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MCPIP1 contributes to the inflammatory response of UVB-treated keratinocytes

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ABSTRACT

Background: Monocyte chemoattractant protein-1-induced protein-1 (MCPIP1), also known as regnase-1, negatively regulates many cellular processes including the cellular response to inflammatory agents, differentiation, viability, and proliferation. It possesses a PilT N-terminus (PIN) domain that is directly involved in regulating the stability of transcripts and miRNAs by recognizing stem loop structures and degrading them by endonucleolytic cleavage.
Objective: We investigated the role of MCPIP1 in the response of human primary keratinocytes to UVB stress.
Methods: Keratinocytes were treated with UVB, siRNA against MCPIP1, pharmacological inhibitors of signaling pathways, or subjected to control treatments. The mRNA and protein levels of MCPIP1 and MCPIP1-dependent changes gene expression were analyzed by quantitative (Q)-RT-PCRs and Western blots. Secretion of TNFα and IL-8 was determined by ELISA.
Results: UVB treatment of keratinocytes induced upregulation of MCPIP1 at the mRNA level after 4–8 h and at the protein level after 8–16 h. MCPIP1 abundance depended on NF-κB activity. Using an siRNA strategy, we found that diminished MCPIP1 resulted in an up-regulation of transcripts coding for IL-8, TNFα, COX-2, and BCL-2, as well as an enhanced release of IL-8. Moreover, decreased phosphorylation of NF-κB and p38 signaling pathways were observed in addition to a slight up-regulation of ERK1/2 directly after UVB treatment. Twenty-four hours later, decreased phosphorylation was observed only for NF-κB and p38. Furthermore, in MCPIP1-suppressed cells, the levels of pro-apoptotic Puma, the phosphorylated form of p53 and the abundance of its target p21 as well as the activity of caspase 3 decreased, while the level of cyclin D1 increased.
Conclusion: MCPIP1 contributes to the UVB response of keratinocytes by altering metabolic and apoptotic processes and the release of inflammatory mediators.

1. Introduction

The skin serves as a barrier that separates the internal organs of our body from the external environment. The outermost layer of the skin, the epidermis, consists of a stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. These layers are composed of keratinocytes in various stages of differentiation [1–3].

One dangerous impact on human skin is solar UV radiation that reaches the skin at high doses particularly during the summer season [4]. Prolonged exposure to UV radiation leads to sunburns, photoaging, inflammation, DNA damage, and, in extreme cases, carcinogenesis [5–9]. On a molecular level, UV exposure is followed by the activation of multiple signaling pathways involved

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in cell growth, proliferation, migration, and chronic inflammation [10–12]. Activated keratinocytes produce a wide repertoire of pro-inflammatory cytokines that profoundly influence both skin-resident and recruited immunocompetent cells [8,13–15]. UVB radiation (280–320 nm) reaches the earth at a significantly lower intensity than UVA (320–400 nm), however, it has more carcinogenic properties than UVA [16,17].

Monocyte chemoattractant protein-1 induced protein-1 (MCPIP1), also known as regnase-1, is a negative regulator of inflammation [18]. This protein is encoded by the *ZC3H12A* gene, which is activated by factors that induce inflammation [19–21]. MCPIP1 possesses a PilT N-terminus (PIN) domain, essential in the degradation of transcripts coding for proinflammatory cytokines IL-1β, IL-6, and IL-12p40 [19,20,22]. Recent studies have shown that MCPIP1 influences miRNA biogenesis by recognizing stem loop regions and degrading pre-miRNA templates [23]. MCPIP1 is also a negative regulator of the transcription factor NF-κB, a key regulator of inflammation [24,25]. In this manner, MCPIP1 interacts with the deubiquitinase, USP10, and, as a complex, diminishes the ubiquitination of TRAF family members, key regulatory proteins in the NF-κB signaling pathway. Thereby, MCPIP1 is able to decrease NF-κB activity [25]. Besides inflammation, MCPIP1 is implicated in angiogenesis [26], adipogenesis [27], and osteoclastogenesis [28].

Although MCPIP1 was described as a potent regulator of inflammation in many physiological settings [19,24,29,30], its regulation and role in keratinocytes stimulated with UVB has not been reported so far.

2. Materials and methods

2.1. Cell isolation and culture

Human primary keratinocytes were isolated from skin biopsies taken from healthy donors during plastic surgery and cultured as previously described [31]. Informed consent and Ethical Committee approval were obtained according to Polish law (No. KBET/72/B/2008). Cells were cultured in 75 cm^2 cell-culture flasks at 37 °C in 5% CO_2 atmosphere in serum free Keratinocyte Growth Medium KGM-Gold™ (Lonza Group Ltd., Basel, Switzerland). This medium is supplemented with bovine pituitary extract, human endothelial growth factor, bovine insulin, hydrocortisone, gentamicin–amphotericin B (GA-1000), epinephrine, and transferrin.

