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Original Article

Protective effects of adipose-derived stem cells secretome on human dermal fibroblasts from ageing damages

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Abstract: Background: Combined effects of intrinsic and extrinsic ageing factors on skin tissue and the therapies have been rarely studied before. ADSCs have gained popularity in anti-ageing field, which may provide promising methods to fight against skin ageing. Objective: To find out the fate of HDFs exposed to intrinsic or extrinsic ageing factors or both of them and further examine the impacts of ADSC-CM on the damaged HDFs. Methods: We irradiated HDFs with UVB at different senescent levels, and then treated them with ADSC-CM. After 48 h, we detected cellular proliferative activity, morphology, SA-β-Gal expression, apoptosis, mRNA expression of collagen I, collagen III and elastin. Results: Intrinsic ageing factors inhibited cellular proliferation, increased senescent ratio and reduced mRNA expression of collagen I, collagen III and elastin, so did UVB, except for its induction of elastin mRNA expression. Furthermore, ADSC-CM treatment can slightly or significantly improve cellular proliferative activity and restore functions both in irradiated and non-irradiated HDFs. Besides, ADSC-CM treatment decreased cellular apoptosis and senescence induced by UVB but had no obvious effect on cellular senescence induced by intrinsic ageing factors. The results were similar in three generations of HDFs, yet in different degrees. Conclusions: The results suggest that ADSCs secretome protect HDFs from ageing damages but with some limitations.

Keywords: Adipose-derived stem cells, secretory factors, anti-ageing, human dermal fibroblasts

Introduction

Skin ageing, which is mostly characterized by loss of elasticity and generation of wrinkles, confuses human for centuries and inspires us to make great efforts to fight against it, yet still remains a conundrum. As exposed directly to the outside environment, skin undergoes extrinsic ageing except for intrinsic ageing; both of them are associated with structural and functional changes in cutaneous tissue [1, 2]. In the present study, we focus on fibroblasts because they are the main source of dermal extracellular matrix (ECM) proteins, mainly are collagens and elastins that are processed to assemble fibers conferring tensile strength and resilience to skin, thus maintains the homeostasis and juvenscence of skin [3, 4]. Ageing damages lead to decline of the amount, life span and function of fibroblasts in dermis and finally result in ageing appearance, which indicates the significance of fibroblasts in researching on mechanisms and further therapies of skin ageing [5, 6]. *In vitro* studies, fibroblasts can be serially passaged until they reach replicative senescence to simulate intrinsic ageing [7, 8]. On the other hand, it is universally accepted that ultraviolet rays serve as a major extrinsic factor (approximately 80%) in skin ageing and ultraviolet radiation B (UVB)-irradiated fibroblasts have been established as an experimental model applied in various studies related to skin ageing [9, 10].

Adipose-derived stem cells (ADSCs) have gained popularity in fields of regeneration medicine and stem cell therapy for reasons that the raw material (adipose tissue) is easy to get and process, the isolated cells expand rapidly and can be induced to differentiate into multiple cell types *in vitro* [11-13]. What's more, ADSCs can synthesize and secrete several cytokines and antioxidants [14-17]. Recent studies have reported ADSCs may act as an effective therapy

for cutaneous photoageing both in vivo and in vitro [18-21]. Besides, ADSCs can increase collagen synthesis and angiogenesis and moreover exhibit protective effect against D-galactose-induced skin ageing in mice [22, 23]. These reports indicate the flourishing application prospect of ADSCs in anti-ageing industry.

Skin ageing results from the combined effects of intrinsic and extrinsic ageing factors, but previous studies preferred to investigate intrinsic or extrinsic ageing separately thus failed to establish a skin ageing model accorded with actual situation and better explore the underlying mechanisms. In addition, few researches focused on effects of ADSCs on intrinsic ageing damages. In our group, human dermal fibroblasts (HDFs) at different senescent levels were exposed to UVB then cultured in conditioned medium of ADSCs (ADSC-CM) that contains the whole raw secretome of and takes advantages of easier storage and safer application over ADSCs. Afterwards we detected related biochemical indexes, aiming at more comprehensively observing the ageing characteristics of HDFs as well as evaluating the anti-ageing effects of ADSCs secretome.

Materials and methods

Isolation and culture of human ADSCs (hADSCs)

Human subcutaneous adipose tissue was harvested from a 20-year-old woman who underwent liposuction surgery with informed patient consent at the approval of the Research Ethical Committee of the First Hospital of China Medical University. The isolation procedures were based on the published methods with minor modifications [24]. Briefly, 150 ml human lipoaspirate was washed in phosphate buffered saline (PBS; Hyclone, Logan, UT, USA) and minced finely, then digested in 0.075% collagenase I (Gibco, New York, NY, USA) at 37.0°C with constant vibration for about 45 min. Digestion was terminated by adding Dulbecco's Modified Eagle Medium/F12 (DMEM; Hyclone) containing 10% Fetal Bovine Serum (FBS; Gibco), then the digested tissue was centrifuged (1200 rpm, 25°C, 5 min). After that, the supernanant was discarded and the pellet containing cells at the bottom was washed in PBS then centrifuged (1000 rpm, 25°C, 5 min) for twice. Cells were resuspended in common culture media (DMEM containing 10% FBS and 1% penicillin-streptomycin) and counted, then inoculated at a density of 5×10^6 cells per 100 mm dish. First culture medium change was conducted 36 h after inoculating and routinely repeated every 72 h. When cells reached 80-90% confluence, the subculture was operated using trypsin (Gibco) containing EDTA.

