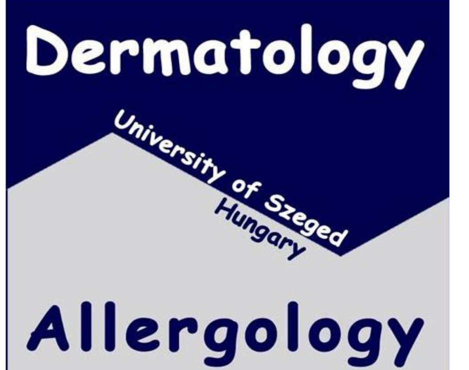
Characterization of UV-B induced cellular processes in a keratinocyte cell line (HPV-KER) immortalized with the HPV-E6 oncogene



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INTRODUCTION, AIMS:

One of the important functions of human skin cells is to protect the organism from the effects of UV-B radiation. Normal human keratinocytes (NHK) are widely used to study the UV-B response but they have restricted availability and a short lifespan in culture. The HaCaT cell line has been considered as a reliable substitute for primary cultures, however these cells exhibit limitations for some applications. In the present work we aimed to establish a valid and easy-to-use *in vitro* model system to study UV-B responses of human keratinocytes.

MATERIALS AND METHODS:

Immortalization and cloning
Normal human adult keratinocytes from a healthy donor were transfected with the HPV16 E6 oncogene in a pCMV vector. Transformants (called HPV-KER cells) were selected with the use of G418 antibiotic.

Cell cultures
HPV-KER cells and NHKs: Keratinocyte Serum Free Medium (Gibco) supplemented with growth factors
HaCaT cells: High glucose Dulbecco's modified Eagle's medium (Lonza) supplemented with 10% fetal bovine serum (PAA).

UV-B irradiation
24h after plating the semiconfluent cultures of cells were irradiated with 20, 40 and 60 mJ/cm^2 UV-B.

MTT assay
To determinate the number of viable cells 48h after the UV-B irradiation.

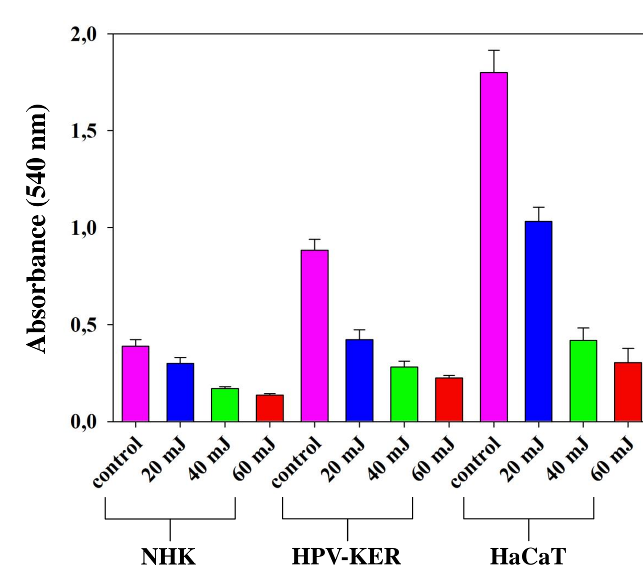
Real Time, Label Free Cellular Analysis (RTCA)
We monitored the changes of cell index (CI) after UV-B irradiation using the xCELLigence System (Roche). We plated 10000 cell/well regardless of cell type. The calculated CI based on the measurement of impedance which depends on the coverage of wells and the quality of cellular interactions with the electrodes.

Real time RT-PCR
The induction of IL-1A, IL-8, COX2 and TNFA gene expressions were analysed by real time RT-PCR. Amplifications were carried out with using the Universal ProbeLibrary-based assay system (Roche). Gene expression data were normalized to the 18S rRNA expression, and relative gene expressions were calculated using the ΔΔCt method.

Western analysis
Cells were irradiated with 40 mJ/cm^2 UV-B and total protein extracts were prepared 8h later. Anti-p53 (DAKO) and anti-actin (Sigma) antibodies were used as primary antibodies.

