Peptides 61 (2014) 93–97

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Short communication

Ultraviolet B radiation-stimulated urocortin 1 is involved in tyrosinase-related protein 1 production in human melanoma HMV-II cells

Yutaka Watanuki[a], Kazunori Kageyama[a,b,*], Shinobu Takayasu[a], Yasushi Matsuzaki[c], Yasumasa Iwasaki[d], Makoto Daimon[a]

[a] Department of Endocrinology and Metabolism, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan
[b] Department of Endocrinology, Metabolism, and Infectious Diseases, Hirosaki University School of Medicine & Hospital, 53 Hon-cho, Hirosaki, Aomori 036-8563, Japan
[c] Department of Dermatology, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan
[d] Health Care Center, Kochi University, Kochi, Kochi 780-8520, Japan

ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form 9 September 2014
Accepted 9 September 2014

Keywords:
Corticotropin-releasing factor
Urocortin 1
Nurr-1
Nur77
Tyrosinase-related protein 1

ABSTRACT

Ultraviolet B (UVB) radiation stimulates cutaneous melanin pigmentation. The melanosomal enzyme tyrosinase-related protein 1 (TRP1) is involved in the modulation of pigment production in response to this stressor. Recent molecular and biochemical analyses have revealed the presence of corticotropin-releasing factor (CRF) and urocortin 1 (Ucn1), together with their corresponding receptors, in mammalian skin. Although CRF and Ucn1 are thought to have potent effects on the skin system, their possible roles and regulations have yet to be determined fully. Our previous findings in human melanoma HMV-II cells suggest that both CRF and Ucn1 regulate TRP1 gene expression via Nurr-1/Nur77, transcription factors that constitute the nuclear receptor 4a subgroup of orphan nuclear receptors. HMV-II cells were found to express mainly Ucn1 mRNA. This study aimed to explore the effects of UVB on Ucn1 mRNA and TRP1 protein levels in HMV-II cells. UVB (30 mJ/cm^2) increased Nurr-1, Nur77, and Ucn1 mRNA levels. UVB also increased TRP1 protein levels. Ucn1 knockdown inhibited the UVB-induced increases in TRP1 protein levels. These data suggest that UVB-stimulated Ucn1 contributes to TRP1 production via the transcription of both Nurr-1 and Nur77. Ucn1, produced in melanoma cells, acts on melanoma cells themselves in an autocrine manner.

Introduction

Corticotropin-releasing factor (CRF), which is synthesized in and secreted from the hypothalamic paraventricular nucleus in response to stress, stimulates adrenocorticotropic hormone (ACTH), which is encoded by the proopiomelanocortin (POMC) gene in pituitary corticotrophs [3,11]. Urocortin 1 (Ucn1), a 40-amino acid peptide originally cloned from the rat Edinger–Westphal nucleus, is a member of the CRF family of peptides [26]. Ucn1 in the Edinger–Westphal nucleus may play a significant role in stress adaptation even in humans [12]. Both CRF and Ucn1 contribute to the stress response and cardiovascular and gonadal functions via G protein-coupled seven transmembrane CRF receptors [6,24,25]. CRF exhibits a high affinity for CRF receptor type 1 (CRF_1 receptor; IC_{50} = 1.6 nM), but not for CRF receptor type 2b (CRF_{2b} receptor; IC_{50} = 42 nM), while Ucn1 exhibits similar affinity for the CRF_1 (IC_{50} = 0.16 nM) and CRF_{2b} (IC_{50} = 0.86 nM) receptors [5]. Recent molecular and biochemical analyses have revealed the presence of CRF and Ucn1, together with their corresponding receptors, in mammalian skin [17,18]. The CRF or Ucn system also may exist in the skin [15,21]. CRF activates POMC gene transcription and ACTH release through CRF_1 receptors in melanocytes or melanoma cells [9,13,23,30]. CRF also regulates cell viability, proliferation, and migration of skin cells [22,29]. In addition, CRF and Ucn1 stimulate the

* Corresponding author at: Department of Endocrinology and Metabolism, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan. Tel.: +81 172 39 5062; fax: +81 172 39 5063.
E-mail address: kkageyama@hkg.odn.ne.jp (K. Kageyama).

http://dx.doi.org/10.1016/j.peptides.2014.09.009
0196-9781/

expression of tyrosinase-related enzymes in human hair follicle melanocytes [7].

Cutaneous melanin pigmentation occurs in response to external and internal stresses [4,19,20]. Ultraviolet B (UVB) radiation stimulates the production of α-melanocyte-stimulating hormone (α-MSH) in human keratinocytes and melanocytes [1,14]. The melanosomal enzyme tyrosinase-related protein 1 (TRP1) is involved in the modulation of pigment production in response to the stressor. Both Nurr-1 and Nur77 are transcription factors that constitute the nuclear receptor 4a subgroup of orphan nuclear receptors [2]. They also have the characteristics of immediate early genes and are induced by a variety of extracellular signals. Both Nurr-1 and Nur77 are known to act via a nerve growth factor-inducible factor-B response element (NBRE) in the TRP1 promoter. Indeed, putative NBREs have also been identified in the TRP1 promoter [27].