Identification of hADSCs

hADSCs (passage 3) were harvested and washed in PBS for thrice. Cell suspension was incubated with FITC-conjugated antibodies against CD13, CD29, CD34 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 37.0°C for 30 min in the dark, followed by being washed and resuspended in PBS then detected by flow cytometer (BD Biosciences, San Jose, CA, USA).

Preparation of ADSC-CM

When hADSCs (Passage 4) reached 80% confluence, the culture medium was changed into DMEM. ADSC-CM was collected after 72 h, then processed by being centrifuged at 300 × g for 5 min and being filtered with a 0.22 μm syringe filter (Jet Bio-Filtration, Guangzhou, China).

Culture, UVB radiation and ADSC-CM treatment of HDFs

On the basis of what Yeo *et al*. reported previously [25, 26] and our preliminary experiments, we picked out cells at passage 5, 15 and 28 as young, intermediate and senescent generation respectively. HDFs isolated from dermis (passage 3) were offered by Department of Dermatology, the First Hospital of China Medical University and cultured in their corresponding dishes and cultured until reaching 70% confluence, then washed once with PBS and immersed in a thin-layer PBS to be irradiated with a single dose (200 mJ/cm^2) of UVB by using a UV lamp (Waldmann, Villingen-Schwenningen, Germany). Once irradiation completed, medium of both irradiated and non-irradiated HDFs was replaced with control medium (DMEM containing 10% FBS) or 100% ADSC-CM. Cells were then cultured in incubator for 48 h.

Cell proliferative activity detection

HDFs were seeded (2×10^3 per well) into 96-well plates and treated with above procedure, then incubated for 3 h after adding a tenth volume Cell Counting Kit-8 solution (CCK-8 kit; Beyotime, Shanghai, China) per well. The opti-

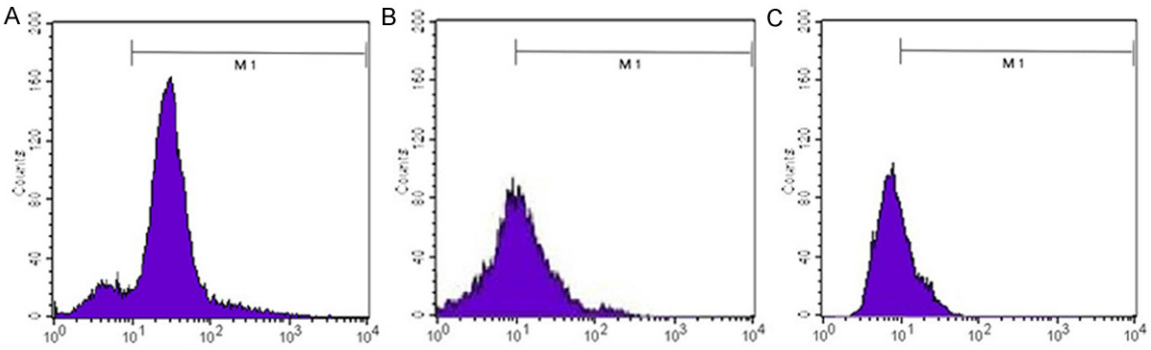

Figure 1. Cell surface markers expression. A. CD13: 87.37%; B. CD29: 86.57%; C. CD34: 2.51%.

Table 1. OD value, senescent ratio, apoptotic ratio and relative mRNA expression of collagen I, collagen III and elastin of HDFs at different senescent levels under different treatments

