Journal of Dermatological Science 81 (2016) 53–60

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Analysis of lncRNAs expression in UVB-induced stress responses of melanocytes

Qinghai Zeng[a,1], Qi Wang[b,1], Xiang Chen[c], Kun Xia[d], Jintian Tang[e], Xiao Zhou[f], Yan Cheng[g], Yong Chen[h], Lihua Huang[i], Hong Xiang[i], Ke Cao[b,**], Jianda Zhou[a,*]

[a] Department of Plastic and Reconstructive Surgery, Third Xiangya Hospital of Central South University, Changsha 410013, China
[b] Department of Oncology, Third Xiangya Hospital of Central South University, Changsha 410013, China
[c] Department of Dermatology, Xiangya Hospital of Central South University, Changsha 410013, China
[d] State Key Laboratory of Medical Genetics, Central South University, Changsha 410078, China
[e] Institute of Medical Physics and Engineering, Department of Engineering Physics, Tsinghua University, Beijing 100084, China
[f] Department of Oncoplastic and Reconstructive Surgery, The Affiliated Tumor Hospital of Xiangya Medical School, Central South University, Changsha 410013, China
[g] School of Pharmaceutical sciences of Central South University, Changsha 410013, China
[h] Key Laboratory of Genetics and Birth Health of Hunan Province, Family Planning Institute of Hunan Province, Changsha 410126, China
[i] Central Laboratory, Third Xiangya Hospital of Central South University, Changsha 410013, China

ARTICLE INFO

Article history:
Received 10 April 2015
Received in revised form 30 September 2015
Accepted 28 October 2015

Keywords:
UVB
Melanocytes
lncRNA
Melanin
Melanoma

ABSTRACT

Background: Long non-coding RNAs (lncRNAs) have close relationships with oxidative stress, nutritional deficiency, DNA damage and other types of cellular stress responses. Previous studies have demonstrated that some non-coding RNAs in melanocytes such as microRNAs can change and contribute to the synthesis of melanin or the development of melanoma after stimulation with UV. However, as an important component of non-coding RNAs, it is unclear what changes occur in lncRNAs during UV-induced stress responses in melanocytes.

Objective: To explore changes in the expression of long non-coding RNAs (lncRNAs) in melanocytes following UVB-induced stress, and to explore if lncRNAs are involved in the synthesis of melanin.

Methods: Primary melanocytes were irradiated by 20 mJ/cm^2 UVB. The MTT method was used to detect cell proliferation. Quantitative real-time PCR was carried out to analyze expression of tyrosinase (*TYR*) and lncRNAs. Dopa colorimetry was performed to analyze *TYR* activity. The expression profile of lncRNAs and mRNAs were confirmed using an Agilent Human lncRNA 4 × 180 K chip. Intracellular ROS levels were detected by flow cytometry. ROS scavenger (NAC) was employed to inhibit the ROS level. *TYR* mRNA expression and activity were re-analysed after transfecting of lnc-CD1D-2:1 siRNA and lnc-SGCG-5:4 siRNA in UVB-irradiated melanocytes to confirm the roles of the two lncRNAs in the synthesis of melanin. phospho-ERK, phospho-p38, and phospho-JNK expressions were detected by Western Blot.

Results: Cell proliferation of the 20 mJ/cm^2 UVB-irradiated melanocytes decreased to 91% of that of the control cells. Twenty-four hours after irradiation with 20 mJ/cm^2 UVB, TYR mRNA expression and activity of the irradiated cells were significantly increased relative to the control group. Chip detection data showed that after irradiation with 20 mJ/cm^2 UVB, the expression of 807 lncRNAs and 69 stress response-related genes had changed by more than two-fold. Expression levels of Lnc-GKN2-1:1, lnc-CD1D-2:1, and lnc-SGCG-5:4 and ROS content were significantly increased after UVB irradiation. NAC reduced UVB-induced ROS generation and inhibited UVB-induced upregulation of lnc-GKN2-1:1 and lnc-CD1D-2:1. Lnc-CD1D-2:1 siRNA significantly suppressed the UVB-induced TYR mRNA expression and tyrosinase activation. Lnc-CD1D-2:1 siRNA inhibited UVB-induced p38 phosphorylation.

* Corresponding author at: Department of Plastic and Reconstructive Surgery, Third Xiangya Hospital of Central South University, 138 Tongzipo Road, Changsha 410013, Hunan, China. Fax: +86 731 88618815.
** Corresponding author at: Department of Oncology, Third Xiangya Hospital of Central South University, 138 Tongzipo Road, Changsha 410013, Hunan, China. Fax: +86 731 88618804.
E-mail addresses: caokehui168@aliyun.com (K. Cao), doctorzhoujianda@163.com (J. Zhou).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jdermsci.2015.10.019

Conclusions: LncRNAs in melanocytes undergo significant changes following irradiation with 20 mJ/cm^2 UVB, suggestting that lncRNAs participate in the UVB-induced stress response. Some lncRNAs expression changes induced by UVB are dependent on ROS generation. ROS-mediated production of lnc-CD1D-2:1 may be involved in the melanogenesis induced by UVB.

