Constitutive Photomorphogenic Protein is involved in the UVB-induced cellular response of human keratinocytes

Barbara Fazekas[1], Hilda Polyánka[2], Attila Bebes[1], Ágnes Kinyó[1], Gábor Tax[1], Ferenc Nagy[3], Lajos Kemény[1,2] Éva Ádám[3], Márta Széll[2,4]

[1.]Department of Dermatology and Allergology, University of Szeged, Hungary
[2.]MTA-SZTE Dermatological Research Group, Hungary
[3.]Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Hungary
[4.]Institute of Medical Genetic, University of Szeged, Hungary

Introduction

The Constitutive Photomorphogenic Protein (COP1) was first described in *Arabidopsis thaliana* (AtCOP1) and defined as a central negative regulator of photomorphogenesis: it functions as an E3 ligase and promotes ubiquitine-dependent degradation.

Others have previously demonstrated that the human orthologue of COP1 (huCOP1) is overexpressed in cancer cells and represses the p53-dependent tumor suppression (Dornan et al.2004, Wertz et al.2004).

Our group have recently shown that huCOP1 is expressed in human keratinocytes in an UV-B regulated manner and regulates the expression of p53. (Kinyó et al.2009) .

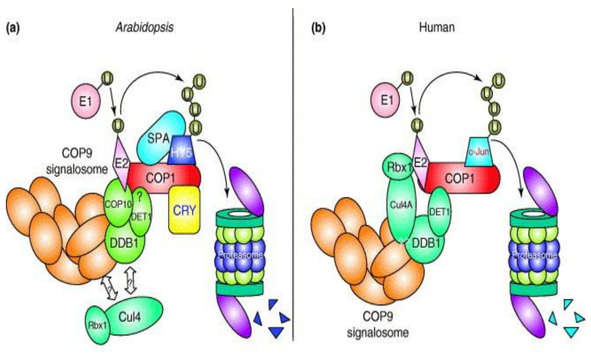

Figure 1. Stuctural and functional analogy between AtCOP1 and mammalian COP1 (Yi et al, Trends Cell Biol, 2005)

Objectives

The aim of our study was to establish keratinocyte cell lines in which the expression of huCOP1 is stably silenced, and to determine the role of huCOP1 in the UVB response using this experimental system.

Materials and methods

- Chimaeric constructs harbouring 53-nt-long oligonucleotides of COP1 were constructed using the pSUPER vector system. (Oligoengine, Seattle, WA, USA)
  5'AGCTTcctcaaggttgcaagaagaTTCAAGAGAtcttcttgcaaccttgaggTTG3'(sense) and
  5'TCGACAACCTCAAGGTTGCAAGAAGATCTCTTGAATCTTCTTGCAACCTTGAGGA3' (antisense).
- Stable transformation of the HPV-immortalized keratinocyte cell line (HPV-KER II/15) was performed using either the empty vector or the vector harbouring the huCOP1 silencing sequences.
- Four cell lines harbouring the empty vector (pS1, pS3, pS4 and pSN) and three cell lines carrying the huCOP1 silencing sequences (Copb8, Copb9 and CopbN) were established.
- HuCOP1 silencing was assessed by Western blot (colorimetric and chemiluminescens detection) and by immunocytochemical stainings. The expression of p53 was followed by immunocytochemistry and by the subsequent semiquantitative analysis using the Metamorph software.
- Cell viability of the established keratinocyte cell lines was measured with the xCELLigence System.
- For the assessment of UV-B regulation of huCOP1 in the established cell lines, 20 mJ/cm^2 irradiation was applied.

Conclusions, future prospects

We have successfully established three cell lines in which the expression of huCOP1 is stably silenced. Our results suggest that the UV-B induced expression of p53 is affected by the silencing of huCOP1.

These cell lines provide a good tool for further investigations to understand the role of huCOP1 in the UVB-induced signaling processes of human keratinocytes.

Results

Demonstration of huCOP1 silencing by different methods

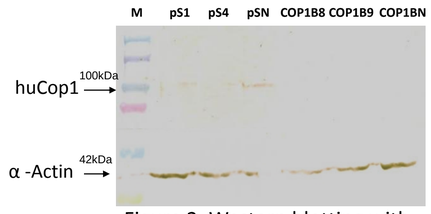

Figure 2. Western blotting with colorimetric detection

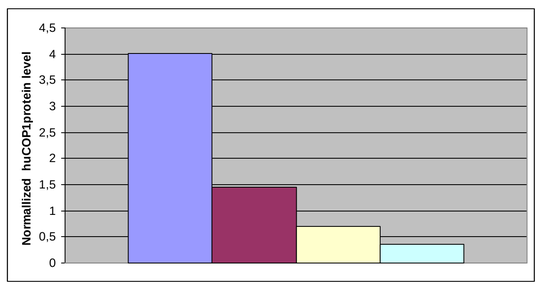

Figure 4. Quantitative analysis of a Western blot with chemiluminescence detection (arbitrary units)

