



Anti-senescence effect and molecular mechanism of the major royal jelly proteins on human embryonic lung fibroblast (HFL-I) cell line*

Chen-min JIANG¹, Xin LIU¹, Chun-xue LI¹, Hao-cheng QIAN¹,
Di CHEN¹, Chao-qiang LAI², Li-rong SHEN^{†‡1}

¹College of Biosystems Engineering and Food Science, Zhejiang University / Key Laboratory of Agro-Products Postharvest Handling of Ministry of Agriculture and Rural Affairs / Zhejiang Key Laboratory for Agri-Food Processing, Hangzhou 310058, China

²USDA ARS Nutritional Genomics Laboratory, JM-USDA Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts 02111, the United States

[†]E-mail: Shenlirong@zju.edu.cn

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Abstract: Royal jelly (RJ) from honeybee has been widely used as a health promotion supplement. The major royal jelly proteins (MRJPs) have been identified as the functional component of RJ. However, the question of whether MRJPs have anti-senescence activity for human cells remains. Human embryonic lung fibroblast (HFL-I) cells were cultured in media containing no MRJPs (A), MRJPs at 0.1 mg/ml (B), 0.2 mg/ml (C), or 0.3 mg/ml (D), or bovine serum albumin (BSA) at 0.2 mg/ml (E). The mean population doubling levels of cells in media B, C, D, and E were increased by 12.4%, 31.2%, 24.0%, and 10.4%, respectively, compared with that in medium A. The cells in medium C also exhibited the highest relative proliferation activity, the lowest senescence, and the longest telomeres. Moreover, MRJPs up-regulated the expression of superoxide dismutase-1 (*SOD1*) and down-regulated the expression of mammalian target of rapamycin (*MTOR*), catenin beta like-1 (*CTNNB1*), and tumor protein p53 (*TP53*). Raman spectra analysis showed that there were two unique bands related to DNA synthesis materials, amide carbonyl group vibrations and aromatic hydrogens. These results suggest that MRJPs possess anti-senescence activity for the HFL-I cell line, and provide new knowledge illustrating the molecular mechanism of MRJPs as anti-senescence factors.

Key words: Major royal jelly protein; Human embryonic lung fibroblast (HFL-I) cell line; Anti-senescence; Relative proliferation activity; Telomere length; Molecular mechanism

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1 Introduction

Royal jelly (RJ), secreted from hypopharyngeal and mandibular glands of nurse honeybees, contains a considerable amount of bioactive substances and has been widely used as a food (Buttstedt et al., 2014).

About 50% of the dry mass of RJ is protein (Malecová et al., 2003), of which 80% belongs to the major royal jelly proteins (MRJPs) (Schmitzová et al., 1998). RJ is the principal food for honeybee queens (Shen et al., 2010). It is believed that exclusive feeding on RJ induces a honeybee larva to develop into a queen, whose lifespan can reach a maximum of eight years much longer than that of worker bees (Remolina and Hughes, 2008). For its beneficial effect on honeybee queens, RJ has become a well-known healthcare product in many countries in Asia and Europe. Consumers anticipate that RJ could exert a similar health benefit in humans.

[‡] Corresponding author

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 ORCID: Li-rong SHEN, <https://orcid.org/0000-0002-3197-9245>

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Previous studies have shown that RJ has several kinds of bioactivity, including anti-oxidation (Jamnik et al., 2007; Cihan et al., 2013), neuronal function protection (Hashimoto et al., 2005; Narita et al., 2009; Hattori et al., 2010; Zamani et al., 2012), and immunoregulatory effects (Šver et al., 1996; Oka et al., 2001; Okamoto et al., 2003; Majtán et al., 2006; Mannoor et al., 2009). Moreover, RJ enhances the reproductivity of many animals such as female sheep, rabbit (Husein and Kridli, 2002), rat, quail, and hen (Shen et al., 2009). Many studies have also found that RJ is effective in extending the lifespans of mice (Inoue et al., 2003) and worms (Honda et al., 2011).

