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A valuable model of UVB-induced oxidative damage in HaCaT cells

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Introduction:

At the moment of "effective skincare", the biological effect of UV has been paid more and more attention. Numerous studies have shown that UV is the main environmental factor leading to skin photoaging. Excessive UV exposure can cause a variety of skin damage, such as sunburn, skin darkening, skin photosensitivity, etc. Many cosmetic companies are working on skin care products that can effectively resist UV light damage.

In order to establish a biological evaluation system for the cell-level evaluation method of cosmetic raw materials stably responding to photoaging, we have developed a UV simulator for cells that contain both UVB and UVA light. This simulator simulates UVA and UVB ultraviolet radiation in sunlight and designed for in vitro evaluation of chemical and cosmetic phototoxicity safety and anti-ROS effect. We measured the oxidative damage marker ROS level, the release level of cellular inflammatory factors, and cell apoptosis, and cell cycle changes in human keratinocyte HaCat after UVB irradiation, and comprehensively evaluated the physiological changes of HaCat cells after UVB irradiation. A model of UV-induced oxidative stress in skin-related cells was successfully established.

Materials & Methods:

Materials

UV simulator

QuantStudio 3, Thermo, USA

iCELLigence, ACEA Biosciences, USA

NovoCyte, ACEA Biosciences, USA

Methods

a) The UVA/UVB simulator uses LED lamp beads, which have the advantages of good wavelength controllability. By setting a number of sensors to collect the radiation intensity, the device can provide real-time feedback and accurately calibrate the irradiation power of the LED lamp beads to realize the uniformity and controllability of the illumination in the irradiated areas.
b) Keratinocytes HaCat were cultured in DMEM medium containing 10% fetal bovine serum. The cells were quantitatively seeded in 6-well plates and cultured in a 37°C, 5% CO2 incubator. Human keratinocytes HaCat were irradiated with a certain dose of UVB after 24 hours.
c) The cell proliferation after UVB irradiation was detected by RTCA technology.
d) The apoptosis degree, cell cycle, and reactive oxygen species level of HaCat cells after UVB irradiation were detected by flow cytometry.
e) The effects of UVB irradiation on the production of inflammatory factors TNF-α, IL-6, and IL-8 in HaCat cells were determined by qPCR.

Results & Discussion:

1. cells were irradiated with UVB at 300, 600 and 900 mJ/cm2 and cell viability after different doses of UVB irradiation was estimated by RTCA assay and Flow cytometry. The results showed that the proliferation curve of HaCaT cells after UVB irradiation was lower than that of the control group(Fig. 1a), and the apoptosis rate of HaCaT cells increased with the increase of UVB irradiation dose(Fig. 1b).

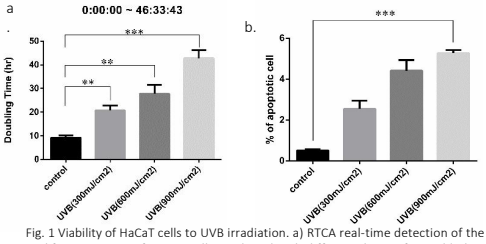

Fig. 1 Viability of HaCaT cells to UVB irradiation. a) RTCA real-time detection of the proliferation curve of HaCaT cells irradiated with different doses of UVB. b) Flow cytometric analysis of Apoptosis.

2. The intracellular ROS level is an important index to evaluate the damages led by UVB exposure. Our research results showed that intracellular ROS increased to varying degrees after different doses of UVB irradiation (Fig. 2a), and high dose UVB irradiation led to a significant increase in ROS in HaCat cells (p=0.0077, Fig. 2b).

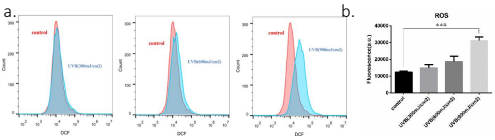

Figure 2. Elevated intracellular ROS level in HaCaT cells after UVB irradiation. a) The results of flow cytometry contain 10,000 ungated events. b)Representative histograms of triplicate experiments plot the relative green DCF fluorescence intensity.

3. A single short-time UVB irradiation did not significantly change the mitotic state of HaCaT cells.
4. High-dose UVB irradiation promoted the release of TNF-α，IL-6 and IL-8 from HaCaT cells, but not significantly.

Conclusions:

We developed a UV simulator for cells and conducted an experimental study of UVB-induced oxidative stress in HaCat cells. The UVB-induced HaCat cell ROS-damage model was successfully constructed, providing a powerful evaluation method for the development of cosmetic raw materials that effectively resist UV damage and photoaging. At the same time, this study further proves that short-term UVB exposure does not change the mitotic cycle of cells and causes irreversible cell damage. We can protect the skin by using sunscreens and avoiding long-term UVB exposure.

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