Our previous findings in human melanoma HMV-II cells suggest that both CRF and Ucn1 regulate TRP1 gene expression via Nurr-1/Nur77 [27]. HMV-II cells were found to express mainly Ucn1 mRNA. This study first aimed to explore the effects of UVB on Ucn1 gene expression and TRP1 protein levels using human melanoma HMV-II cells. To elucidate further the possible effects of endogenous Ucn1, TRP1 protein levels following UVB stimulation were examined further using small interfering RNA (siRNA) for Ucn1.

Materials and methods

Cell culture

HMV-II cells were cultured as follows using a cell line provided by the RIKEN BRC through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan. Human melanoma HMV-II cells were cultured in a T_{75} culture flask with Dulbecco's modified Eagle's medium/F-12 HAM supplemented with 10% fetal bovine serum, 50 μg/mL streptomycin, and 50 U/mL penicillin at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Culture media were changed every 48 h, and the cells were subcultured once a week. For each experiment, the cells were plated in 6-well plates at 2.0×10^5 cells/well and the medium was changed every 48 h. At the end of each experiment, total cellular RNA or protein was collected and stored at −80 °C until the relevant assay was performed.

UVB irradiation

UVB irradiation was performed as reported previously [10]. Cells in phosphate-buffered saline (PBS) were irradiated with UVB using two FL20S-E lamps (Toshiba, Tokyo, Japan) that emitted wavelengths of 280–320 nm with an emission peak at 312.5 nm and an intensity of 500 μW/cm^2 at the target area. The irradiation dose was 30 mJ/cm^2 (25 cm distance for 70 s). After UVB irradiation, the cells were cultured in fresh growth medium. The irradiance of the UVB rays was determined using an UVR-3036/S2 radiometer and a UVB detector (Clinical Supply, Kakamigahara, Japan).

RNA extraction

The cells were incubated with medium for the indicated times following UVB stimulation. At the end of each experiment, total cellular RNA was extracted using an RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. cDNA was synthesized from total RNA (0.5 μg) using random hexamers as primers with the SuperScript First-Strand Synthesis System for Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions.

Real-time RT-PCR

Total cellular RNA extraction and cDNA synthesis were performed as described above. First strand cDNAs were then subjected to real-time PCR as follows. The expression levels of human Nurr-1, Nur77, and Ucn1 mRNA were evaluated using quantitative real-time PCR based on specific sets of primers and probes (TaqMan Gene Expression Assays; Applied Biosystems, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene to standardize the obtained values, because GAPDH mRNA levels did not change in any of the treatments. Each reaction consisted of 1× TaqMan Universal PCR Master Mix (Applied Biosystems), 1× TaqMan Gene Expression Assays Products (Hs00174941_m1 for human CRF, Hs01845155_s1 for human Ucn1, Hs00264218_s1 for human Ucn2, Hs01118813_m1 for human Nurr-1, Hs00374230_m1 for human Nur77, and Hs99999905_m1 for human GAPDH), and 1 μL cDNA in a total volume of 25 μL using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems) as follows: 95 °C for 10 min and then 40 cycles at 95 °C for 15 s and 60 °C for 1 min.

The above assays involved specific sets of primers and a TaqMan probe spanning the exon–exon junction and should not, therefore, have been affected by DNA contamination. Data were collected and recorded with ABI PRISM 7000 SDS software (Applied Biosystems) and expressed as a function of the threshold cycle (C_T). The amplification efficacies for each gene of interest and the housekeeping gene amplimers were found to be identical when analyzed with diluted samples.

RNA interference experiments

Ucn1 and control siRNAs were designed and purchased from QIAGEN. The cells were transfected with siRNA and HiPerFect transfection reagent (QIAGEN) according to the manufacturer's protocol. For the measurement of Ucn1 mRNA levels, the cells, seeded into 6-well plates at a density of 2.0×10^5 cellswell, were incubated for 2 days in 1 mL culture medium containing siRNA for either control (siControl) or Ucn1 (siUcn1, Hs_UCN_2), and then incubated with medium for 48 h after UVB stimulation. The expression levels of TRP1 and β-actin proteins were examined by western blotting.

Western blotting

HMV-II cells were plated in 6-well plates at approximately 70% confluence. At the end of the incubation period, the cells were washed twice with PBS and lysed with Laemmli sample buffer. Cell debris was pelleted by centrifugation, and the supernatant was recovered. Twenty micrograms of extract were boiled and used for electrophoresis on a gradient (4–20%) polyacrylamide gel. The proteins were then transferred to a polyvinylidene fluoride membrane (Daiichi Kagaku, Tokyo, Japan). After blocking with Detector Block® Buffer (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA), the membrane was incubated for 1 h, detected with anti-TRP1(1:5000 dilution) (sc-25543; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-β-actin antibodies (1:10,000 dilution) (ab8227; Abcam, Cambridge, MA, USA), washed with PBS containing 0.05% Tween 20, and incubated