Ten proteins with a molecular weight of 49–87 kDa have been reported in the MRJP family (Schmitzová et al., 1998; Drapeau et al., 2006). MRJP1, the most abundant component in the MRJP family, is the primary active substance in RJ (Kamakura, 2011). MRJPs can stimulate DNA synthesis and increase albumin production in hepatocytes (Kamakura et al., 2001). They also protect cells from apoptosis by the action of several important intracellular signaling factors (Kamakura, 2002). MRJPs extracted from RJ can stimulate the proliferation of several cell lines, including hepatocytes (Kamakura et al., 2001), human myeloid cell line U-937 (Watanabe et al., 1998), human monocytes (Kimura et al., 2003), and Tn-5B1-4 insect cells (Salazar-Olivo and Paz-González, 2005). Our recent study suggests that MRJPs can increase mean lifespan and reproductivity in *Drosophila* (Xin et al., 2016) and partially replace fetal bovine serum (FBS) for culturing several human cell lines and increase the cell viability (Chen et al., 2016).

The question of whether MRJPs have anti-senescence activity for human cells such as human embryonic lung fibroblast (HFL-I) cells remains. Therefore, in this study we examined the effects of MRJPs on the proliferation activity, population doubling level (PDL), senescence-associated β -galactosidase (SA- β -gal) activity, telomere length, expression of age-related genes, and Raman spectra of HFL-I cells.

2 Materials and methods

2.1 Cell line and reagents

The HFL-I cell line was obtained from Shanghai Sixin Biotechnology Co., Ltd. (Shanghai, China).

Primers were supplied by TaKaRa Co., Ltd. (Beijing, China). Mammalian DNA extraction and SA- β -gal activity kits were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Reverse transcription polymerase chain reaction (RT-PCR) and real-time quantitative PCR (qPCR) kits were supplied by TaKaRa Co., Ltd. (Beijing, China). A 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay kit was purchased from Nanjing Keygen Biotech. Co., Ltd. (Nanjing, China). FBS was purchased from HyClone Laboratories, Inc. (Logan, USA). F12K media were supplied by Gino Biotechnology Co., Ltd. (Hangzhou, China). All biochemical reagents were of the highest purity commercially available.

2.2 Preparation of MRJPs

Fresh RJ was provided by the Hangzhou Biyun-tian Heath Food Co., Ltd. (Hangzhou, China) and stored at -80°C . For extraction, RJ was diluted in phosphate buffered saline (PBS) and extracted at 4°C for 12 h. Then the extraction was centrifuged at 12000g for 30 min at 4°C . MRJPs were obtained from the upper layer of the extraction, which was dialyzed against 500 volumes of double-distilled water (ddH₂O) using a 1000 Da cutoff dialysis membrane at 4°C (Salazar-Olivo and Paz-González, 2005). The concentration of MRJPs was measured by the Bradford method using bovine serum albumin (BSA) as a standard (Bradford, 1976).

2.3 HFL-I cultivation

The HFL-I cell line was cultured in F12K medium supplied with 10% (v/v) FBS at 37°C in a humid environment with 5% (v/v) CO₂. MRJPs and BSA solution were diluted with F12K medium by 5 mg/ml, and added to media as calculated. Five treatments were prepared as follows: (A) no MRJPs, (B) 0.1 mg/ml MRJPs, (C) 0.2 mg/ml MRJPs, (D) 0.3 mg/ml MRJPs, and (E) 0.2 mg/ml BSA. Cells were exposed to the various concentrations of MRJPs or BSA and cultured until they reached cellular senescence.

2.4 Morphological observations

Cells were observed every day and cell images were taken using an inverted microscope. The shape, size, and refractivity of HFL-I cells were considered as indicators to evaluate the condition of the cells.

2.10 Raman spectrum analysis

With different frequencies of incident light, Raman spectroscopy can detect and analyze scattering spectra, molecular vibration, and rotation information from live cells. This method is often used as a diagnostic tool to study cancer cell lines or a single cancer cell (Yan et al., 2005; Li et al., 2013). It has been used to analyze the senescence of cells in vivo (Bai et al., 2015). Here we used Raman spectroscopy to estimate the biochemical changes in HFL-I cells in media treated with different concentrations of MRJPs.

After the culture media were removed, the cells were washed twice with PBS gently. Raman spectral data were obtained using a laser Raman microscope. An excitation laser of 785 nm with power of 50 mW was focused on a single cell each time through a 10× objective lens. Raman scattering light from the sample was collected and detected by a liquid nitrogen supplied charge coupled device (CCD) with a grating (600 gr/mm) under the visible region. Each cell was exposed to the laser for 3 s, and the spectrum of this targeted cell was drawn and analyzed using Microsoft Excel. The spectral resolution error was less than 5 cm^{-1} , and the diameter of the laser spot at the focus point was less than $1\text{ }\mu\text{m}$.