Parameters		Con	Con+ADSC-CM	UVB	UVB+ADSC-CM
OD value (n=5)	P5	0.5670 ± 0.0318	0.7654 ± 0.0482**	0.3330 ± 0.0396**	0.4034 ± 0.0297#
	P15	0.5250 ± 0.0400	0.6772 ± 0.0502**	0.3170 ± 0.0404**	0.3762 ± 0.0356#
	P28	0.4496 ± 0.0440	0.4711 ± 0.0301	0.3096 ± 0.0390**	0.3180 ± 0.0310
Ratio of senescent cells (n=5)	P5	0.0008 ± 0.0011	0.0004 ± 0.0009	0.8932 ± 0.0353**	0.3668 ± 0.0204##
	P15	0.3980 ± 0.0231	0.3940 ± 0.0183	0.9560 ± 0.0113**	0.7000 ± 0.0271##
	P28	0.8908 ± 0.0213	0.8916 ± 0.0155	0.9740 ± 0.0134**	0.9336 ± 0.0385
Ratio of apoptotic cells (n=5)	P5	0.0004 ± 0.0009	0.0000 ± 0.0000	0.5164 ± 0.0392**	0.1132 ± 0.0178##
	P15	0.0012 ± 0.0018	0.0004 ± 0.0009	0.3052 ± 0.0263**	0.1080 ± 0.0191##
	P28	0.0000 ± 0.0000	0.0004 ± 0.0009	0.0616 ± 0.0096**	0.0460 ± 0.0084#
Expression of collagen I mRNA (n=6)	P5	1.0260 ± 0.0400	1.1231 ± 0.0423**	0.9104 ± 0.0138**	0.9513 ± 0.0342#
	P15	0.9713 ± 0.0181	1.0302 ± 0.0358**	0.8040 ± 0.0362**	0.8074 ± 0.0152
	P28	0.9807 ± 0.0160	1.0132 ± 0.0114**	0.7110 ± 0.0088**	0.6991 ± 0.0117
Expression of collagen III mRNA (n=6)	P5	1.0293 ± 0.0329	1.0774 ± 0.0402*	0.9799 ± 0.0167*	1.0012 ± 0.0144#
	P15	0.9646 ± 0.0196	1.0016 ± 0.0334*	0.8888 ± 0.0444**	0.9150 ± 0.0173
	P28	0.9821 ± 0.0142	1.0074 ± 0.0111**	0.8876 ± 0.0192**	0.8855 ± 0.0283
Expression of elastin mRNA (n=6)	P5	1.0111 ± 0.0238	1.0243 ± 0.0349	1.0920 ± 0.0221**	1.0847 ± 0.0527
	P15	0.9661 ± 0.0203	0.9755 ± 0.0203	1.0571 ± 0.0523**	1.0168 ± 0.0173
	P28	1.0354 ± 0.0203	1.0371 ± 0.0243	1.0677 ± 0.0111**	1.0724 ± 0.0246

Values are mean ± sd. *P < 0.05 and **P < 0.01 compared with con group. #P < 0.05 and ##P < 0.01 compared with UVB group.

cal density (OD) values were measured at 450 nm with a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Cellular senescence detection

HDFs were seeded (5×10^4 per well) into 6-well plates and treated with above procedure, then cells were fixed and stained with senescence associated β-galactosidase (SA-β-Gal) Staining Kit solution (Beyotime). After being incubated in a humidified, without CO_2 injection, constant temperature (37.0°C) environment overnight, the cellular morphology and staining condition were observed and photographed using a light microscopy (OLYMPUS, Tokyo, Japan) at magnification of 100 and 200, senescent cells were counted and their ratio was figured out.

Cellular apoptosis detection

HDFs were seeded (5×10^4 per well) into 6-well plates and treated with above procedure, then cells were fixed and stained with Hoechst staining solution (Beyotime). After discarding stain solution, cells were washed twice with PBS then observed and photographed using a fluorescence microscopy (OLYMPUS) at magnification of 100 and 200, apoptotic cells were counted and their ratio was figured out.

Quantitive real time polymerase chain reaction (qRT-PCR)

Relative expression of type I collagen, type III collagen and elastin were measured by qRT-

Table 2. Differences of impacts of UVB or ADSC-CM treatment on HDFs at different senescent levels

Parameters		A	B	C
OD value (n=5)	P5	0.1984	-0.234	0.0704
	P15	0.1522	-0.208	0.0592
	P28	0.0215	-0.14	0.0084
Ratio of senescent cells (n=5)	P5	-0.04%	89.24%	-52.64%
	P15	-0.4%	55.8%	-25.6%
	P28	0.08%	8.32%	-4.04%
Ratio of apoptotic cells (n=5)	P5	-0.04%	51.6%	-40.32%
	P15	-0.08%	30.4%	-19.72%
	P28	0.04%	6.16%	-1.56%
Expression of collagen I mRNA (n=6)	P5	0.0971	-0.1156	0.0409
	P15	0.0589	-0.1673	0.0034
	P28	0.0326	-0.2697	-0.0119
Expression of collagen III mRNA (n=6)	P5	0.0480	-0.0494	0.0213
	P15	0.0370	-0.0759	0.0263
	P28	0.0252	-0.0945	-0.0022
Expression of elastin mRNA (n=6)	P5	0.0132	0.0809	-0.0072
	P15	0.0093	0.0910	-0.0403
	P28	0.0017	0.0323	0.0047

Differences of average values of detected indexes between groups (A. Control group and con+ADSC-CM group; B. Control group and UVB group; C. UVB group and UVB+ADSC-CM group) at different senescent levels (Negative value means inhibitory effects of the treatment factor, positive value means promoting effects of the treatment factor).

