Time-dependent investigation of UVB-induced cellular mechanisms in human keratinocytes using mRNA encoding cyclobutane pyrimidine dimer-specific photolyase

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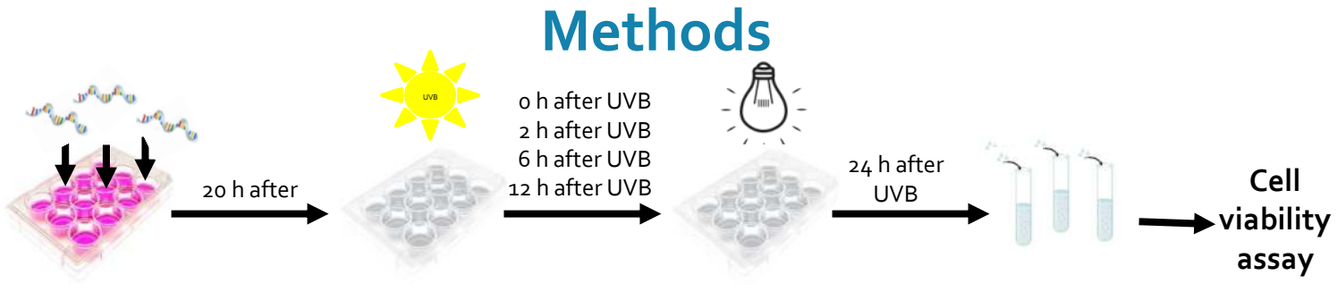
Introduction

In vitro-synthesized mRNA containing nucleotide modifications has distinguished therapeutic potential to transiently express physiologically important proteins. One such protein is photolyase, which accelerates the removal of UV-induced DNA damages, including cyclobutane pyrimidine dimers (CPD) using the energy of visible light (photoreactivation). Photolyase however is absent in humans.

Aims

Our aims were to investigate the UVB-induced cellular mechanisms in human keratinocytes using mRNA encoding cyclobutane pyrimidine dimer-specific photolyase (CPD-specific photolyase) in a time-dependent experiments.

Methods



1. HaCaT cells were transfected with 0,25 µg pseudouridine-modified mRNA encoding CPD-specific photolyase.

2. 20 mJ/cm² UVB irradiation in Dulbecco's phosphate buffer saline using TL-20W/12 tubes

3. HaCaT cells were either exposed in DMEM without phenol red to photoreactivating light (active CPD-photolyase) or kept in the dark (inactive CPD-photolyase) for one hour.

4. Cells were harvested 24 after UVB irradiation.

Our previous outcomes

In our previous work, mRNA encoding CPD-specific photolyase has been effectively transfected into HaCaT cells. The encoded protein was visualized in the nuclei. Using CPD-specific ELISA, we showed that CPD-photolyase removed 90% of CPDs in the mRNA transfected cells at one hour after photoreactivation.

Immunofluorescence detection of UVB-induced CPDs

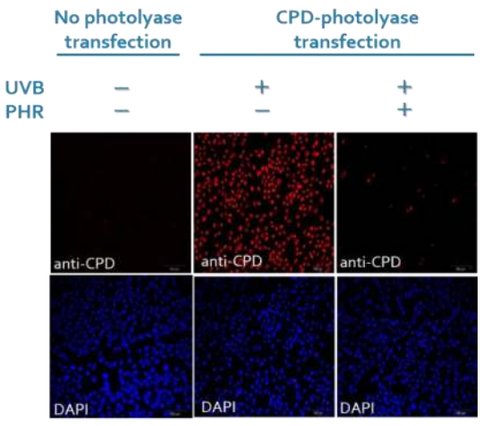


Figure 1. HaCaT keratinocytes were transfected with lipofectamine-complexed pseudouridine-modified-mRNA encoding CPD-photolyase, or untransfected (no photolyase transfection). 20 hours later, cells were irradiated with 20 mJ/cm² UVB and immediately exposed to photoreactivating light (PHR) or kept in the dark for 1 h. Immediately after photoreactivation or incubation in the dark, CPDs were detected by immunofluorescent staining with anti-CPD-specific antibody and Alexa-conjugated secondary antibody (red). Nuclei were visualized by DAPI staining. Images are representatives of three independently performed experiments (original magnification ×20).

Rapid repair of CPDs promotes the survivals of cells

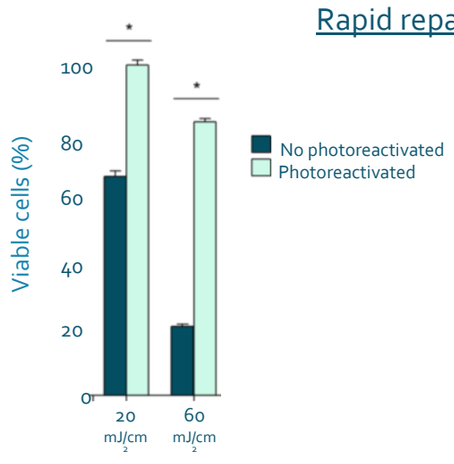
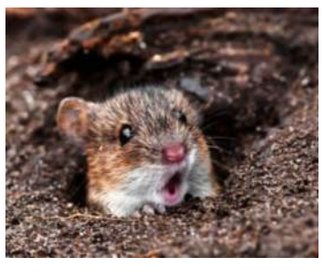


Figure 2. HaCaT cells were irradiated with 20 or 60 mJ/cm² UVB 20 h after delivery of pseudouridine-modified-mRNAs and immediately exposed to photoreactivating light (Photoreactivated) or kept in the dark (No photoreactivated) for 1 h. After photoreactivation or incubation in the dark cell viability was determined by EZ4U assay at 24 h after UVB irradiation. The values were calculated relative to those obtained with cells that were not UVB irradiated. Significance was assessed by paired t-test, asterisk, $p<0.05$. Error bars represent the standard error of the mean. The results are means of three independent experiments.