2.11 Statistical analysis

The data reported represent average values from at least three independent experiments and were analyzed using Statistical Product and Service Solutions

(SPSS) software. One-way analysis of variance (ANOVA) was used to evaluate differences between each group. If there was a significant difference ($P < 0.05$) between groups, Duncan analysis was used to compare all the groups in pairs. The values are shown as mean \pm standard deviation (SD), and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Morphological phenotypes of senescent cells

Cellular morphological observations showed that the manifestation of HFL-I cells was influenced by MRJPs. As shown in Fig. 1a, most of the cells in medium A (no MRJPs) became flat, and the cellular boundary was obscure, a cellular phenotype typical of senescent cells. Many HFL-I cells displayed the unattached state, losing their spindle shapes. However, most cells in medium C (0.2 mg/ml MRJPs; Fig. 1c) and medium D (0.3 mg/ml MRJPs; Fig. 1d) had a healthy shape and normal size. The cell boundaries were distinct. The condition of the cells in medium B (0.1 mg/ml MRJPs; Fig. 1b) and medium E (0.2 mg/ml BSA; Fig. 1e) was similar, and some of the cells in both media were in a senescent state.

3.2 Lifespan-extending effect of MRJPs

Fig. 2 shows that the average PDL of cells differed significantly among the treatments ($P < 0.001$).

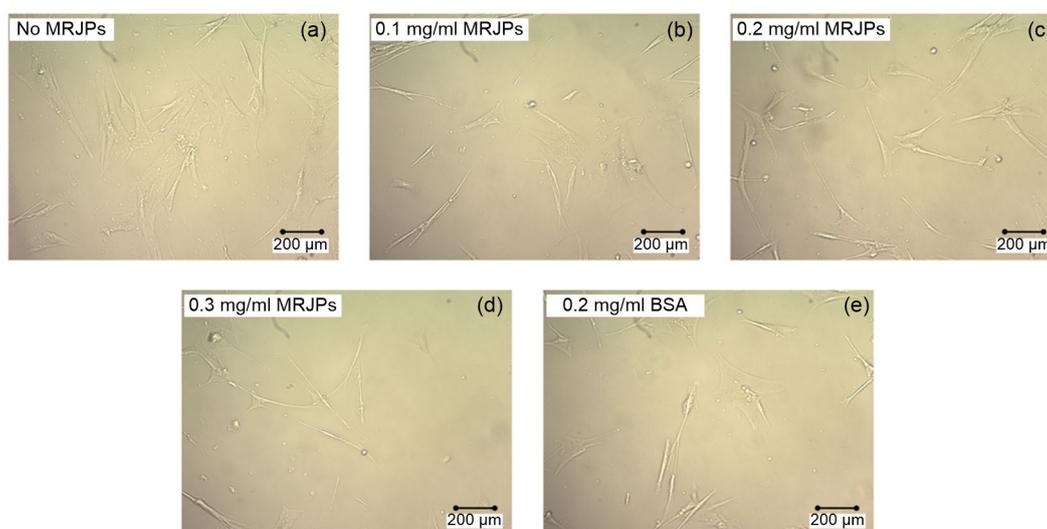


Fig. 1 Cellular morphology of HFL-I cells supplied with different concentrations of MRJPs or BSA at Day 59

Compared with the cells in medium A, the PDL of the cells in media B, C, D, and E increased by 12.4%, 31.2%, 24.0%, and 10.4%, respectively. Compared with the cells in medium E, the PDL of the cells in media B, C, and D increased by 1.8%, 18.8%, and 12.4%, respectively. The cells in medium C had the highest PDL, followed by the cells in media D, A, and E. Thus, a concentration of 0.2 mg/ml MRJPs (medium C) was the optimal dose for lifespan-extension of HFL-I cells.

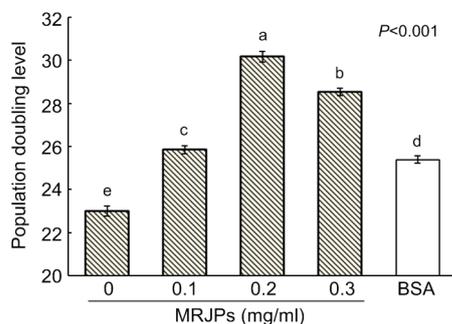


Fig. 2 Lifespans of HFL-I cells supplied with different concentrations of MRJPs or BSA

The PDL represents the lifespan of HFL-I. Data are expressed as mean±SD ($n=3$). Different lowercase letters (a–e) above bars indicate a significant difference from each other at $P<0.05$ by Duncan's multiple range tests

