Int J Clin Exp Med 2016;9(5):8846-8851
www.ijcem.com /ISSN:1940-5901/IJCEM0015786

Original Article

Establishment of a photoaging model in human dermal fibroblasts and study on photoaging-related biomarkers

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Received September 7, 2015; Accepted March 24, 2016; Epub May 15, 2016; Published May 30, 2016

Abstract: Objective: We established the photoaging model in human dermal fibroblasts and studied the influence of ultraviolet irradiation on the target biomarkers related to photoaging. Method: Human dermal fibroblasts were proliferated in vitro and photoaging was induced by UVB irradiation. The mRNA expressions of precollagen (pro-COL1α1) and human telomerase reverse transcriptase (hTERT) were detected using real-time fluorescence quantitative RT-PCR, and changes of telomere DNA length were detected as well. The expressions of metalloprotease (MP)-1 and MP-3 were characterized by Western Blot technique. Results: The mRNA expressions of both pro-COL1α1 and hTERT were upregulated significantly in the photoaging model. The telomere DNA length was shortened considerably in the experimental group compared with the control group. The expressions of MP-1 and MP-3 were upregulated significantly. Conclusion: UV irradiation has a substantial impact on 5 photoaging-related biomarkers in photoaging model in human dermal fibroblasts.

Keywords: Photoaging, human dermal fibroblasts, biomarkers

Introduction

The mechanism of skin photoaging is still unclear so far, and many relevant researches are underway [1]. Establishing the model of skin photoaging is the basis of the experiments [2]. As the outermost barrier of the human body, skin is exposed to multiple environmental factors, among which UV irradiation is the primary cause of accelerated photoaging [3]. Human dermal fibroblasts obtained from circumcision were irradiated by UV light to induce photoaging in this study. Using the established model of skin photoaging, mRNA expressions of pro-COL1α1 and hTERT, protein expressions of MP-1 and MP-3, and telomere DNA length were detected as the biomarkers of induced photoaging. Based on the results, the mechanism of UV-induced photoaging was discussed.

Materials and methods

Materials

Human dermal fibroblasts (HDFs) were obtained from one case receiving circumcision at Southwest Hospital. The cells were isolated and cultured before experiment.

Reagents: DMEM/F12 medium (GIBCO, US), Trizol reagent (Invitrogen, US); RT reagent, fluorescent quantitative PCR reagent, sybr green I, HRP-conjugated secondary antibody, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal reference (Western Biotechnology, US); matrix metalloproteinase 1 (MMP1) and MMP3 (CapitalBio, Beijing).

Primary culture and passage of dermal fibroblasts and establishment of skin photoaging model: The cells were cultured by referring to

Table 1. Primers for qRT-PCR

Amplified fragment	Name	Sequence	Length of product	Source
pro-COL1α1	hCOL1α1 F	GTGCGATGACGTGATCTGTGA	108 bp	Literature [6]
	hCOL1α1 R	TGGTCGGTGGGTGACTCTG		
hTERT	hTERT F	GCTGCTCAGGTCTTTCTTTTATG	115 bp	Literature [7]
	hTERT R	CAAGTGCTGTCTGATTCCAATG		
TelomereDNA	Tel 1	GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT		The present study
	Tel 2	TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA		
Beta-actin	h actin F	TGACGTGGACATCCGCAAAG	205 bp	Literature [8]
	h actin R	CTGGAAGGTGGACAGCGAGG		

previous literature [4]. The DMEM/F12 medium supplemented with 10% fetal bovine serum was used. For primary culture, 2 cm of tissue resected by circumcision was taken and washed with culture medium twice. Then the tissue was cut into small pieces on a surface dish, washed with culture medium once, and soaked in fetal bovine serum. The pieces were transferred to the culture flask and grew to a compact monolayer. Then the cells were passaged by conventional method.

The fifth generation of cells were harvested and inoculated to a 6-well plate. When the adherence rate reached 70-80% at 48 h, the cells were harvested and the control cells were set up. The experimental cells were irradiated with a UVB tube at 30 mJ/cm^2 for 24 h. Both the experimental cells and the control cells were cultured in a CO_2 incubator, and the cell morphology was observed under the light microscope after 24 h.

