Effects of temperature on the expression of redox genes in human dermal fibroblasts

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1. Introduction

While core temperature of human bodies is maintained around 37°C, skin temperature is subject to the environment and changes at a wide range. Skin surface temperature is 33°C and lower than the core temperature in normal condition. Especially skin temperature of legs can be very low in cold winter.

Temperature is very important to the human common cells and only a few degrees of change from 37°C will have negative effects on the cells. However, the effect of temperature on skin cells has not been fully investigated. In order to elucidate the effects of low temperature on dermal fibroblasts, the effects of culture temperature on the gene expressions of dermal fibroblasts were investigated. In this study, effects on redox-related genes were targeted.

Fig. 1 Facial skin temperature in different room temperature (Thermography)

2. Methods

① Proliferation assay of dermal fibroblasts

1×10⁴ cells of normal human dermal fibroblasts (new born) were seeded in each well of 6-well plates, and cultured in DMEM with 10% FBS at 37°C for 24 hours. Then the plates were transferred to 30°C~42°C. After 0, 3, and 6 days, the cells were trypsinized and the numbers of the cell were counted.

② Quantification of gene expression of redox-factors

3×10⁵ cells of normal human dermal fibroblasts (new born) were seeded in each well of 6-well plates, and cultured in DMEM with 10% FBS at 37°C for 24 hours. Then the plates were transferred to 30°C ~37°C. 24 hours later, RNA was prepared by RNeasy Mini Kit (Qiagen).

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From the collected RNA, cDNA was synthesized by SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen). Then expression level of the following redox-related genes was quantified by qPCR.

- Heme Oxygenase 1 (HO-1)
- Thioredoxin Reductase 1 (TXNRD-1)
- NAD(P)H Quinone Dehydrogenase 1 (NQO1)
- Glutathione-Disulfide Reductase (GSR)
- Thioredoxin (TXN)
- NF-E2 Related Factor 2 (Nrf-2)

③ Thioredoxin reductase activity assay

Dermal fibroblasts were cultured at 30°C or 37°C for 24 hours. Activity of thioredoxin reductase was evaluated by using "Thioredoxin Reductase Activity Colorimetric Assay Kit" (bioVision)

④ Detection of Reactive Oxygen Species

Dermal fibroblasts were cultured at 30°C or 37°C for 24 hours. Oxidative stress in live cells was measured by using CellROX® Green Reagent. After adding CellROX® Green Reagent into the culture medium, cells were incubated at 37°C for 30min. Then cells were fixed with formalin and fluorescence was observed under a microscopy.

3. Results

① Effects of culture temperature on the cell proliferation rate of dermal fibroblasts

Culture temperature of dermal fibroblasts was shifted from 37°C to 30, 33, 37, 40, or 42°C. The numbers of the cells were after 3 and 6 days are shown in Fig. 2. Cell proliferation rate was highest at 37°C and suppressed at both high and low temperatures.

Fig. 2 Effects of temperature on the proliferation rate

② The expressions of redox-related genes at low culture temperatures

Dermal fibroblasts were cultured at 30, 33, or 37°C for 24 hours. The expression levels of redox-related genes were quantified. The results for Heme Oxygenase 1 (HO-1), Thioredoxin Reductase 1 (TXNRD-1), NAD(P)H Quinone Dehydrogenase 1 (NQO1), Glutathione-Disulfide Reductase (GSR), Thioredoxin (TXN) are shown in Fig. 3. The expression levels of Nrf-2, the transcription factor of these of redox-related genes, was also quantified and is shown in Fig.3f.

As a result, the expressions of HO-1, TXNRD-1, NQO-1, and GSR decreased in 30°C and 33°C compared with 37°C. The expression of Nrf-2 also decreased in low temperatures.

Fig. 3 The expression of redox-related gene under the culture at low temperatures

③ Thioredoxin reductase activity at 30°C

Dermal fibroblasts were cultured at 30 or 37°C for 24 hours and then the activity levels of thioredoxin reductase was evaluated.

The result showed that low temperature suppressed thioredoxin reductase at enzymatic activity level, as well as gene expression level (Fig. 4).

Fig. 4 Thioredoxin reductase activity of the dermal fibroblasts cultured at 30°C and 37°C

④ Reactive oxygen species production

Dermal fibroblasts were cultured at 30 or 37°C for 24 hours. Then both cells were placed at 37°C and oxidative stress in the cells was measured by using CellROX green.

The result showed that cells pre-cultured at 30°C produced more ROS than the cells pre-cultured at 37°C (Fig. 5).

Fig. 5 Reactive oxygen species production of dermal fibroblasts pre-cultured at 30°C and 37°C

⑤ Time-course analysis of redox-related gene expression in dermal fibroblasts after heating

Dermal fibroblasts were cultured for 24 hours at 33°C, which is close to the normal skin surface temperature. Then culture temperature was increased to 37°C~42°C. Expressions of HO-1, TXNRD-1, NQO1, TXN, and GSR were investigated over time. The results are shown in Fig. 6.

The expression of HO-1 kept increasing from the beginning. On the other hand, the expressions of TXNRD-1 and NQO1 decreased at first and recovered at around 6 hours, then increased at 12 hours. The expression of GSR kept decreased for more hours than TXNRD-1 and NQO1. And the temperature shift had less effects on TXN.

Thus, time-course expression patterns for redox-related genes which were downstream genes of the same transcription factor Nrf-2, were not the same.

4. Conclusions

• Gene expression of redox-related genes such as HO-1, TXNRD-1, and NQO-1 decreased in low temperatures. Thioredoxin reductase activity also decreased in 30°C and ROS production increased in the cells pre-cultured at 30°C. These results indicated that low temperature lowers the antioxidant capacity of dermal fibroblasts.

• Because the expression of Nrf-2 also change in temperature change, the expressional change of redox-related genes in temperature shift were thought to be caused by the change of the expression of Nrf-2.

• The time-course patterns of the expression of redox-related factors after heating are different. Other mechanisms than Nrf-2 also may be involved.