3.3 Proliferation effect of MRJPs

On the fourth day, the cells in media A, B, C, D, and E displayed differential cellular proliferation activity (absorbance): 0.39, 0.40, 0.45, 0.44, and 0.42, respectively (Fig. 3). Compared with the negative control (medium A), the relative proliferation activity (RPA) of the cells in media B, C, D, and E had increased by 1.0%, 11.9%, 11.1%, and 7.7%, respectively. In addition, the cells in medium B showed no significant difference ($P>0.05$) when compared with those in medium A after 4 d. However, the RPA of the cells in media C and D had increased significantly ($P<0.001$) compared with that of cells in medium A after 4 d.

3.4 SA- β -gal activity measurement

The percentage of positive cells measured by SA- β -gal activity assay (Fig. 4) showed the extent of the senescence of HFL-I cells. The cells in medium C

had the lowest percentage of positive cells (19.7%), followed by the cells in media D (41.0%) and B (33.7%). Cells in medium A had the highest percentage (63.7%), followed by those in medium E (43.7%). The percentage of positive cells in media B, C, D, and E had decreased by 47.1%, 69.1%, 35.6%, and 31.4% respectively, compared with that in medium A.

3.5 Effect of MRJPs on telomere length

Fig. 5 shows the effects of supplemental MRJPs of different concentrations in media on the relative telomere length in HFL-I cells. The cells in medium A

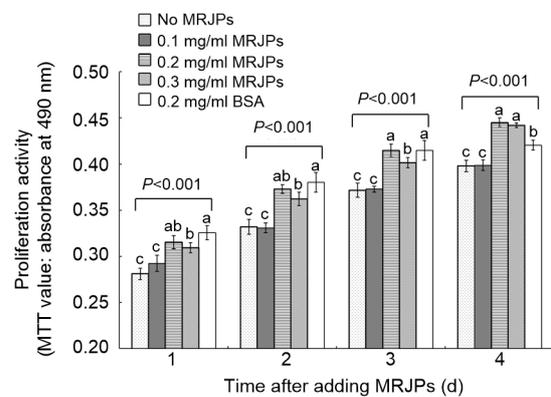


Fig. 3 Proliferation effect of MRJPs after 4 d

The proliferation activity represents the relative proliferation of HFL-I. Data are expressed as mean±SD ($n=8$). Different lowercase letters (a–c) above bars within the same day indicate a significant difference from each other at $P<0.05$ by Duncan's multiple range test

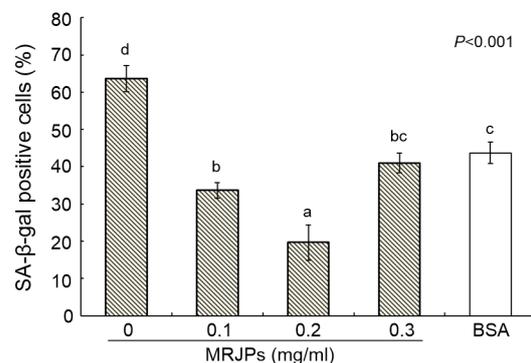


Fig. 4 Percentages of senescent cells after consecutive culturing for 59 d

Cells stained blue were defined as senescent. Data are expressed as mean±SD ($n=3$). Different lowercase letters (a–d) above bars indicate a significant difference from each other at $P<0.05$ by Duncan's multiple range test

clearly exhibited the lowest relative length (RL). The RL of HFL-I cells in medium C was significantly different from the RLs of cells in the other media. The RLs of cells in all the media containing MRJPs were higher than those of media E and A. Compared with the RL of cells in medium A, the RLs of the cells in media B, C, D, and E increased by 6%, 50%, 10%, and 4%, respectively.

