the multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

GQP promotes the proliferation of WI-38 cells

The effects of GQP on the growth and proliferation of WI-38 cells were first investigated. WI-38 cells were treated with 0.00064, 0.0032, 0.016, 0.08, 0.4, and 2 mg/ml GQP, respectively, for 48 h, and then the cell proliferation was detected by the MTT assay. As shown in **Figure 1**, the promoting effects of GQP on the proliferation of WI-38 cells showed up at the concentration of 0.0032 mg/ml ($P < 0.05$), and peaked at 0.016 mg/ml ($P < 0.05$), after which the promoting effects started to decline. These results suggest that GQP treatment could promote the proliferation of WI-38 cells, in a dose-dependent manner. Accordingly, the treatment concentration of GQP was set at 0.016 mg/ml in the following experiments.

H_2O_2 treatment induces premature senescence in WI-38 cells

Cellular premature senescence model was established in WI-38 cells by H_2O_2 treatment. WI-38 cells were treated with 100 μl H_2O_2 at indicated concentrations (0, 1, 10, 100, 200, 400, 800, 1600, 3200, and 6400 μmol/L) for 2 h. Then MTT assay was performed to assess the cell viability and survival rate. Our results showed that the fibroblast proliferation activity was decreased along with the increasing doses of H_2O_2 treatment. The logarithmic doses of H_2O_2 and related cell survival death rates were shown in **Figure 2A**, indicating a LD50 value between 2.6 (400 μmol/L) and 2.9 (800 μmol/L). The cell survival rates for H_2O_2 treatment concentrations of 10, 100, 200, and 400 μmol/L were 104.1%, 101%, 94.0%, and 85%, respectively. Further observation of the cellular morphology under H_2O_2 treatment was carried out. WI-38 cells were treated with H_2O_2 (10, 100, 200, and 400 μmol/L, respectively) for 2 h, and then cultured with normal medium. On Day 5, cellular morphology was detected under an inverted microscope. Significantly changed cellular morphology was observed after H_2O_2 treatment (**Figure 2B**). Based on these results, 100 μmol/L H_2O_2 was chosen as the optimal treatment concentration to induce senescence in the following experiments.

GQP restores the cellular morphology of H_2O_2-treated WI-38 cells

Effects of GQP on the morphology of H_2O_2-treated WI-38 cells were next investigated. As shown in **Figure 3**, normal control cells were small and fusiform in shape, with clear and continuous edges. In contrary, H_2O_2-treated WI-38 cells were enlarged in size, and filled with vacu-