PCR. HDFs were seeded (5×10^4 per well) into 6-well plates and treated with above procedure, then total RNA was extracted using the RNAiso Plus (TaKaRa, Shanghai, China) and quantitated by spectrophotometer (NanoDrop products, Wilmington, DE, USA). Afterwards, the obtained RNA was converted to cDNA using PrimeScriptTM RT Master Mix (TaKaRa) by PCR thermal cycler (Applied Biosystems, Darmstadt, Germany). The qRT-PCR assay was performed using SYBR Premix Ex TaqTM II (TaKaRa) by LightCycler 480 System (F. Hoffmann-La Roche, Ltd, Basel, Switzerland). Sequences of Primers used in this study were as followed:

Human GAPDH (Forward: 5'-ACATCGCTCAGACACCATG-3'; Reverse: 5'-TGTAGTTGAGGTCAATGAAGGG-3'); human type I collagen (Forward: 5'-CCCCTGGAAAGAATGGAGATG-3'; Reverse: 5'-TCCAAACCACTGAAACCTCTG-3'); human type III collagen (Forward: 5'-AAGTCAAGGAGAAAGTGGTCG-3'; Reverse: 5'-CTCGTTCTCCATTCTTACCAGG-3'); human elastin (Forward: 5'-CCTGGCTTCGGATTGTCTC-3'; Reverse: 5'-CAA AGGGTTTACATTCTCCACC-3').

Statistical analysis

Data were analyzed using SPSS package 19.0 (SPSS Inc., Chicago, IL, USA). Comparisons among the groups were analyzed with independent sample t-test. $P < 0.05$ was considered statistically significant, $P < 0.01$ was considered obviously statistically significant.

Results

Characterization and identification of hADSC

At passage 3, hADSCs displayed CD13 and CD29 (both are stromal stem cell surface markers) positive expression, whereas CD34 (hematopoietic stem cell marker) negative expression (**Figure 1**).

Comparison of cellular proliferative activity

In young HDFs (P5) culture, OD values of UVB group were obviously lower than that of control group ($P < 0.01$); ADSC-CM treatment groups exhibited higher OD values than common media treatment groups in non-irradiated ($P < 0.01$) and irradiated ($P < 0.05$) HDFs. Intermediate HDFs (P15) displayed similar trends to young HDFs. In senescent HDFs (P28) culture, OD values of UVB group were obviously lower than that of control group ($P < 0.01$); ADSC-CM treatment had no obvious impacts both in non-irradiated and irradiated HDFs ($P > 0.05$) (**Tables 1**, **2** and **Figure 2A**).

Comparison of cellular senescence

In young HDFs culture, senescent HDFs ratio of UVB group was obviously higher than control group ($P < 0.01$); Compared with common media, ADSC-CM treatment had no obvious effect on non-irradiated HDFs ($P > 0.05$), but can obviously decrease senescent ratio in irradiated HDFs ($P < 0.01$). Intermediate and senescent HDFs exhibited similar trends to young HDFs, except for the slight reduction of senescent ratio in irradiated senescent HDFs (P28) treated with ADSC-CM ($P>0.05$) (**Tables 1**, **2** and **Figures 2B**, **3**).

