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Original Article

p38 but not p53 is responsible for UVA-induced MCPIP1 expression

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ABSTRACT

MCPIP1 (Monocyte Chemotactic Protein-1 Induced Protein) is an important regulator of inflammation and cell apoptosis, but its role in UVA-induced stress response in the epidermis has never been studied. We have found that moderate apoptosis-inducing dose of UVA (27 J/cm^2) increases the level of MCPIP1 expression in HaCaT cells and normal human keratinocytes (NHEK) within 6–9 h after the treatment. MCPIP1 upregulation was dependent on the induction of p38, but not p53, as demonstrated by using p38 inhibitor SB203580 and p53 inducer RG7388, respectively. This increase was also blocked by antioxidants (α-tocopherol and ascorbic acid), suggesting the involvement of MCPIP1 in UVA-induced oxidative stress response. Si-RNA-mediated down-regulation of MCPIP1 expression in HaCaT cells resulted in increased sensitivity to UVA-induced DNA damage and apoptosis. This was accompanied by decreased phosphorylation of p53 and p38 in MCPIP1-silenced cells following UVA irradiation. The activation of p38 in response to low doses of ultraviolet radiation was postulated to be protective for p53-inactive cells. Therefore, MCPIP1 may favor the survival of p53-defective HaCaT cells by sustaining the activation of p38. This creates a loop of mutual positive regulation between p38 and MCPIP1 protein in HaCaT cells, providing the protection against the consequences of UVA irradiation.

1. Introduction

UVA radiation (315/320–400 nm), a major part of solar ultraviolet light (> 90%) and of the radiation emitted by artificial UV tanning devices (99–95%), is a well-established oxidizing agent (Svobodova et al., 2006; Xing et al., 2008; Bossi et al., 2008). Exposure to sunlight can induce erythema, inflammation, pigmentary disorders, premature skin aging (photoaging), and even skin cancers (Svobodova et al., 2006; Xing et al., 2008; Bossi et al., 2008; Pfeifer and Besaratinia, 2012). In the high risk group there are not only people with increased sensitivity to light, but all Caucasians, especially with phototype I and II of the skin.

The UVA mutagenic effect is thought to be due to DNA damage, mediated by the generated ROS. The most frequent mutagenic DNA lesion produced by oxidative stress is 8-oxo-2′-deoxyguanosine (8-oxo-dG), which causes G to T transversion mutations. This fingerprint mutation was found to be harbored more frequently than UVB (C to T) mutation in the basal layer in human squamous cell tumors, which suggested a role for UVA-induced oxidized guanines in human skin carcinogenesis (Pfeifer and Besaratinia, 2012; Agar et al., 2004)

MCPIP1 (Monocyte Chemotactic Protein-1 Induced Protein), also known as regnase-1, is a multi-functional protein encoded by the *ZC3H12A* gene (Jura et al., 2012). The protein is engaged in such processes as the regulation of inflammatory state (Miao et al., 2013; Liang et al., 2008), the induction of autophagy (Kolattukudy and Niu, 2012) and angiogenesis (Roy and Kolattukudy, 2012), the activation of programmed cell death (Zhou et al., 2006; Qi et al., 2011) and the defense against viral infections (Lin et al., 2013). The protein possesses two distinct molecular activities: it acts as a ribonuclease, targeting specific transcripts encoding proinflammatory cytokines (Matsushita et al., 2009; Mizgalska et al., 2009), and deubiquitinase, responsible for the inactivation of such proteins as TRAF2 or TRAF6 (Liang et al., 2010). By degrading the transcripts coding for IL-6, IL-1β and IL-2, MCPIP1 inhibits the activation of macrophages and T cells (Li et al., 2012; Iwasaki et al., 2011). MCPIP1 also decreases transcriptional activity of NF-κB and AP-1, which additionally interfere with the activating processes inside immune cells (Liang et al., 2010; Skalniak et al., 2009). Due to its immunosuppressive, proapoptotic and antiviral properties the protein was studied in several disease-related states, such as rheumatoid arthritis (He et al., 2013), cardiovascular disease (Niu and Kolattukudy, 2009), psoriasis (Monin et al., 2017), HIV infection (Liu et al., 2013) and cancer (Lu et al., 2016). Our recent study showed

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that MCPIP1 level increases after UVB exposure in NHEK cells and contribute to pro-apoptotic and pro-inflammatory responses *in vitro* (Bugara et al., 2017). However, the possible role of MCPIP1 UVA-induced stress response and apoptosis in the epidermis remains unknown.