Detection of mRNA expression of pro-COL1α1 and hTERT by real-time fluorescence quantitative PCR (qRT-PCR) and telomere DNA length: Total RNA extraction was performed for the control cells and experimental cells using the method in literature [5] with Trizol reagent, chloroform, isopropylalcohol and ethanol. Each group had 3 replicates. The extracted RNA was reverse transcribed into cDNA using random primers. QRT-PCR was performed using specific primers of pro-COL1α1, hTERT and telomere DNA in **Table 1** and sybr green I, with human beta-actin as internal reference. The reverse transcription system (20 μl) consisted of the followings: 2× RT buffer 10 μL, 6N random primers (100 pmol/μL) 1 μl, RT mix 1 μl, template 5 μl, DEPC-treated water 3 μl. Reaction conditions: 25°C for 10 min, 42°C for 50 min, 85°C for 5 min. qQT-PCR system (50 μl) consisted of 2× PCR buffer 25 μl, primers (25 pmol/μl) 1 μl ×2, Sybr green I (20×) 0.5 μl, template 2 μl, DEPC-treated water 20.5 ul. Reaction conditions: 94°C for 4 s, 94°C for 20 s, 60°C for 30 s, 72°C for 30 s, 35 cycles; signals were detected at 72°C.

Relative copy number of each biomarker was calculated from the standard curve. The ratio of their values to relative copy number of internal was taken as the corrected value of mRNA content of each biomarker. The difference between the experimental group and the control group was measured by this corrected value. For testing of the significance of differences between the two groups, t-test was adopted.

Detection of protein expressions of MMP-1 and MMP-3 by Western blot: As described in literature [9], experimental group irradiated by UVB and control group without UVB irradiation were set up, each having 4 replicates. The cells were added with RIPA buffer (0.1 ml for every 10^6 cells) after treatment and centrifuged. The supernatant was taken and analyzed by 4% SDS-PAGE. The proteins were then transferred to membrane, which was sealed with TBST buffer containing 5% defatted milk powder for 1 h. They were incubated with 1:500 diluted primary antibodies (MMP1, MMP3), internal reference (GAPDH, 1:2000 diluted) at 4°C overnight. The cells were washed and cultured with HRP-conjugated secondary antibodies (1:2000 diluted) for 1.5 h. The target protein was detected in the bands by ECL system Amersham Biosciences.

Statistical analysis

SPSS11.7 was used for all statistical analyses. Count data were analyzed with χ^2 test. Measurement data were expressed as X±s,