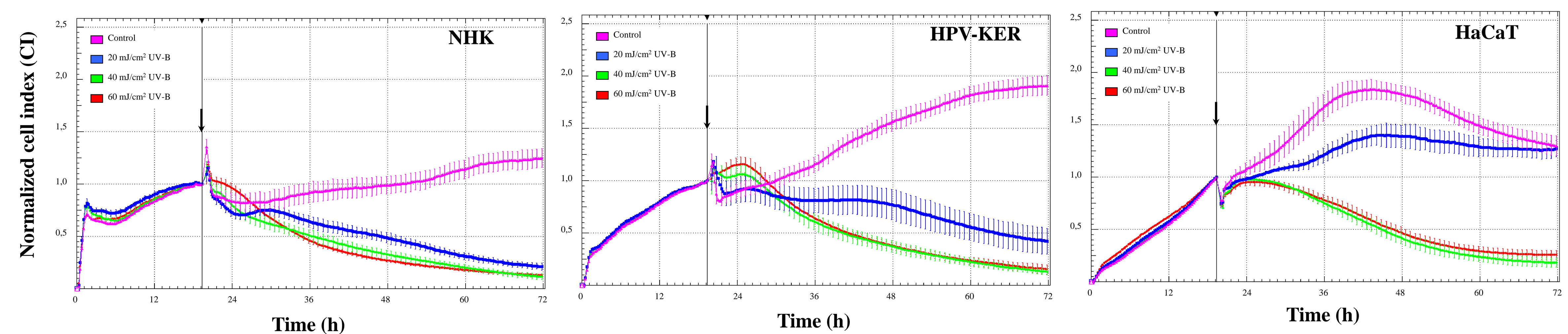
RESULTS:

MTT assay



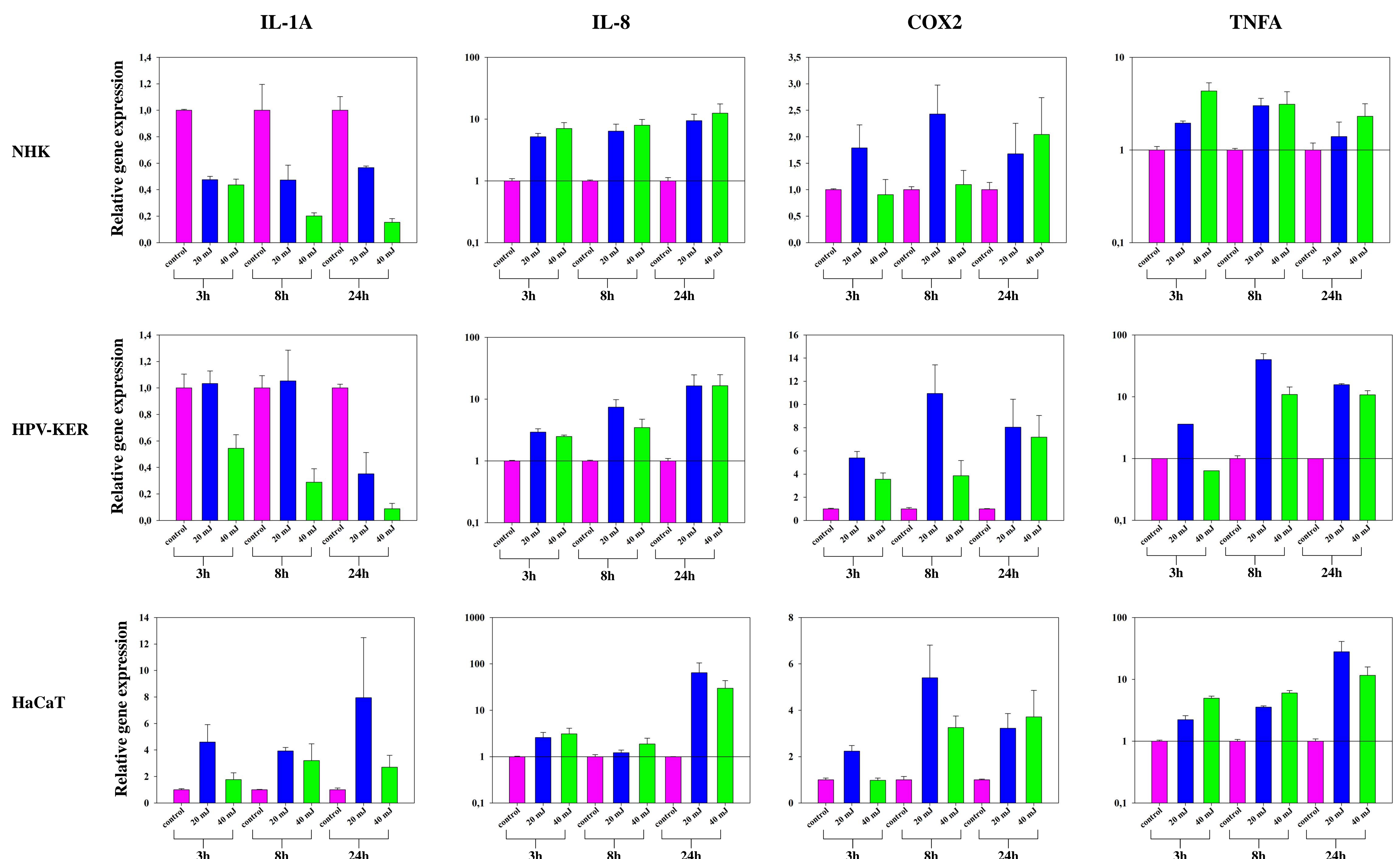
All three cell types exhibited a dose dependent decrease of cell viability 48h after UV-B irradiation. (Average of 2 independent experiments, with an average of 5 technical replicates in each. Error bars: SEM)