2.2. UVB irradiation

Keratinocytes were cultured at a seeding density of 2×10^5 cells per 35 mm culture dish for 24 h in the medium described above. The medium was then replaced by PBS supplemented with 0.01% $MgCl_2$ and 0.01% $CaCl_2$, and keratinocytes were irradiated with a bank of four Philips UVB TL/40 W/12/RS sunlamps (280–320 nm, max 311 nm). The cells were irradiated with 0.5 mW/cm^2 UVB (as measured by UVX 31 digital radiometer; Ultra-Violet Products, California, USA) for 30 or 60 s (equivalent of 15 and 30 mJ/cm^2 of UVB). After irradiation, the original medium was returned to each culture dish, and the cells were subsequently used for further experiments.

2.3. Cell transfection

Keratinocytes (2×10^5 cells per 35 mm culture dish) were seeded 24 h before transfection. Two types of small interfering RNA (siRNA) were used: a pool of four different siRNAs specific for MCPIP1 coding gene (Dharmacon; cat. M-014576-01-0020) and Non-Targeting siRNA (Dharmacon; cat. D-001206-13-20), serving as a negative control of the experiment. Cells were transfected with 60 pmol of siRNA in 0.5 ml Opti-MEM media (Life Technologies, Carlsbad, CA, USA) using 4 μl of Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. After 24 h, the medium was changed, and 48 h after transfection, the cells were treated with UVB radiation. The ability of siRNA to inhibit *ZC3H12A* gene expression was assessed by Western blot analysis.

2.4. Treatment with inhibitors

All inhibitors were purchased from Sigma Aldrich (St. Louis, MO, USA) and used as follows: actinomycin D and cycloheximide were applied 1 h before UVB irradiation in the concentrations of 2 μg/ml and 20 μg/ml, respectively. Bay11-7082 was applied 24 h before UVB irradiation in the concentrations of 20 nM. SB203580 was applied 2 h before UVB irradiation in the concentration of 20 μM. SP600125 was applied 2 h before UVB irradiation at the concentration of 30 μM. UO126 was applied 1 h before UVB irradiation at a concentration of 10 μM. In all experiments, cells treated with 0.1% DMSO (vehicle) served as a control. Medium used for cell culturing before UVB irradiation was returned to each culture dish after UVB treatment.

2.5. Western blot analysis

Cells were lysed in RIPA buffer (25 mM Tris–HCl, pH 7.6; 150 mM NaCl; 1% NP-40; 1% sodium deoxycholate; 0.1% SDS) containing a protease inhibitor cocktail (Sigma Aldrich). For phosphorylated protein detection, a phosphatase inhibitor cocktail (PhosSTOP; Roche, Basel, Switzerland) was added to the RIPA buffer. For caspase 3 activation analysis, adherent and floating cells (collected by centrifugation of the supernatant) were combined. The protein concentration in cell lysates was measured with a bicinchoninic acid assay. Equivalent amounts of total protein were separated on 8–12% polyacrylamide gels and then transferred to Immobilon PVDF membranes (Millipore Corporation). As a blocking agent, 3% milk in Tris-buffered saline containing 0.05% Tween (Sigma Aldrich) was applied. Membranes were incubated with primary antibody at 4 °C overnight. After three washes, secondary antibodies were added and an additional three washes were performed. Detection was performed using a Luminata Crescendo (Millipore) substrate and the chemiluminescence detector ChemiDoc (BioRad). Specific proteins were detected using the following antibodies: rabbit polyclonal anti-MCPIP1 (1:2000, cat. GTX110807; GeneTex), rabbit monoclonal anti-phospho-p65 (Ser536) (1:1000, cat. 3033; Cell Signaling), rabbit polyclonal anti-p65 (1:1000; Cell Signaling, cat. 4764), rabbit polyclonal anti-phospho-JNK (1:500, cat. 9251; Cell Signaling), rabbit polyclonal anti-JNK (1:500, cat. 9252; Cell Signaling), rabbit polyclonal anti-phospho-p38 (1:1000, cat. 9211; Cell Signaling), rabbit polyclonal anti-p38 (1:500, cat. 9212; Cell Signaling), rabbit polyclonal anti-phospho-ERK1/2 (1:1000, cat. 9101; Cell Signaling), rabbit polyclonal anti-ERK1/2 (1:1000, cat. 9102; Cell Signaling), anti-caspase 3 (1:1000, Cell Signaling, cat. 9662), rabbit anti-phospho-p53 (Ser15) (1:1000, cat. 9284S; Cell Signaling), rabbit anti-Puma (1:750, cat. 4976; Cell Signaling), rabbit anti-Bax (1:1000, cat. 2772; Cell Signaling), rabbit anti-p21 (1:1000, cat. 2947 T), rabbit anti-Cyclin D1 (1:1000, cat. 2978S, Cell Signaling), mouse anti-β-actin (1:3000, cat. A1978, Sigma Aldrich), goat peroxidase-conjugated anti-rabbit (1:3000, cat. 7074; Cell Signaling), and goat peroxidase-conjugated anti-mouse IgG (1:20,000, cat. 554002; BD Pharminogen). Densitometry analysis was performed with ImageJ 1.40 G software. All measured values were normalized to β-actin expression.