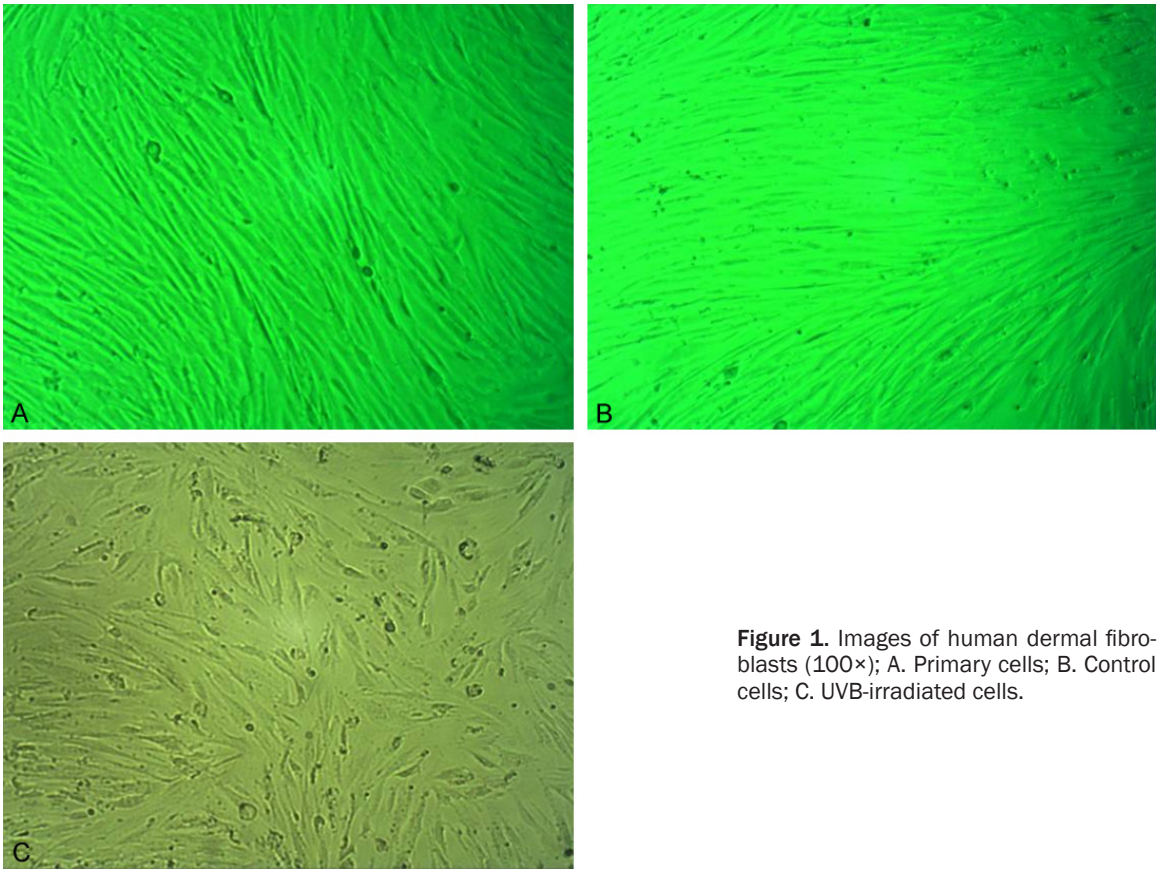

Figure 1. Images of human dermal fibroblasts (100×); A. Primary cells; B. Control cells; C. UVB-irradiated cells.

Table 2. Copy number of pro-COL1α1 and hTERT in UVB group and control group

	pro-COL1α1		hTERT		β-actin
	Average relative copy number (×10^7)	Ratio	Average relative copy number (×10^7)	Ratio	Average relative copy number (×10^7)
Control group	1.23±0.23	0.221	2.04±0.53	0.366	5.57±0.62
UVB group	2.3±0.37*	0.708	4.48±0.97*	1.378	3.25±0.58

Note: *P<0.05 compared with the control group.

Table 3. Telomere DNA length detected by qRT-PCR

	Telomere DNA		β-actin
	Average relative copy number (×10^7)	Ratio	Average relative copy number (×10^7)
Control group	0.99±0.34	0.49	2.03±0.64
UVB group	0.26±0.03*	0.13	1.99±0.28

Note: *P<0.05 compared with the control group.

and t test and repeated measures analysis of variance were adopted. P<0.05 was considered as statistically significant.

Results

Establishment of a photoaging model in human dermal fibroblasts

Primary human dermal fibroblasts were large in the shape of long spindle and had clear contour (**Figure 1A**). The fifth generation of cells (**Figure 1B**) did not change greatly in morphology compared with primary cells. However, the experimental cells after UVB irradiation (**Figure 1C**) were irregular in shape and presented the signs of photoaging such as shrinkage.

MRNA expression of pro-COL1α1 and hTERT

The ratio of relative copy number of pro-COL1α1 mRNA in the experimental group and control group was 0.221 and 0.708, respectively; the ratio was 4.48±0.97E+07 and 2.04±0.53 E+07 for hTERT in the experimental group and control group, respectively. Thus UVB irradiation in the

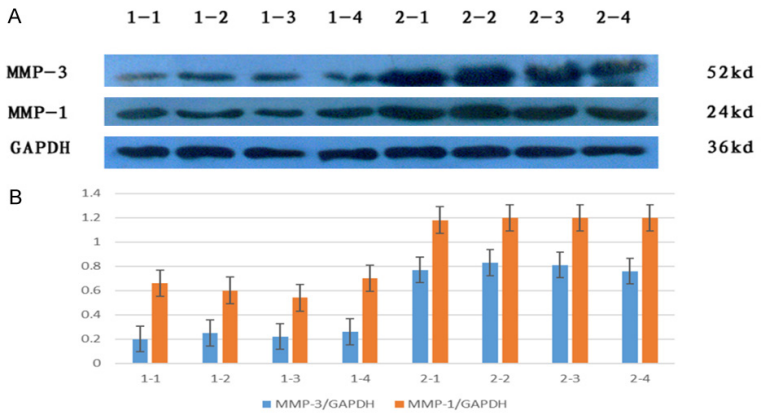

Figure 2. Comparison of relative expressions of MMP-1 and MMP-3 in UVB group (2-1, 2-2, 2-3, 2-4) and control group (1-1, 1-2, 1-3, 1-4).