In the present study we investigated the influence of moderate apoptotic dose of UVA on MCPIP1 expression level in HaCaT cells. To explore MCPIP1 function, RNA interference technique was employed. Our results show that silencing of MCPIP1 by specific siRNA increases the sensitivity of HaCaT cells to UVA-induced oxidative DNA damage and apoptosis. This study shows for the first time that MCPIP1 is a component of the cell response to UVA.

2. Materials and methods

2.1. Cell culture

HaCaT cells were purchased from CLS (Cell Lines Service GmbH, Eppelheim, Germany), normal, adult, primary human keratinocytes (NHEK) from Lonza (Lonza). HaCaT cells were cultured in DMEM containing 4.5 g/l glucose and 10% FBS (Lonza), U2OS and SAOS-2 in McCoy medium with 10% FBS (Lonza). NHEK cells were cultured in Keratinocyte Growth Medium KGM-Gold™ supplemented with bovine pituitary extract, human endothelial growth factor, bovine insulin, hydrocortisone, epinephrine, gentamicin-amphotericin B (GA-1000) and transferring (Lonza). The cells were cultured in a humidified atmosphere at 37 °C and 5% CO_2 and passaged at c.a. 70–80% of confluence. The cells were cultured without antibiotics, and were routinely tested for mycoplasma using PCR mycoplasma test (AppliChem, Darmstadt, Germany) according to manufacturer's protocol.

2.2. UVA irradiation and antioxidants and p38 inhibitor treatment

3×10^5 cells were seeded on 35 mm culture dish 24 h before the treatment. Thereafter, the medium was removed and the cells were irradiated with UVA in PBS, supplemented with 0.01% $MgCl_2$ and 0.01% $CaCl_2$ at room temperature via a lid, using UVA (TL-09 UV-A) fluorescent tubes (Philips HB 311 lamp, the Netherlands) (Wolnicka-Głubisz et al., 2005; Wolnicka-Glubisz et al., 2010). The integrated irradiance of the lamp measured using a UVX 36 digital radiometer (Ultra-Violet Products, California, USA) was 6.2–6.3 mW/cm^2. Control cells were kept in the dark under similar conditions.

To investigate the effect of antioxidants on intracellular H_2O_2 level and the expression of MCPIP1, the cells were preincubated overnight with α-tocopherol (40 μM, Sigma Aldrich St. Louis, MO, USA) and irradiated with UVA in the absence or presence of ascorbic acid (100 μM, Sigma). p38 inhibitor, SB203580 (Sigma Aldrich) was applied 1 h before UVA irradiation at the concentration of 1 μM in the culture medium, withdrawn for the exposure and added back after the treatment.

2.3. Cell transfection

For MCPIP1 silencing the cells were transfected with MCPIP1-specific Stealth RNAi (Thermo Fisher Scientific) siRNA sequences (AGCUGGGCUAUUCAUCCACGGAGAU, AUCUCCGUGGAUGAAUAGCCCAGCU), or negative control, Stealth RNAi Neg Med GC (Thermo Fisher Scientific, Waltham, USA) using Lipofectamine RNAiMAX, according to manufacturer's recommendations. Briefly, 100 000 cells were seeded on 35 mm cell culture dishes 24 h before the transfection. For the transfection, 60 pmol of siRNA was mixed with 500 μl of Opti-MEM medium, and separately 7.5 μl of Lipofectamine RNAiMAX was mixed with 500 μl of Opti-MEM and incubated for 5 min at room temperature. After that, Lipofectamine-containing Opti-MEM was added to siRNA-containing Opti-MEM and incubated at room temperature for additional 20 min. Then, the prepared mixture was added dropwise to the culture dish, and incubated in cell culture incubator for 6 h. The medium was exchanged with the fresh culture medium, and cells were cultured for additional 42–48 h.