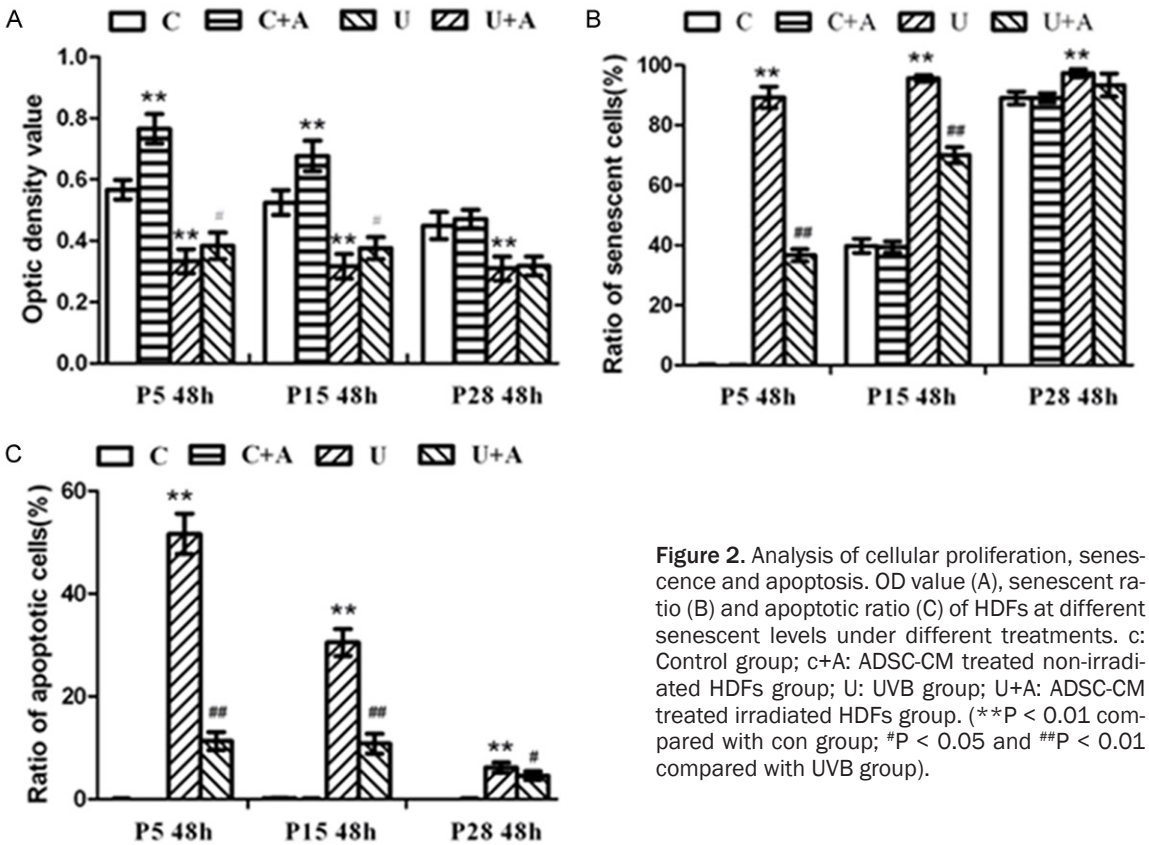

Figure 2. Analysis of cellular proliferation, senescence and apoptosis. OD value (A), senescent ratio (B) and apoptotic ratio (C) of HDFs at different senescent levels under different treatments. c: Control group; c+A: ADSC-CM treated non-irradiated HDFs group; U: UVB group; U+A: ADSC-CM treated irradiated HDFs group. (**P < 0.01 compared with con group; #P < 0.05 and ##P < 0.01 compared with UVB group).

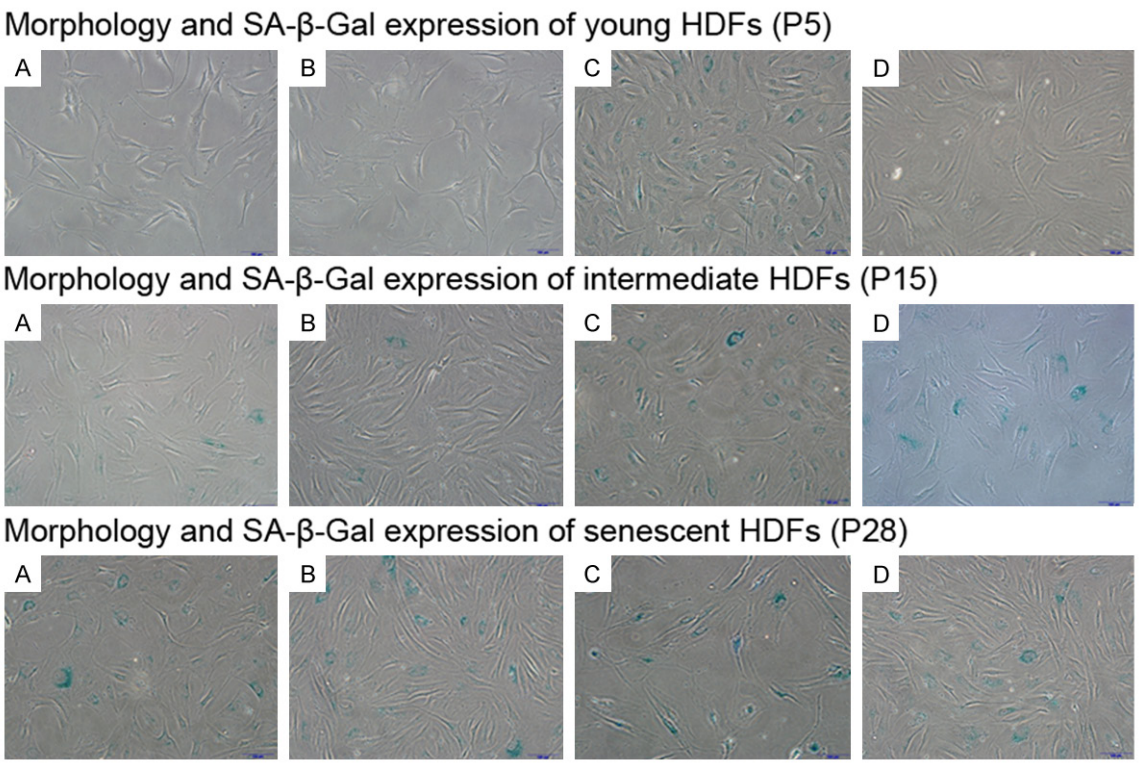

Figure 3. Morphology and SA-β-Gal expression. A: Control group; B: ADSC-CM treated non-irradiated HDFs group; C: UVB group; D: ADSC-CM treated irradiated HDFs group. (magnification 200×).