experimental group resulted in significant differences in mRNA expressions of pro-COL1α1 and hTERT (P<0.0, **Table 2**).

Detection of telomere DNA length by qRT-PCR

Telomere DNA length differed greatly between the UVB group and control group (*P*<0.05) (**Table 3**), the length being shorter in the UVB group.

Expression of MMP-1 and MMP-3

The gel images of SDS-PAGE are shown in **Figure 2A**. The target bands of MMP-1 and MMP-3 in UVB group were obviously thickened compared with the control group. The gray value of each band was calculated using the gel imaging system, and the ratio of gray value between each biomarker and the internal reference was estimated. It can be seen from **Figure 2B** that MMP-1 and MMP-3 expressions in the UVB group were significantly upregulated compared with the control group.

Discussion

Fibroblasts are the most common cells of dermis [10], which play an important role in the process of photoaging [11]. Establishing photoaging model in human dermal fibroblasts is the first step in the understanding of mechanism of photoaging and prevention and treatment of photoaging [12]. UV light that can induce photoaging is mainly the long-wavelength light (UVA320-400 nm) and medium-wavelength light (UVB290-320 nm). UVB exposure is the main reason of skin damage [13]. Photoaging model in human dermal fibroblasts has been established in some literature [14-16], and the studies over the influence of UV radiation on the biomarkers related to photoaging are carried out. We chose 5 biomarkers as objects of research, among which mRNA expression of hTERT was first reported in photoaging model in human dermal fibroblasts.

Type I and III collagen are predominant in adult skin. Accounting for 85% of the dry weight of collagen in skin, type I collagen maintains skin tension and bears the pulling force. It is also the substance responsible for skin flexibility [17]. According to one study, the synthesis of type I collagen in fibroblasts will be affected by UVB radiation [18]. But in our study, the mRNA of pro-COL1α1 was upregulated, probably due to self-repair. Fisher et al. [19] showed that generation of MMPs was induced by UV radiation, which facilitated the degradation of nearly all extracellular components (e.g. type I collagen), leading to photoaging. According to our results, MMP-1 and MMP-3 proteins were upregulated significantly in human dermal fibroblasts after UVB irradiation. The expression of hTERT mRNA is positively correlated with the activity of telomerase, and hTERT is the rate-limiting enzyme of telomerase [20]. Therefore, the activity of hTERT directly determines the telomere DNA length [21]. According to one report [22], hTERT was upregulated considerably in photoaging and progeria compared with normal skin and aged skin. We found that mRNA expression of hTERT in UVB group was markedly higher than that in the control group, which agreed with the previous reports. Telomere DNA length reflects the ability of cell division, and its shortening is associated with cellular aging [23]. In our study, the telomere DNA length in UVB group was obviously shorter than that in the control group, the former being 1/4 of the latter on average. The mechanism underlying this phenomenon may be the UV-induced inflammation and oxidative stress, which leads to DNA damage and shortening of telomere DNA during photoaging.

To conclude, 5 biomarkers related to photoaging were detected. The upregulation of MMP-1,

MMP-3 and shortening of telomere DNA were the direct outcomes of UVB irradiation, all of which facilitated photoaging. Meanwhile, mRNA expressions of pro-COL1α1 and hTERT were upregulated, probably due to self-repair after photoaging. A complex molecular regulatory network is involved in photoaging process. Although the knowledge about this network is still limited, it is promising to find ways to repair and prevent photoaging in the future.

Acknowledgements

This study was from Project No. 12521318, Supported by Special Foundation from education of Heilongjiang Province of China.

Disclosure of conflict of interest

None.

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