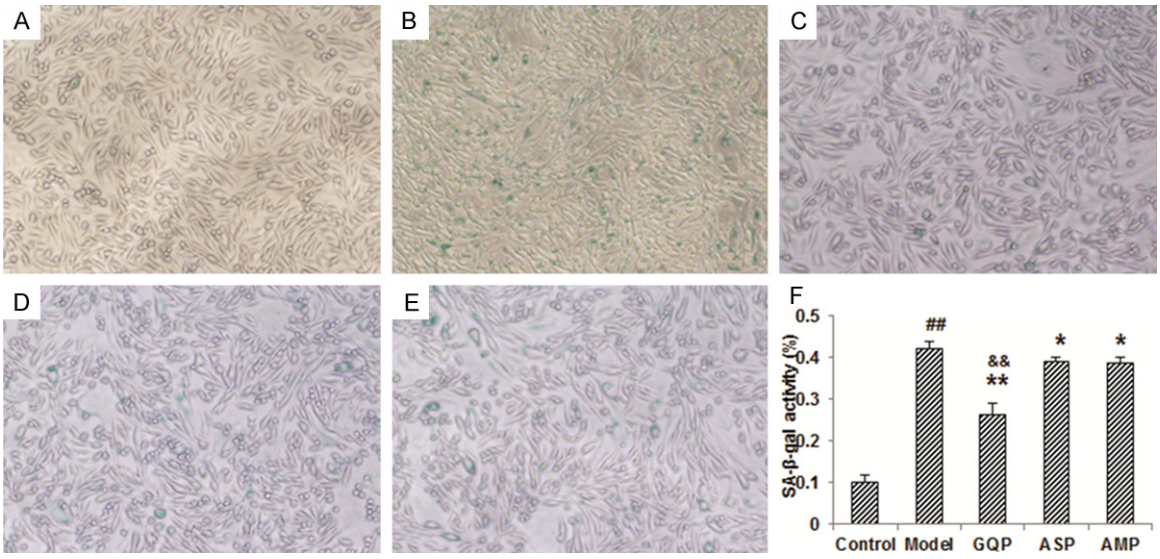

Figure 4. Effects of GQP on the SA-β-gal activity in H_2O_2-treated WI-38 cells. WI-38 cells were subsequently incubated with polysaccharides and X-gal substrate. Then the expression of SA-β-gal activity was detected. Blue-stained cells were identified as senescent cells. A. The normal control group. B. The H_2O_2-induced model group. C. The GQP group. D. The ASP group; E. The AMP group (× 200). F. Statistical analysis of SA-β-gal activities. Compared with the normal control group, $^{\#\#}P < 0.01$; compared with the model group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with the ASP and AMP groups, $^{\&\&}P < 0.01$.

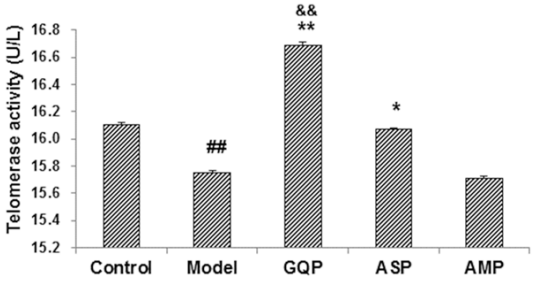

Figure 5. Effects of GQP on the telomerase activity in H_2O_2-treated WI-38 cells. H_2O_2-treated WI-38 cells were pretreated with 0.016 mg/ml GQP, ASP, or AMP for 2 h. Ultraviolet spectrophotometry was performed to evaluate the telomerase activities in these cells. Compared with the normal control group, $^{\#\#}P < 0.01$; compared with the model group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with the ASP and AMP groups, $^{\&\&}P < 0.01$.

oles, implying that H_2O_2 induced senescence in these cells. On the other hand, senescent cells treated with 0.016 mg/ml GQP, *Angelica sinensis* polysaccharide (ASP), or *Astragalus membranaceus* polysaccharide (AMP) displayed fusiform appearance, with oval nuclei. Unlike H_2O_2-treated cells, senescent cells in the drug treatment groups no longer show senescent characteristics with accumulation of granular cytoplasmic inclusions. These results suggest that GQP, ASP, and AMP could restore the morphological changes in WI-38 cells induced by H_2O_2 treatment.

GQP decreases the SA-β-gal activity in H_2O_2-treated WI-38 cells

To further evaluate the protective effects of GQP on H_2O_2-induced senescent WI-38 cells, these cells were subsequently incubated with polysaccharides and X-gal substrate. Then the expression of SA-β-gal activity, another cellular senescence marker, was detected. As shown in **Figure 4**, normal control cells expressed low SA-β-gal activity. In H_2O_2-treated cells, as least 80% cells were positive for SA-β-gal activity, indicating that H_2O_2 enhanced SA-β-gal activities in WI-38 cells (**Figure 4**). When cells were pretreated with GQP, AMP, and ASP, compared with H_2O_2-treated cells, significant declined SA-β-gal activities were observed ($P < 0.01$). These results indicate that GQP treatment could decrease the elevated SA-β-gal activity in H_2O_2-treated WI-38 cells.