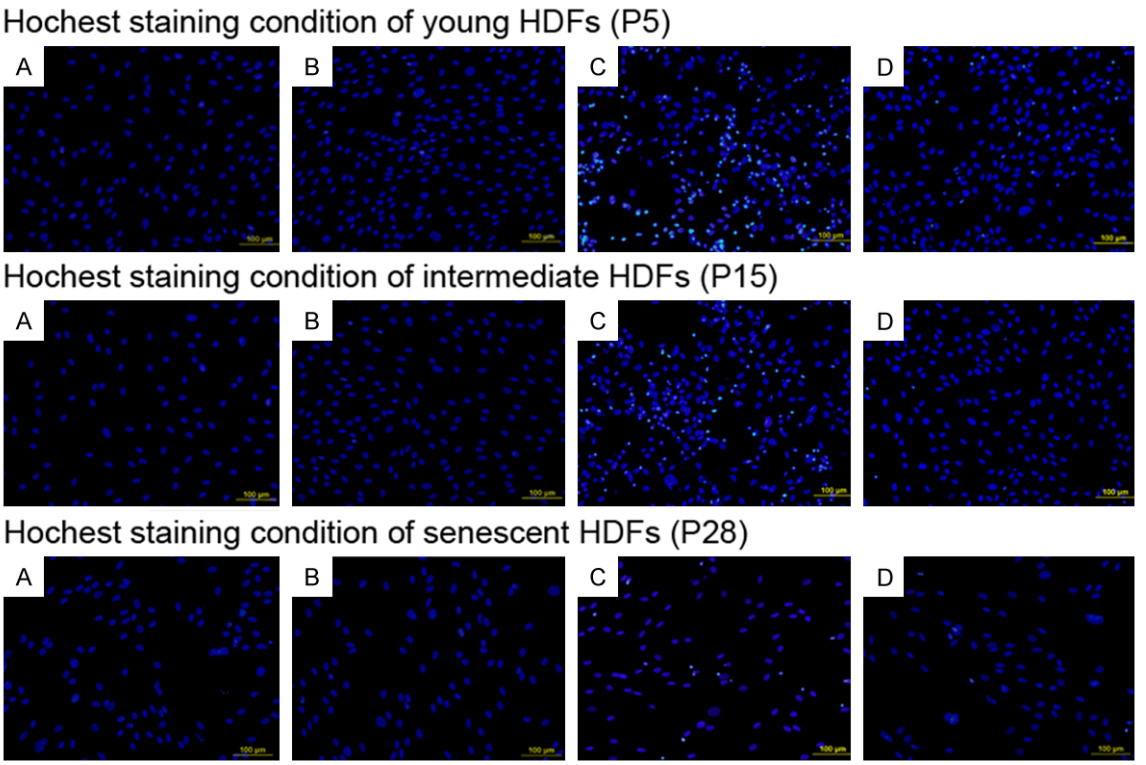

Figure 4. Hochest staining condition. A: Control group; B: ADSC-CM treated non-irradiated HDFs group; C: UVB group; D: ADSC-CM treated irradiated HDFs group. (magnification 200×).

Comparison of cellular apoptosis

In young HDFs culture, apoptotic HDFs ratio of UVB group was obviously higher than control group ($P < 0.01$); Compared with common media, ADSC-CM treatment had no obvious effect on non-irradiated HDFs ($P > 0.05$), but can obviously decrease apoptotic HDFs ratio in irradiated HDFs ($P < 0.01$). Intermediate and senescent HDFs shared similar trends with young HDFs, except for the moderate reduction of apoptosis in irradiated senescent HDFs (P28) treated with ADSC-CM ($P < 0.05$) (**Tables 1**, **2** and **Figures 2C**, **4**).

Comparison of relative expression type I collagen, type III collagen and elastin at mRNA level

In young HDFs culture, relative expression of type I collagen mRNA in UVB group obviously decreased compared with control group ($P < 0.01$); ADSC-CM treatment can increase its expression of either non-irradiated ($P < 0.01$) or irradiated HDFs ($P < 0.05$) compared with common media. Intermediate HDFs displayed similar changing tendency to the young generation, except for its slight improvement in irradiated HDFs treated with ADSC-CM ($P > 0.05$). Senescent HDFs shared the similar trends with intermediate HDFs (**Tables 1**, **2** and **Figure 5**).

In young HDFs culture, relative expression of type III collagen mRNA in UVB group decreased compared with control group ($P < 0.05$); ADSC-CM treatment can increase its expression of either non-irradiated or irradiated HDFs compared with common media ($P < 0.05$). In intermediate HDFs culture, type III collagen mRNA in UVB group obviously decreased compared with control group ($P < 0.01$); ADSC-CM treatment can increase its expression in non-irradiated HDFs compared with common media ($P < 0.05$), but had no obvious effect on irradiated HDFs ($P > 0.05$). Senescent HDFs shared similar trends with intermediate HDFs (**Tables 1**, **2** and **Figure 5**).