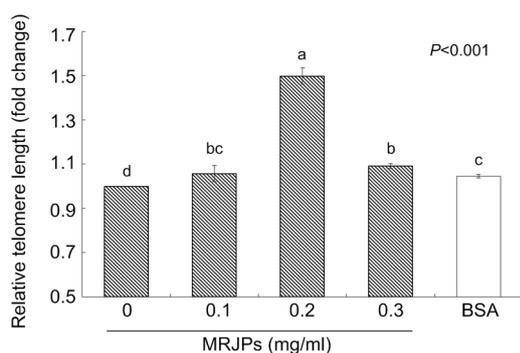


Fig. 5 Telomere length using qPCR

Data are shown as fold-changes of the negative control group (medium A). Data are expressed as mean \pm SD ($n=3$). Different lowercase letters (a–d) above bars indicate a significant difference from each other at $P<0.05$ by Duncan's multiple range test

3.6 Effect of MRJPs on age-related gene expression

To understand the molecular mechanism underlying the anti-senescence effect of MRJPs on HFL-I cells, we measured the RE of mammalian target of rapamycin (*MTOR*), superoxide dismutase-1 (*SOD1*), catenin beta like-1 (*CTNNB1*), and tumor protein p53 (*TP53*) in HFL-I cells (Fig. 6).

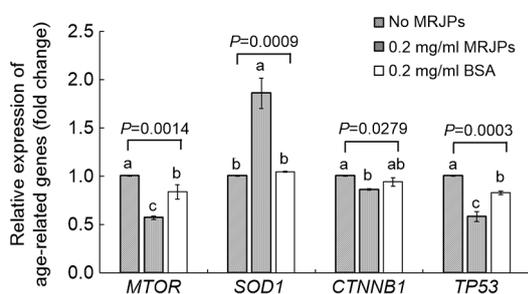


Fig. 6 Age-related gene expression using qPCR

Data are shown as fold-changes of the negative control group (medium A). Data are expressed as mean \pm SD ($n=3$). Different lowercase letters (a–c) above bars within the same gene indicate a significant difference from each other at $P<0.05$ by Duncan's multiple range test

Cells in medium C (0.02 mg/ml MRJPs) were used to evaluate the effect on gene expression exerted by MRJPs as they showed the longest lifespan. Cells in media A (no MRJPs) and E (0.02 mg/ml BSA) were used as negative and positive controls, respectively. Significant differences ($P<0.05$) in RE of four age-related genes were observed between cells in media C and A (negative control). Cells in medium C showed lower RE of *MTOR*, *CTNNB1*, and *TP53* than those in medium A, decreasing significantly ($P<0.05$) by 43%, 14%, and 42%, respectively. A significant increase ($P=0.0009$) of about 86% in *SOD1* expression was observed in cells in medium C compared with that in medium A.

On the other hand, cells in medium C had a higher RE level of *SOD1*, and lower RE levels of *MTOR* and *TP53* than those in medium E (positive control), while there was no significant difference in the RE of *CTNNB1* ($P>0.05$). Compared with medium E, media supplemented with MRJPs showed stronger bioactivity than BSA to some extent.

3.7 Raman spectrum analysis

To estimate the biochemical changes induced in HFL-I cells in media with different concentrations of MRJPs, we measured the mean spectra of treated cells normalized by the Raman peak of phenylalanine at 1002 cm^{-1} . The contributions of peaks, as identified by Li et al. (2013) and Movasaghi et al. (2007), are listed in Table 2. The mean spectra from the different treatments were roughly similar (Fig. 7). However, bands at 1342 and 1536 cm^{-1} were detected only on the spectra of MRJP-treated groups and were assigned to purine (guanine and adenine) and amide carbonyl group vibrations and aromatic hydrogens, respectively.