2.4. Western blotting

For the detection of proteins, total cell lysates were prepared using RIPA buffer (25 mM Tris HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS), supplemented with protease inhibitor cocktail (Sigma Aldrich). For the analysis of phosphorylated proteins, RIPA buffer was additionally supplemented with phosphatase inhibitor cocktail (PhosSTOP, Sigma Aldrich). Twenty μg of total lysates were separated on 4–12% SDS-Page polyacrylamide gel (Life Technologies), western blot procedure was performed as described before (Skalniak et al., 2013). The following antibodies and dilutions were used: rabbit polyclonal anti-MCPIP1 (1:3 000,GeneTex, cat. 110807), anti-p-p53 (S15) (1:1 000, Cell Signaling Technology (CST), cat. 9284), rabbit polyclonal anti-p53 (1:200, Santa Cruz, cat. sc-6243), rabbit anti-p-p38 (1:1 000, CST, cat. 4511), rabbit polyclonal anti-p38 (1:1 000, CST, cat. 9212), rabbit monoclonal anti-p21 (1:1 000, CST, cat. 2947), mouse anti-α-tubulin (1:2 000, Calbiochem, cat. 554002), rabbit monoclonal anti-GAPDH (1:4 000, CST, cat. 2118), goat peroxidase-conjugated anti-rabbit (1:3 000, CST, cat. 7074), and goat peroxidase-conjugated anti-mouse (1:20 000, BD Pharminogen). The densitometry analysis was performed with ImageLab software (Bio-Rad, California, USA).

2.5. DHR 123 and DNA damages

The detection of intracellular production of H_2O_2 was performed using dihydrorhodamine 123 (DHR123; Sigma Aldrich) directly after UVA-irradiation as described before (Wolnicka-Glubisz et al., 2015). The level of DNA damage was determined by the electrophoresis of single cells in agarose gel as earlier described (Kapiszewska et al., 2005, 2007). To measure UVA-induced oxidative DNA damage we used the combination of comet assay and specific enzymes, i.e. endonuclease III (Endo III), which specifically recognizes oxidized pyrimidines (i.e. thymine glycol, 5,6-dihydroxy thymine) and formamidopyrimidine DNA glycosylase (Fpg), which specifically recognizes oxidized purines (i. e. 8-oxo-2′-deoxyguanosine, 8-oxo-2-deoxyadenine) (Kapiszewska et al., 2005). Briefly, the cell suspension was mixed with low melting point agarose, set on slides, lysed and neutralized in appropriate buffers. To detect oxidative damage, endonuclease III (Endo III; 0.1 U/slide) and formamidopyrimidine DNA glycosylase (Fpg; 0.1 U/slide) were applied and incubated for 45 min at 37 °C in humidified chamber. Electrophoresis was performed at 23 V (0.74 V/cm, 300 mA) for 30 min at 4 °C. All stages of the experiment were carried out in the dark to avoid any additional DNA damage. Prior to the analysis, the slides were stained with propidium iodide (2.5 μg/ml). The analysis of DNA damage was carried out with COMET PLUS 2.9 software (Comet Plus, Theta System Gmbh, Germany). The percentage content of DNA in the comet's tail (DNA damage) was determined from 100 random images of comets per slide. Each experimental point was run in triplicate.

2.6. Cell cytotoxicity and apoptosis assays

For MTT cell viability assay, Thiazolyl Blue Tetrazolium Bromide (MTT, Sigma) was added for 60 min at a final concentration of 500 ng/ml. The medium was removed by suction and MTT crystals were dissolved in DMSO: EtOH (1:1). The absorbance was measured at 560 nm in a plate reader (Tecan Genios, Männedorf, Switzerland).

Caspase 3/7 activity was measured using Caspase-Glo 3/7 Assay (Promega, Madison, USA). Between 6–9 h following UVA irradiation the cells were washed with PBS, scraped, lysed in 90 μl RIPA buffer, and stored at −20 °C. Then, 4 μg of protein adjusted to 40 μl with RIPA was mixed with 40 μl of Caspase-Glo 3/7 Reagent on white 96-well. The plates were shaken for 1 min at 300 rpm and kept in the dark at room temperature for 90 min. Luminescence was measured with Infinite