In young HDFs culture, relative expression of elastin mRNA obviously increased ($P < 0.01$) in UVB group compared with control group; ADSC-CM treatment had no obvious effect on elastin mRNA expression of either non-irradiated or

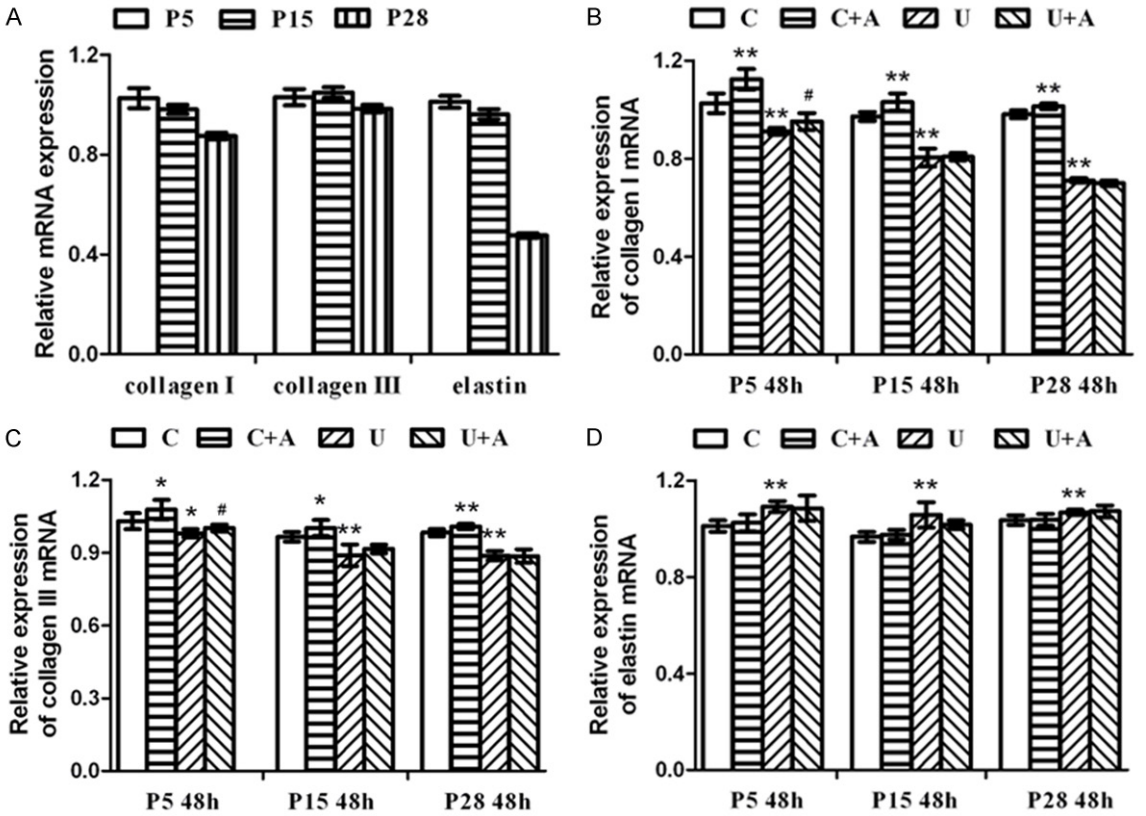

Figure 5. Analysis of mRNA expression. (A) Changing tendency of collagen I, collagen III and elastin mRNA expression of HDFs in control group at different senescent levels. mRNA expression of collagen I (B), collagen III (C) and elastin (D) of HDFs at different senescent levels under different treatments. c: Control group; c+A: ADSC-CM treated non-irradiated HDFs group; U: UVB group; U+A: ADSC-CM treated irradiated HDFs group. (*P < 0.05 and **P < 0.01 compared with con group; #P < 0.05 compared with UVB group).

irradiated HDFs compared with common media (P > 0.05). Intermediate and senescent HDFs displayed similar changing tendency to the young generation (**Tables 1**, **2** and **Figure 5**).