1. Introduction

Long non-coding RNA (lncRNA) is a type of non-protein RNA with complex biological functions [1]. LncRNAs can be divided into sense, antisense, bidirectional, intronic and intergenic lncRNAs according to their location and characteristics [2]. Recent studies showed that lncRNAs participate in various biological processes such as embryogenesis, cell proliferation, cell differentiation, apoptosis, invasion, metastasis, and angiogenesis [3,4]. LncRNAs can not only act as activators or inhibitors to regulate the expression of genes at the transcriptional level, but also can regulate genes at the post-transcriptional and post-translational level [4,5]. LncRNAs also have close relationships with oxidative stress, nutritional deficiency, DNA damage and other types of cellular stress responses [6,7].

Cellular stress responses are a series of changes in cells under various internal or external stimuli such as ultraviolet (UV) radiation, ionizing radiation, starvation, hypoxia, and oxidative stress. They play very important roles in maintaining normal physiological activities and in the occurrence and development of diseases [8,9]. On the one hand, the cellular stress response forces cells to make adaptive changes, leading to changes in gene expression to enhance damage resistance ability and viability under adverse conditions [10,11], while on the other, it is also an important factor that induces diseases such as cancer [12].

UV radiation is one of the major environmental factors for cellular stress, and is closely related to changes in skin color and the occurrence of skin tumors [13]. When melanocytes are exposed to UV radiation, gene expression related to the synthesis of melanin such as that of tyrosinase (*TYR*) is enhanced, causing an increase in melanin synthesis, thereby further resisting UV damage to the skin [14]. However, persistent or high doses of UV radiation can cause malignant changes in melanocytes, which can result in malignant melanoma [15].

Dynoodt et al. [14] found that melanocytes dosed with UVB stimulation resulted in changes in the expression profile of microRNAs, and also found that miR-145 can regulate the synthesis of melanin via modulating the expression of *TYR*, *SOX9*, *MITF* and other genes. However, as an important component of non-coding RNAs, it is unclear what changes occur in lncRNAs in the process of UV-induced stress responses in melanocytes.

This current study explored the expression differences of lncRNAs in melanocytes following UVB irradiation. We found that after stimulation of melanocytes with 20 mJ/cm^2 UVB irradiation, no significant cell death was observed, and the expression level and activity of *TYR* was increased. At the same time, chip detection of lncRNAs expression profile showed that the expression of 807 lncRNAs and 995 mRNAs was altered more than two-fold. Through GO analysis, this phenomenon was revealed to be closely related to the cellular stress response, which was further confirmed by the detection of ROS in response to stress. Furthermore, our results shed some light on the link among ROS, lncRNAs, and melanin synthesis, and indentified that ROS-mediated production of lnc-CD1D-2:1 was involved in the melanogenesis induced by UVB.

2. Materials and methods

2.1. Cell culture

Primary melanocytes were purchased from Shanghai XinYu Biological Technology Co., Ltd (Shanghai, China). Cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS; Corning) and 1% double-antibody (Gibco) in a humidified incubator containing 5% CO_2 in 37 °C. For experiments, 3–6 generations of cells in logarithmic growth phase were used.

2.2. UV irradiation treatment

UVB irradiation was provided using the SS-07 light therapy device from Sigma, with an irradiation power of 36W, and an exposure time that can be automatically adjusted with the irradiation dose. The wavelength of UVB used in the experiment was ranged in 280–320 nm. The UVB irradiation treatment was undertaken when the cell confluency reached approximately 70%. Before UVB irradiation, the culture medium was removed and the cells were washed twice with PBS, and then covered with a thin layer of PBS. Vertical irradiation of cells with UVB was administered immediately upon lifting of the culture dish lid; complete culture medium was then immediately added and cells were maintained in the normal culture environment. Twenty-four hours after UVB irradiation, RNA was extracted from cells or other experiments were performed. Control cells were treated similarly to the UVB group with the exception that they did not undergo UVB stimulation.

2.3. Chemical treatments

N-acetyl-L-cysteine (NAC), the ROS scavenger, was purchased from Beyotime (Shanghai, China). Cells were pre-treated with NAC (10 mM) for 1 h before UVB irradiation and were cultured continuously in complete culture medium with NAC (10 mM) 24 h after UVB irradiation. Lnc-CD1D-2:1 siRNA (5′-GCAACUCAC-CAAGA-AGAAUTT-3′), lnc-SGCG-5:4 siRNA (5′-UGUAACCCA-GAAUUGGAAUTT-3′), and control siRNA were purchased from GeneChem Biotechnology Company (Shanghai, China). Cells with 30–50% confluency were transfected with siRNAs in serum-free medium using Lipofectamine 3000 (Life Technologies). Following incubation for 6 h, the transfection medium was replaced with fresh medium. when transfected melanocytes reached approximately 70% confluency, the UVB irradiation treatment was undertaken as described above.

2.4. Detection of cell proliferation activity by MTT assay and cell apoptosis by AnnexinV/PI

Cell proliferation was detected according to the methods of Liu et al. [16]. Cells were seeded into 96-well plates at a density of 10^4 cells per well in 200 μl medium. Cells were serum-starved for 24 h by the addition of serum-free DMEM, and then underwent UVB irradiation. Normal culture medium was replaced and cells were cultured for 24 h. Then, 20 μl MTT (Sigma) was added to the culture medium for 4 h, and the supernatant was removed followed by the addition of 150 μl DMSO (Sigma). Finally, absorbance was