GQP elevates the telomerase activity in H_2O_2-treated WI-38 cells

Telomerase can elongate telomeres and delay cellular aging, whose activity is regulated by many factors. 'To further evaluate the effects of

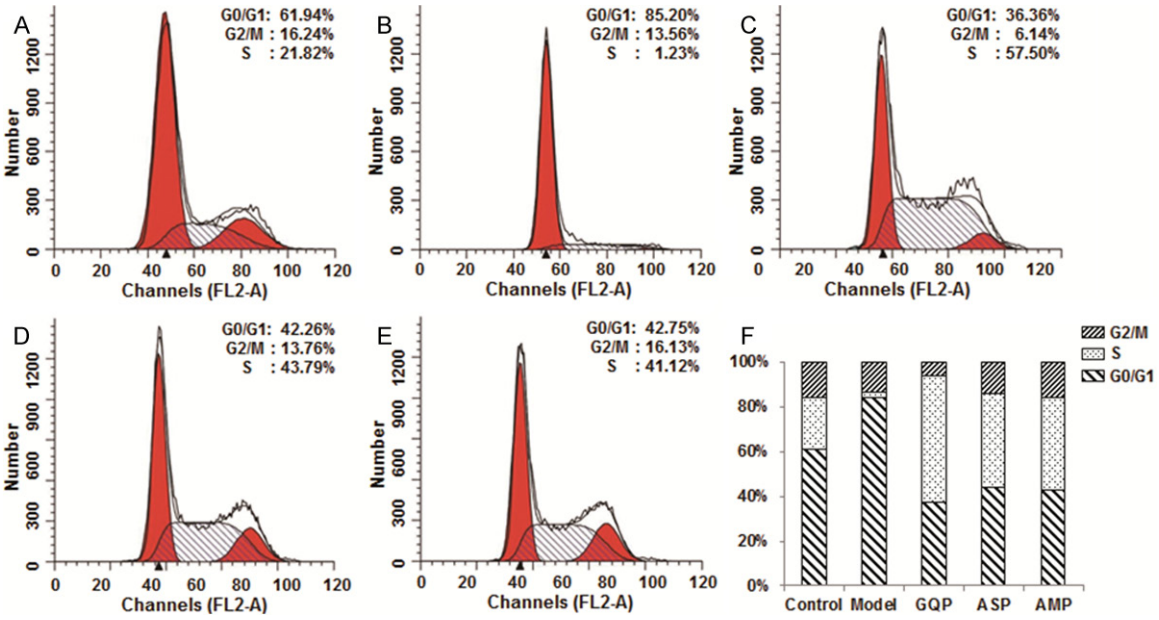

Figure 6. Effects of GQP on cell cycle of H_2O_2-treated WI-38 cells. H_2O_2-treated WI-38 cells were pretreated with 0.016 mg/ml GQP, ASP, or AMP for 2 h. Cell cycle analysis was performed with flow cytometry. Representative figures were shown. A. The normal control group. B. The H_2O_2-induced model group. C. The GQP group. D. The ASP group; E. The AMP group (× 200). F. Statistical analysis of cell cycle fraction.

GQP on telomerase activity in senescence, ultraviolet spectrophotometry was performed in H_2O_2-treated WI-38 cells. Our results showed that, compared with the normal control, H_2O_2 treatment significantly inhibited the telomerase activity and the cell proliferation of WI-38 cells ($P < 0.01$). In contrast, the telomerase activities in premature senescent WI-38 cells were obviously increased by ASP ($P < 0.05$) and AMP treatments, respectively, which was further elevated by GQP treatment (compared with the normal control group, $P < 0.01$; compared with the ASP and AMP groups, $P < 0.05$) (**Figure 5**). These findings suggest that GQP could effectively elevate the telomerase activities in senescent cells, which is superior to ASP or AMP treatment alone.