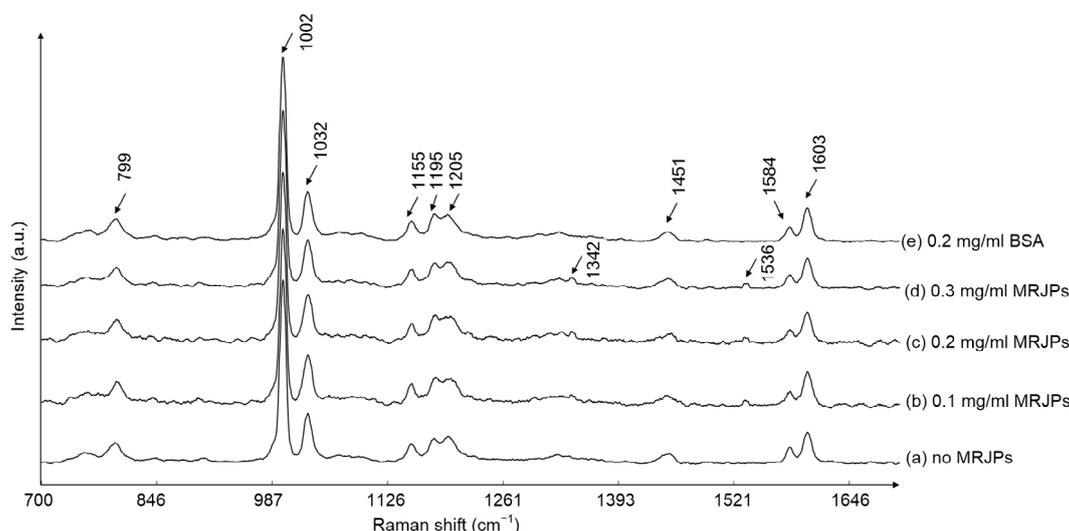
4 Discussion

In this study, we discovered that MRJPs may exert an anti-senescence effect on HFL-I cells. The cells in MRJP media experienced no or delayed typical cell aging, whereas cells developed senescence in MRJP-free media. The PDL assay showed that MRJPs enabled extension of the lifespan of HFL-I cells. The cells in MRJP media also had higher RPA, lower SA- β -gal activity, and longer telomere length

Table 2 Band positions and contributions

Raman shift (cm ⁻¹)	Contribution
799	Backbone geometry and phosphate ion interactions
1002	Phenylalanine
1032	C–H/C–C stretching of phenylalanine
1155	C–C and C–N stretching of proteins
1195	Anti-symmetric phosphate vibrations
1205	Differences in collagen content
1342	Guanine and adenine
1451	CH ₂ bending and CH ₂ CH ₃ deformation of proteins and collagens
1536	Amide carbonyl group vibrations and aromatic hydrogens
1584	C=C bending of phenylalanine
1603	C=C vibration of phenylalanine and tyrosine

The content references from Li et al. (2013) and Movasaghi et al. (2007)

**Fig. 7 Mean Raman spectra of HFL-I cells**

Substance changes as shown through Raman spectra. Each number in this figure represents one position of a band, as explained in Table 2. a.u.: arbitrary unit

than cells in MRJP-free media. In MRJP-supplied media, the expression of *SOD1* increased and the expression of *MTOR*, *CNNB1*, and *TP53* decreased. In addition, Raman spectra analysis showed that peaks assigned to purine and amide carbonyl groups changed when MRJPs were added to the media.

The HFL-I cell line has been widely used in studies investigating cell senescence, apoptosis, proliferation, growth factors, and toxicological tests. Matsuo (2004) revealed that the lifespan of HFL-I cells is perturbed under oxidative stress. HFL-I is also used as a model organism in cell apoptosis tests (Xu et al., 2012; Ren et al., 2013; Chen et al., 2014; Ershova et al., 2016) and proliferation tests (Vietti et al., 2013; Zeng et al., 2015). We previously found that FBS culture media partially replaced by MRJPs could exert a proliferation effect on several cell lines,

including the HFL-I cell line (Chen et al., 2016). Cell senescence is followed by a series of phenomena, including morphological changes, the loss of proliferation ability, and an increase in SA- β -gal activity (Cristofalo and Pignolo, 1993; Dimri et al., 1995; Toussaint et al., 2000; Liu et al., 2007; Debacq-Chainiaux et al., 2008; Hwang et al., 2009). Morphological changes are widely used to identify senescent cells. One of the most apparent morphological hall-marks of senescent human diploid cells is that they usually have irregular flat and enlarged shapes (Cristofalo, 1988; Cho et al., 2004). Moreover, some senescent fibroblasts retract their branches resulting in a higher number of cells per unit area devoid of cell–cell contacts (Foyer et al., 2009). In the morphological assay, senescence-related cellular morphologies partially disappeared after adding 0.2 or 0.3 mg/ml MRJPs. Thus, we discovered

that MRJPs might have an anti-senescence effect on HFL-I cells. As for proliferation ability, the results of PDLs in this study imply that supplementation with MRJPs could significantly extend the mean lifespan of HFL-I cells. MTT assay after consecutive cultivation of HFL-I cells with or without different MRJPs concentrations also suggested that MRJPs might affect the proliferation of HFL-I cells.