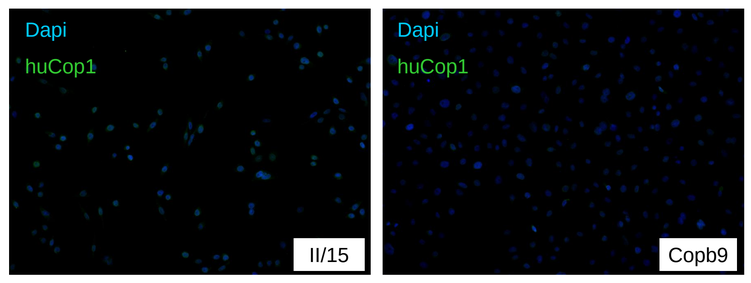

Figure 3. Immunocytochemistry

Investigation of the cell viability of the huCOP1 silenced keratinocytes

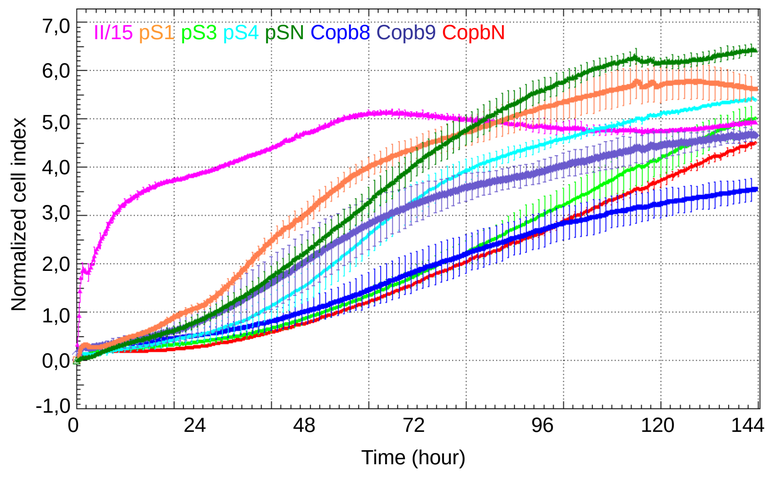

Figure 5. Cell viability of the established keratinocyte cell lines was measured with the xCELLigence System

We investigated the proliferation profiles of the cell lines and found that one of them (CopbN) had a significantly reduced growth rate, suggesting that the silencing of huCOP1 affected crucial growth regulatory pathways in this cell line.

Investigation huCOP1 and p53 expression upon UV-B irradiation

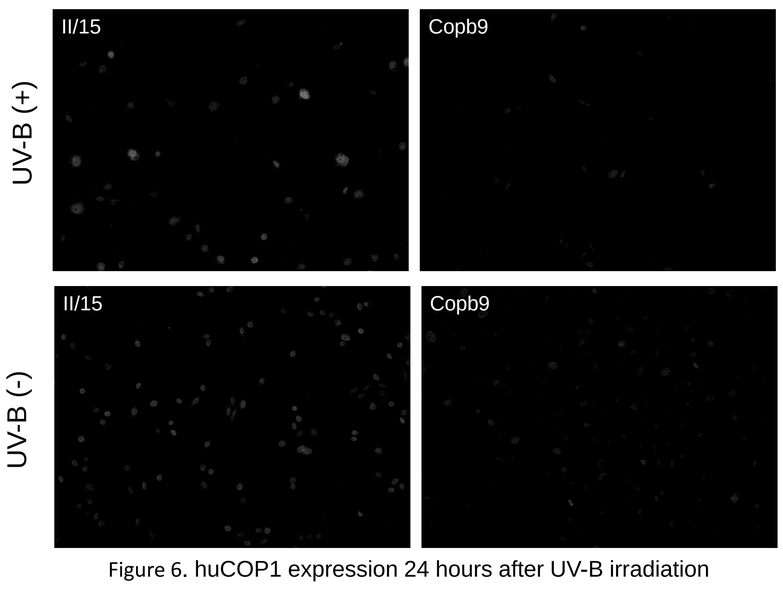

Figure 6. huCOP1 expression 24 hours after UV-B irradiation

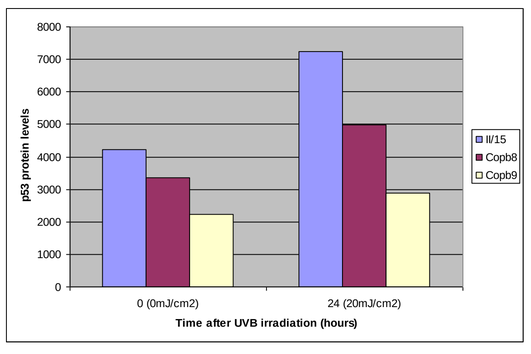

Figure 7. p53 expression 24 hours after the UV-B irradiation (semiquantitative analysis of immunocytochemical stainings; results of one representative experiment)

The expression of huCOP1 is decreasing upon UV-B irradiation in the control cells (HPV-KER II/15). In the huCOP1-silenced cells (Copb9) the basal expression huCOP1 is lower compared to that of seen in the control cells (II/15) and is further decresed upon UV-B irradiation.

UV-B irradiation resulted in a 1.7-fold induction of p53 protein expression in the control keratinocytes (HPV-KER II/15) while the level of induction was somewhat lower in the case of the two huCOP1-silenced keratinocyte cell lines (1.3 and 1.4 in the Copb9 and Copb8 cell lines, respectively).

barbara.fazekas8@gmail.com

TÁMOP 4.2.2.A-II/I/KONV-2012-0035

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."