Real time, label free cellular analysis (RTCA)



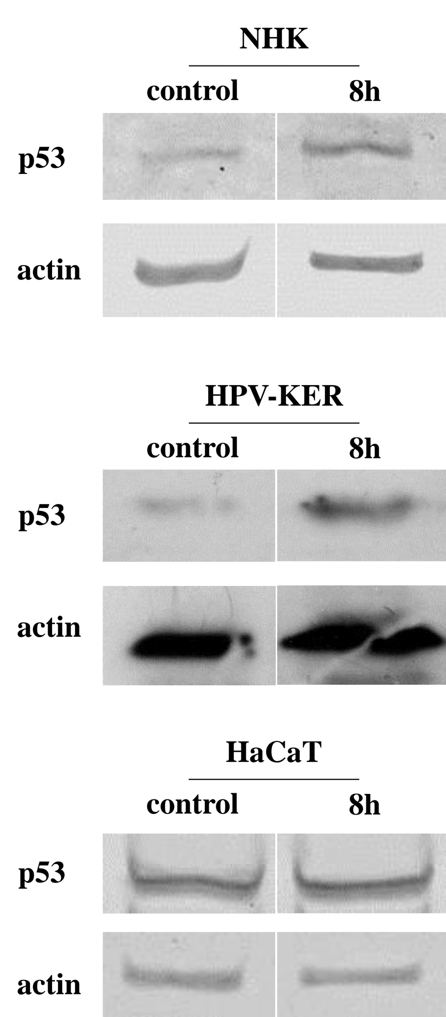
After plating of the cells, the CI value increased due to their proliferation. Twenty hours later the cultures were irradiated (black arrows). In the control cultures the CIs increased until the cells reached confluency, and showed cell-type specific characteristics: In case of the treatment with 20 mJ/cm^2 UV-B dose the CI values slowly decreased in NHK and HPV-KER cultures. In HaCaT cells, CIs reached a steady state level after a transient increase. Treatments of 40 and 60 mJ/cm^2 UV-B caused a gradually decreasing CIs in all three cell types. These results were in agreement with the results of the MTT assay. (Representative of two parallel experiments with 4 technical replicates each.)

Gene expression analysis



The expressions of selected UV-B target genes (IL-1A, IL-8, COX2 and TNFA) were analysed by real time RT-PCR. The pattern of gene expression changes were the same in NHKs and HPV-KER cells, while HaCaT cells exhibited significantly different patterns of IL-1A and IL-8 gene expressions compared either to NHKs or HPV-KER cells. All data were compared to the time matched, untreated control values. (Average of 2 independent experiments, with an average of 2 technical replicats in each. Error bars: SEM)

Western blot analysis



Inducible p53 expression was detected in NHKs and HPV-KER cells upon UV-B irradiation. In contrary, HaCaT cells exhibited high basal p53 expression and that was not further inducible upon UV-B irradiation.

CONCLUSION:

HaCaT cells are widely used to study the effect of UV-B irradiation, however these cells have mutated p53 protein. Our data suggest that HPV-KER cells show more similar characteristics in their induced gene and protein expression patterns after UV-B irradiation to NHKs then HaCaT cells. As a result they may provide a suitable *in vitro* model for studying keratinocyte UV-B responses.

FUNDING: OTKA K83277, OTKA NK 105369, TÁMOP-4.2.2/A-II/I/KONV-2012-0035, SZÉCHENYI PLAN TÁMOP-4.2.2/B-10/1-2010-0012 project: "Broadening the knowledge base and supporting the long term professional sustainability of the Research University Center of Excellence at the University of Szeged by ensuring the rising generation of excellent scientists." HUNGARY'S RENEWAL

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