Cellular senescence occurs following cell proliferation failure or exposure to a variety of cellular stresses, e.g. expression of activated oncogenes, oxidation, or DNA damaging agents. Studies have pointed out that proliferation rate is an essential parameter to judge the senescence level of cells (Toussaint et al., 2000) and that the SA- β -gal assay is a robust method to distinguish senescent cells from non-senescent cells in vitro (Itahana et al., 2004, 2007; Krishnamurthy et al., 2004; Liu et al., 2007; Debacq-Chainiaux et al., 2009). Our results showed that four-day incubation with addition of 0.2 or 0.3 mg/ml MRJPs significantly delayed the increase in senescent HFL-I cells, implying that MRJPs were likely to have promoted proliferation activity. From the results of SA- β -gal activity measurement, we found that the experimental MRJPs concentrations reduced the proportion of stained (senescent) cells and the best MRJP concentration was 0.2 mg/ml. Thus, MRJPs may play an important role in inhibiting the overexpression of β -galactosidase in HFL-I cells, and seem to exhibit an anti-senescence effect on HFL-I cells.

Telomeres are specialized functional complexes located at the ends of eukaryotic chromosomes (Blackburn, 2001). The shortening of telomeres could induce the DNA damage stress response and cell apoptosis. Without telomeres, terminal attrition of chromosomal DNA would lead to loss of genetic information and prevent cells from multiplying (Smogorzewska and de Lange, 2002; D'Adda di Fagnana et al., 2003; Takai et al., 2003). Numerous studies have proved that telomeres shorten with the aging of cells and tissues (Allsopp et al., 1992; Kitada et al., 1995; Jeanclous et al., 1998; Okuda et al., 2000). These phenomena all support the hypothesis that telomere length limits cell mitosis (Harley, 1991; Chan and Blackburn, 2004; Debacq-Chainiaux et al., 2008) and indicates the replicative potential and age of cells (Harley et al., 1990; Toussaint et al., 2000;

von Zglinicki et al., 2005). It has been reported that telomere length is positively associated with proliferative potential in fibroblasts (Allsopp et al., 1992; Murillo-Ortiz et al., 2013). The finding that cells in MRJP media have longer telomeres shows that MRJPs have the ability to inhibit the shortening of telomeres in HFL-I cells and proves the anti-senescence effect of MRJPs on HFL-I cells.

Based on gene expression analysis of age-related genes and Raman spectroscopy analysis, we speculate that the regulation of age-related gene expression and the promotion of DNA and protein syntheses by MRJPs might be responsible for our observed results. *MTOR*, *SOD1*, *CTNNB1*, and *TP53* are four age-related genes. *MTOR* plays a vital role in the mTOR/S6K pathway. The mTOR/S6K signal pathway can be regulated by age-related factors such as stress, growth factors, nutritional conditions, and energy supplements (Sarbasov et al., 2005; Kapahi et al., 2010). Previous studies have indicated that the lifespan of yeasts, worms, fruit flies, and mice can be extended by inhibiting mTOR/S6K signal pathway through gene knockout, rapamycin treatment, or dietary restriction (Kapahi et al., 2010). MRJPs may exert the anti-senescence effect through inhibiting the expression of *mTOR* and signal transduction in the mTOR/S6K pathway. The oxidative stress theory claims that the accumulation of oxidative damage induced by reactive oxygen species (ROS) will lead to senescence and apoptosis (Salmon et al., 2010). However, overexpression of *SOD1*, an enzyme located mainly in the cytosol to remove highly toxic superoxide radicals, extends the lifespan of *Drosophila*, while *sod1* knockdown causes a reduction of lifespan in mice and flies (Phillips et al., 1989; Elchuri et al., 2005; Landis and Tower, 2005). Previous reports revealed that many age-related genes are involved in the oxidative stress response (Zahn and Kim, 2007; Zhan et al., 2007; Salmon et al., 2010). Our results showed an overexpression of the *SOD1* gene in the cells supplied with MRJP media. This finding supports the idea that MRJPs may protect HFL-I cells from oxidative damage through promoting the expression of the *SOD1* gene and scavenging the ROS in vivo.

β -Catenin encoded by the *CTNNB1* gene is a signal molecule in the Wnt/ β -catenin pathway. This pathway is one of the most essential signal transduction

pathways in the growth process of vertebrate creatures. It has been proposed that the transduction level of the Wnt/ β -catenin pathway increases with age (Brack et al., 2007; Liu et al., 2007; Xu et al., 2008). Moreover, overexpression of the p53 protein is associated with overexpression of β -catenin (Damalas et al., 2001) and the activation of the Wnt/ β -catenin pathway (Mao et al., 2001; Hiyama et al., 2010). MRJPs might inhibit β -catenin through the down-regulation of *CTNNB1* gene expression, and the Wnt/ β -catenin pathway may be suppressed as a result of the lack of the signal molecule (β -catenin). p53, encoded by the *TP53* gene, plays a vital role in cell cycle control, cell stress reaction, and cell gene protection (May and May, 1999). p53 can be activated by cell stress factors such as genotoxicity, shortening of telomeres, hypoxia, and tumor gene activation (Kern et al., 1991). Senescence is induced by the accumulation of ROS oxidative damage, which leads to cell cycle arrest caused by *p21* and *E2F* (the target genes of *p53*) (Gewirtz et al., 2008). Our findings suggest that MRJPs down-regulate the expression of the *TP53* gene, which results in inhibition of *p21* and lifespan extension of HFL-I cells. These results are consistent with the results above, showing that MRJPs induce a longer lifespan in HFL-I cells.