GQP abrogates cell cycle arrest in H_2O_2-treated WI-38 cells

Another well-known feature of cellular senescence is G1 cell cycle arrest. Our results indicated that GQP could promote cell proliferation. To investigate whether the promoting effects of GQP on cell proliferation is based on its influence on cell cycle, H_2O_2-treated WI-38 cells were subjected to flow cytometry analysis. Our results indicated that H_2O_2-treated WI-38 cells showed predominant G1 phase fraction (85.20%) and minimal S phase fraction (13.56%), compared with normal control cells with G1 phase fraction of 61.94% and S phase fraction of 21.82%, respectively (**Figure 6**). However, GQP pretreatment resulted in a significant decrease in G1 phase fraction (36.36%) and a concomitant increase in S phase fraction (57.5%). Similar results in cell cycle were observed in WI-38 cells treated with AMP (G1 phase fraction of 42.75% and S phase fraction of 41.12%) and ASP (G1 phase fraction of 42.46% and S phase fraction of 43.79%), even though not so potent as GQP treatment. These results indicate that GQP treatment could effectively promote the cell cycle re-entry into S phase in senescent cells, which might contribute to its anti-senescence effects.

GQP down-regulates the expression of senescence markers in H_2O_2-treated WI-38 cells

The p53-p21 and p16-pRb pathways have been shown to be closely correlated with cellular senescence [26]. We next examined the effects of GQP on the expression levels of p53 and p16 in H_2O_2-treated WI-38 cells. Western blot analysis showed that, compared with the normal control group, the protein expression levels of

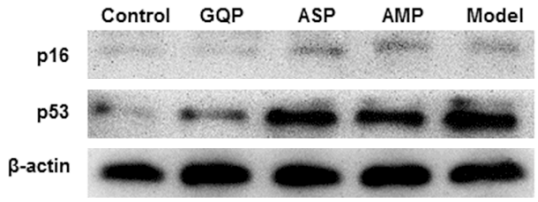

Figure 7. Effects of GQP on the expression of senescence markers in H_2O_2-treated WI-38 cells. H_2O_2-treated WI-38 cells were pretreated with 0.016 mg/ml GQP, ASP, or AMP for 2 h. The protein expression levels of p53 and p16 were detected by Western blot analysis.

p53 and p16 were dramatically up-regulated in H_2O_2-treated WI-38 cells, indicating the activation of the p53-p21 and p16-pRb signal transduction pathways in these cells, which was consistent with the H_2O_2-induced premature senescence. When these H_2O_2-treated cells were subjected to ASP or AMP treatment, the expression levels of p53 and p16 were significantly decreased. Moreover, GQP treatment resulted in potent declined expression levels of p53 and p16 in these senescent cells, which were comparable to the normal control group (**Figure 7**). These results suggest that GQP could significantly down-regulate the p53-p21 and p16-pRb pathways in oxidative stress-induced senescence.

Discussion

Aging is a complex physiological process, which is always characterized by progressive memory loss, contributing to dementia pathogenesis like Parkinson's and Alzheimer's diseases [27]. Nowadays, there are several popular theories trying to explain the aging process [26], one of which is the free radical theory [28]. Increasing evidence has indicated that oxidative stress plays an important role in the aging process [29]. Cellular senescence induced by H_2O_2 has been widely used to evaluate the anti-aging effects of drugs [30]. H_2O_2 could induce oxidative stress in cells, which may involve the overproduction and accumulation of oxygen free radicals [31].

Among multiple components of *Angelica sinensis* and *Astragalus membranaceus*, polysaccharides have been considered as the most important gradient with therapeutic activities [32, 33]. However, so far, there are few reports in the literature regarding the anti-aging effects of GQP. In the present study, we investigated the anti-aging activities of GQP in WI-38 cells treated by H_2O_2 and the related mechanisms. We found the optimal concentration of H_2O_2 treatment in inducing premature senescence in WI-38 cells, and established H_2O_2-induced premature senescent WI-38 cell model. H_2O_2 treatment at 100 μmol/L could cause significant changes in cellular morphology and cytoplasmic inclusions, which were typical senescent features. Our results showed that GQP could not only dramatically restore the altered morphology, but also significantly increase the cell viability and proliferation. The protective effects of GQP against H_2O_2-induced cellular senescence were further confirmed by the detection of senescence markers. SA-β-gal activity is the most widely used indicator for cellular senescence [34, 35]. Our results showed that H_2O_2 led to an increase in SA-β-gal activity in WI-38 cells, which could be inhibited by GQP treatment.