Future plans



In vitro time-dependent investigation:
- CPD-dimers
- DNA damage
- ROS production

In vivo delivery of mRNA encoding CPD photolyase in to mouse keratinocytes

Results

Time-dependent investigation of cell viability after UVB irradiation followed by CPD-specific photolyase transfection

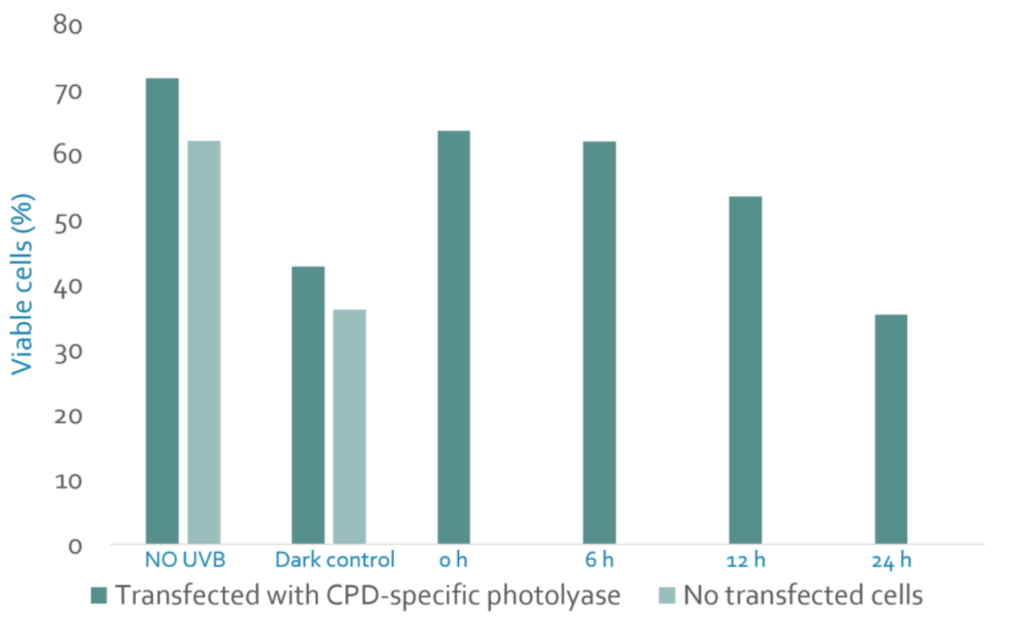
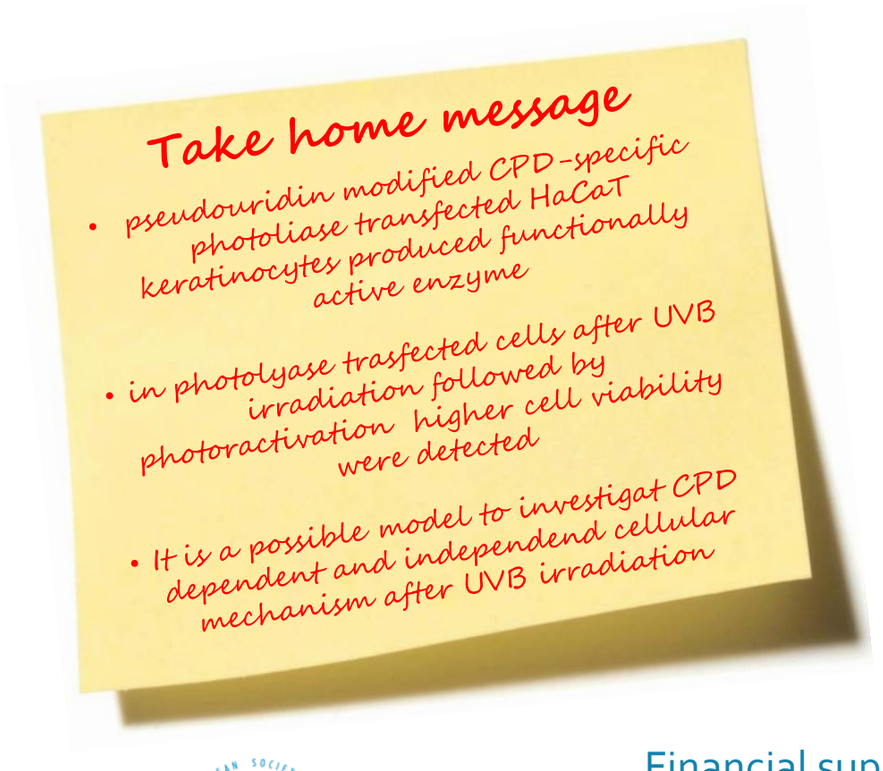


Figure 3. HaCaT cells were irradiated with 20 mJ/cm² UVB 20 h after delivery of pseudouridine-modified-mRNAs and immediately (0 h), 6 h, 12 h or 24 h after UVB irradiation exposed to photoreactivating light. No UVB exposed HaCaT cells (NO UVB), and in dark kept HaCaT keratinocytes (Dark control) were used as controls. Cells were stained with Annexin V Alexa Fluor™ 488 & Propidium Iodide (PI). Detection the cell viability was determined using FACSCalibur. Results were processed using Flowing Software. These results indicate that the viability of photoreactivated cells was higher at 6 h or 12 h after UVB irradiation compared to dark control. However, no difference could be observed between the groups when the photoreactivation was delivered at 24 h after UVB irradiation.



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