Raman spectroscopy is a rapid and non-invasive technique, which enables us to analyze cells and tissues in vivo without additional labeling or staining (Buschman et al., 2000; Swain et al., 2008). Guanine and adenine are the materials of DNA replication. Amide carbonyl group and aromatic hydrogens are compounds of protein synthesis. From changes in peaks representing purine bases and amide carbonyl groups in the Raman spectroscopy assay, we inferred that MRJPs might promote the syntheses of DNA and proteins in HFL-I cells. Our findings are coincident with previous studies which suggested that MRJPs stimulate hepatocyte DNA synthesis, prolong the proliferation of hepatocytes, and increase albumin production (Kamakura et al., 2001; Kamakura, 2002). However, since only small peaks representing purine bases and amide carbonyl groups were observed, they might also represent products from catabolism. So in the future, more studies are needed to prove whether these changes are related to DNA and protein syntheses. In general, we successfully used Raman spectroscopy to explore differences between cells

growing in media with or without MRJPs. Our results might provide a new method to characterize cellular senescence.

5 Conclusions

We found that MRJPs could extend the lifespan of HFL-I cells when supplied in media. The anti-senescence effects of MRJPs were associated with up-regulation of *SOD1* and down-regulations of *MTOR*, *CTNNB1*, and *TP53*, and had a stimulatory effect on DNA and protein syntheses. The anti-senescence effect of MRJPs on the HFL-I cell line may indicate a beneficial effect of MRJPs on human health.

Compliance with ethics guidelines

Chen-min JIANG, Xin LIU, Chun-xue LI, Hao-cheng QIAN, Di CHEN, Chao-qiang LAI, and Li-rong SHEN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题目: 蜂王浆主蛋白对人胚肺成纤维细胞HFL-I的抗衰老作用及其作用机制研究

目的: 探讨不同浓度蜂王浆主蛋白(MRJPs)对人胚肺成纤维细胞HFL-I的抗衰老作用,并对其可能的分子机理进行预测。

创新点: 本实验采用拉曼光谱法探究添加MRJPs对HFL-I细胞内成分的影响。实验发现添加MRJPs的

HFL-I与对照组的HFL-I的拉曼光谱图中,与DNA和蛋白质合成相关的拉曼峰发生了变化。该发现在未来可作为鉴定衰老HFL-I的一种手段。

方法: 实验以蜂王浆为原料,利用透析法得到MRJPs。通过对不同浓度MRJPs处理的HFL-I进行细胞形态学观察,测定HFL-I生命周期以及检测其 β -半乳糖苷酶活性,评价了MRJPs的抗衰老效果。并且通过MTT法考察了不同浓度MRJPs对HFL-I的促增殖作用。同时,实验一方面采用实时定量聚合酶链反应(qPCR)测量了不同浓度MRJPs处理的HFL-I的细胞端粒相对长度以及*MTOR*、*SOD1*、*CTNIB1*、*TP53*四种衰老相关基因的相对表达量;另一方面还通过拉曼光谱实验法,进一步探索了MRJPs抗衰老的分子机理。

结论: 本实验结果显示,添加MRJPs能降低HFL-I衰老细胞所占比例,显著延长HFL-I的生命周期,一定量的MRJPs还能够促进HFL-I的增殖。此外,MRJPs还能减缓细胞端粒长度的缩短,上调衰老相关基因*SOD1*的表达,下调抗衰老基因*MTOR*、*CTNIB1*和*TP53*的表达。通过MRJPs添加组细胞的拉曼光谱上出现的独特的碱基、酰胺羰基和芳香氢峰,推测MRJPs的抗衰老作用可能与促进DNA及蛋白质在细胞中的合成密切相关。

关键词: 蜂王浆主蛋白;人胚肺成纤维细胞;抗衰老;拉曼光谱;端粒长度;分子机理