To further investigate the mechanism underlying the anti-senescence effects of GQP, the cell cycle distribution was examined by flow cytometry. Our results demonstrated that H_2O_2 treatment could cause G1 cell cycle arrest and decrease S phase fraction cells. However, GQP treatment could promote the cell cycle re-entry into S phase in senescent cells. In addition, GQP could down-regulate the protein expression levels of p53 and p16 in H_2O_2-treated WI-38 cells, which might also contribute to its proliferation-promoting and anti-senescence effects. Moreover, our results revealed that the effectiveness of GQP was much stronger than AMP or ASP treatment alone. However, how these signaling pathways could be activated by GQP, ASP, and AMP is still not clear and further investigations are still needed.

In conclusion, our results showed that GQP could restore morphological changes, decrease SA-β-gal activity, elevate telomerase activity, abrogate cell-cycle arrest, and inhibit p53-p21 and p16-pRb pathways in H_2O_2-treated WI-38 cells, superior to AMP or ASP treatment alone, suggesting that GQP could exert protective effects in oxidative stress-induced senescence. These results suggest the promising role of GQP as an attractive and bio-safe agent with the potential to retard senescence and attenuate senescence-related diseases.

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Disclosure of conflict of interest

None.

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References

[1] Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL and van Deursen JM. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 2011; 479: 232-236.

[2] Rando TA. Stem cells, ageing and the quest for immortality. Nature 2006; 441: 1080-1086.

[3] Yang D, Song J, Wu L, Ma Y, Song C, Dovat S, Nishizaki T and Liu J. Induction of senescence by adenosine suppressing the growth of lung cancer cells. Biochem Biophys Res Commun 2013; 440: 62-67.

[4] Acosta JC and Gil J. Senescence: a new weapon for cancer therapy. Trends Cell Biol 2012; 22: 211-219.

[5] Hayflick L and Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961; 25: 585-621.

[6] Marcotte R and Wang E. Replicative senescence revisited. J Gerontol A Biol Sci Med Sci 2002; 57: B257-269.

[7] Serrano M and Blasco MA. Putting the stress on senescence. Cur Opin Cell Biol 2001; 13: 748-753.

[8] Roninson IB. Tumor cell senescence in cancer treatment. Cancer Res 2003; 63: 2705-2715.

[9] Campisi J and d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. Nature reviews. Mol Cell Biol 2007; 8: 729-740.

[10] Chen QM, Tu VC and Liu J. Measurements of hydrogen peroxide induced premature senescence: senescence-associated beta-galactosidase and DNA synthesis index in human diploid fibroblasts with down-regulated p53 or Rb. Biogerontology 2000; 1: 335-339.

[11] Schmid I, Uittenbogaart C and Jamieson BD. Live-cell assay for detection of apoptosis by dual-laser flow cytometry using Hoechst 33342 and 7-amino-actinomycin D. Nature Protoc 2007; 2: 187-190.

[12] Kim CO, Huh AJ, Han SH and Kim JM. Analysis of cellular senescence induced by lipopolysaccharide in pulmonary alveolar epithelial cells. Arch Gerontol Geriatr 2012; 54: e35-41.

[13] Kosar M, Bartkova J, Hubackova S, Hodny Z, Lukas J and Bartek J. Senescence-associated heterochromatin foci are dispensable for cellular senescence, occur in a cell type- and insult-dependent manner and follow expression of p16(ink4a). Cell Cycle 2011; 10: 457-468.

[14] Nie W and Zhang YX. Progress of the immuno-modulating effect of polysaccharides and their mechanisms. Chinese Pharmacological Bulletin 1999; 15: 484-487.

[15] Tian GY. Research and development of traditional Chinese medicine (TCM) immune regulator. Chinese Journal of New Drugs 1999; 8: 721-724.