Discussion

ADSCs stand out as an upstart in the field of anti-ageing mostly owing to their secretome that have been identified by using proteomics, including transforming growth factor-beta (TGF-β), platelet derived growth factor (PDGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF), pigment epithelium-derived factor (PEDF), interleukins and many antioxidants such as insulin-like growth factor-binding proteins (IGFBPs), Superoxide Dismutase$_2$ (SOD_2) and so on [14-17]. ADSCs have been proven to communicate with and modify the surrounding cells through a paracrine mechanism, thus ADSC-CM, which not only contains the whole bioactive components secreted by ADSCs but also takes advantages of easier storage and safer application over ADSCs, has received considerable attention. Numerous studies have verified ADSCs and their secretome can fight against photoageing. Kim *et al.* demonstrated subcutaneous injection of ADSCs can improve UVB-induced wrinkles and increase dermal thickness in mice [19]. Song *et al.* reported ADSC-CM, better than ADSCs co-culture, improved proliferation and converted necrotic or late apoptotic cells to early apoptotic cells in UVB-irradiated HDFs [21]. These results were consistent with that of the present study: ADSC-CM increased proliferative activity but decreased apoptotic cell ratio in UVB-irradiated HDFs. In addition, our team reported ADSC-CM decreased UVB-irradiated cellular senescence in HDFs for the first time. As Xu and Kim *et al.* interpreted in their studies, ADSC-CM kept HDFs out of UVB-induced G1 phase arrest [19, 20], in which condition proliferation stops, senescence occurs and apoptosis is triggered,

through repressing p16 expression [21, 27]. However, further investigation and more related markers are required to explain these results.

Very few studies reported impacts of ADSCs on intrinsic ageing damages, since Shen X *et al.* demonstrated in a latest study that ADSCs can increase SA-β-Gal mRNA expression of co-cultured HDFs that were extracted from young and aged volunteers. As we know, SA-β-Gal has been confirmed as a reliable biomarker of cellular senescence, both its mRNA and protein expression increase significantly in senescent cells, as does its enzymatic activity. Shen X *et al.* presumed ADSCs co-culture may accelerate fibroblast senescence [28], which to some extent deviated from our results: ADSC-CM treatment had no impact on SA-β-Gal activity in the serially passaged HDFs *in vitro*, this may imply the existing and accumulated damages at genetic level can hardly be reversed. We suppose the inconsistence in these results indicates that ADSCs don't simply act on HDFs via paracrine factors, there exists a feedback between HDFs and ADSCs through cell-to-cell cross talk. We are looking forward to interpreting the differences and underlying mechanisms in subsequent experiments.

Results of the present study showed that UVB irradiation inhibited proliferation, induced apoptosis in HDFs. On the other hand, along with the passage of HDFs, cellular proliferative activity declined but cellular apoptosis remained low and what interested us was, the proliferation-inhibition and apoptosis-induction effect of UVB declined. These suggest UVB interacts with intrinsic ageing factors rather than simply superimposes on them to result in fibroblast senescence, this finding verifies the necessity and significance of our ageing model establishment. UVB-induced cellular apoptosis decreased in senescent HDFs, which, according to Yeo *et al.*, is probably ascribed to diminished activation of SAPK/JNK, moreover, p21waf1 protein, whose reduction was related to cellular apoptosis induced by DNA-damage, was certified to increase in senescent cells [26]. After all, these results just involve a tiny part of the whole ageing process of fibroblasts, much need to be revealed. In spite of the differences in action patterns and approaches, intrinsic ageing and photoageing share some important molecular features, among which the reactive oxygen species (ROS) may play a critical role in both of their processes [29, 30]. Consequently, the cytokines and other substances that possessed anti-oxidative effects existing in ADSC-CM take effects in fighting against ageing damages to stimulate cellular proliferation and reduce apoptosis. Besides, cytokines in ADSC-CM or other active components induced by the treatment have mitogenic effects, such as TGF-β1 and TIMP-1 are assumed to manage a potent activity [31, 32]. On the other hand, we found the protective effects of ADSC-CM declined with the passage of HDFs, probably due to damages accumulating and weaker response of senescent HDFs.

Remodeling of collagens and elastins, the most important components among ECM proteins, is a key feature of both intrinsic ageing and photoageing [3, 4] and has become the target of most anti-ageing researches pertaining to the skin. Previous researches displayed diverse or even controversial results about expression of these proteins. Our team found that along with the passage of HDFs, type I collagen and elastin mRNA expression gradually decreased, type III collagen mRNA expression changed slightly, this reveals the attenuation of HDFs function. Besides, UVB-induced reduction in type I and III collagen mRNA expression aggravated and increasing of elastin mRNA expression caused by UVB firstly enhanced slightly and then attenuated significantly. The differences in changing tendency of the three indexes reflect their role and significance in maintaining skin youth. Slightly or significantly, ADSC-CM treatment can promote their expression both in irradiated and non-irradiated HDFs, but the synthesis-promoting effect of ADSC-CM decreased with the passage of HDFs. In addition to the anti-oxidative activity of ADSC-CM, TGF-β1 may play a dominant role in stimulating their mRNA synthesis through its following cascade reactions, among which the restoration of Wnt/β-catenin pathway may hold sway [20, 32-34].

We conclude ageing damages caused by UVB and intrinsic factors aren't simply superimposed, ADSCs secretome protect HDFs from ageing damages, yet with some limitations. Our ADSC-CM is hoped to be a safe and valuable material against skin ageing. However, much needs to be improved to research on mechanisms and develop applications.

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Disclosure of conflict of interest

None.

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