[16] Zuo ZY and Liu ZX. New biological response regulator, angelica polysaccharides (ASDP) combined antitumor effects and impact on a tumor-burdened little mouse immune function. Chinese Journal of Immunology 2000; 16: 545-546.

[17] He WJ, Yuan ZJ and He XS. Research progress on pharmacological effects of Astragalus polysaccharide. Chinese Journal of Biochemical Pharmaceutics 2012; 33: 692-694.

[18] Cai L and Zhu J. Research Status and Development of Astragalus Polysaccharide. Chinese Journal of Clinical Oncology 2007; 34: 896-900.

[19] Pu XY, Li Y, Zhang WJ, Wang HR and Wang P. Study on Anti-aging Effect of Guiqi Polysaccharides. Natural Product Research and Development 2012; 24: 1630.

[20] Pu XY, Li Y and Wang P. Study on Anti-oxidation of Guiqi Polysaccharides in Vitro. China Food Industry 2011; 1: 64-66.

[21] Pu XY, Li Y, Zhou LY. Deferring senile effect of polysaccharides from Angelica and Astragalus on aging mice. International Conference on Human Health and Biomedical Engineering HHBE 2011; 289-292.

[22] Pu XY, Wang HR, Li Y, Fan WB, Yu S. Antiviral activity of GuiQi polysaccharides against enterovirus 71 in vitro. Virologica Sinica 2013; 28: 352-359.

[23] Sun Y, Wang S, Li T, Li X, Jiao L and Zhang L. Purification, structure and immunobiological activity of a new water-soluble polysaccharide from the mycelium of Polyporus albicans (Imaz.) Teng. Bioresour Technol 2008; 99: 900-904.

[24] Dubois M, Gilles KA and Hamilton JK. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 1956; 28: 350-356.

[25] Kim SY, Kang HT, Choi HR and Park SC. Biliverdin reductase A in the prevention of cel-

lular senescence against oxidative stress. Exp Mol Med 2011; 43: 15-23.
[26] Kumar A, Prakash A and Dogra S. Protective effect of curcumin (Curcuma longa) against D-galactose-induced senescence in mice. J Asian Nat Prod Res 2011; 13: 42-55.
[27] Lan Z, Liu J, Chen L, Fu Q, Luo J, Qu R, Kong L and Ma S. Danggui-Shaoyao-San ameliorates cognition deficits and attenuates oxidative stress-related neuronal apoptosis in d-galactose-induced senescent mice. J Ethnopharmacol 2012; 141: 386-395.
[28] Harman d. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 11: 298-300.
[29] Nohl H. Involvement of free radicals in ageing: a consequence or cause of senescence. Bri Med Bull 1993; 49: 653-667.
[30] Roudkenar MH, Halabian R, Ghasemipour Z, Roushandeh AM, Rouhbakhsh M, Nekogoftar M, Kuwahara Y, Fukumoto M and Shokrgozar MA. Neutrophil gelatinase-associated lipocalin acts as a protective factor against H(2)O(2) toxicity. Arch Med Res 2008; 39: 560-566.
[31] Ha JS, Sung HY, Lim HM, Kwon KS and Park SS. PI3K-ERK1/2 activation contributes to extracellular H_2O_2 generation in amyloid β toxicity. Neurosci Lett 2012; 526: 112-117.
[32] Jin M, Zhao K, Huang Q and Shang P. Structural features and biological activities of the polysaccharides from Astragalus membranaceus. Int J Biol Macromol 2014; 64: 257-266.
[33] Hua Y, Xue W, Zhang M, Wei Y and Ji P. Metabonomics study on the hepatoprotective effect of polysaccharides from different preparations of Angelica sinensis. J Ethnopharmacol 2014; 151: 1090-1099.
[34] Kurz DJ, Decary S, Hong Y and Erusalimsky JD. Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. J Cell Sci 2000; 113: 3613-3622.
[35] Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I and Pereira-Smith O. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A 1995; 92: 9363-9367.