Cellular Signalling 28 (2016) 25–33

Contents lists available at ScienceDirect

Cellular Signalling

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

Gene expression profiling reveals the role of RIG1 like receptor signaling in p53 dependent apoptosis induced by PUVA in keratinocytes

Shruti Chowdhari, Neeru Saini *

Functional Genomics Unit, CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi -110007, India

ARTICLE INFO

Article history:
Received 21 August 2015
Accepted 24 October 2015
Available online 27 October 2015

Keywords:
PUVA
Psoriasis
Apoptosis
RIG-1 like receptor signaling
p53
miR-4516/UBE2N axis

ABSTRACT

Photochemotherapy using 8-methoxypsoralen in combination with UVA radiation (PUVA) is an effective treatment for various skin dermatosis including psoriasis however its molecular mechanism is not clear. Previously we demonstrated that PUVA differentially regulates miRNA expression profile with a significant up-regulation of hsa-miR-4516. To study in detail the molecular mechanism of PUVA in keratinocytes, we investigated the genome wide transcriptomic changes using Illumina whole genome gene expression beadchip. Microarray analysis revealed 1932 differentially expressed gene and their Insilico analysis revealed Retinoic Acid Inducible Gene-I (RIG-1) signaling, apoptosis and p53 pathway to be associated with PUVA induced effects. We demonstrate that miR-4516 mediated down-regulation of UBE2N promotes p53 nuclear translocation and pro-apoptotic activity of PUVA is independent of IRF3 but is mediated by the RIG-I in a p53 and NFκB dependent manner. Additionally, PUVA inactivated the AKT/mTOR pathway in concert with inhibition of autophagy and suppressed cell migration. Taken together this study broadens our understanding about the mechanism of action of PUVA providing possible new strategy targeting proapoptotic function of RIG-1, a regulator of innate immune response or p53 for psoriasis therapy.

1. Introduction

Psoriasis an autoimmune disease is characterized as red scaly patches on the skin (psoriatic plaques) and its pathogenesis is incompletely understood. Multiple pathways regulating epidermal proliferation, inflammation, distinct miRNA expression as well as cytokine dysregulation are implicated [1–3]. During psoriasis there is activation of mitogen activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K)-AKT-mTOR pathways, Signal Transducer and activator of transcription (STAT) and Nuclear Factor-Kappa B (NFκB) transcription factors [4–6] and pathogen recognition receptors (PRRs-Toll Like Receptors (TLRs), RIG-1 Like Receprots (RLRs), NOD like Receptors (NLRs) and C-type lectin receptors) initiate type 1 interferon response along with aberrant expression of proinflammatory cytokines [7–11].

8-Methoxypsoralen (8-MOP) plus UVA irradiation (PUVA therapy) has shown considerable clinical efficacy [12], however its mechanism of action is poorly understood. Psoralen derivatives 8-methoxypsoralen (8-MOP) 5-methoxypsoralen (5-MOP) etc. form UV-induced photo-adducts and induce apoptosis via tumor protein p53 (p53) activation, mitochondrial depolarization, reactive oxygen species (ROS) production and activation of FAS and caspases [13–15].

Previously we reported that PUVA up regulates hsa-miR-4516 [16] in human keratinocytes and directly targets Signal Transducer and Activator of Transcription 3 (STAT3) by binding to its 3'Untranslated region (UTR). We also demonstrated suppression of phosphorylated STAT3, cyclin D1, Bcl xl proteins and up-regulation of pro-apoptotic proteins p53 and BAX.

In the current study, genome-wide transcriptome profiling revealed significant upregulation of innate immune sensor Retinoic acid inducible gene 1 (RIG-1), miR-4516/Ubiquitin Conjugating Enzyme E2N (UBE2N) mediated p53 activation, inactivation of AKT/mTOR pathway, inhibition of autophagy and suppression of cell migration in keratinocytes in response to PUVA. Furthermore, RIG-I initiated apoptosis was found to be p53 dependent and required Noxa.

2. Materials and methods

2.1. Cell culture and treatments

Human keratinocytes (HaCaT) were a kind gift from Dr. Sudhir Krishna's lab from National Center for Biological Sciences, Bangalore and maintained in DMEM F12 containing 10% (*v*/*v*) fetal calf serum,

Abbreviations: PUVA, 8-methoxypsoralen plus UVA; RIG-1, retinoic acid induced gene 1; MDA5, melanoma differentiation associated gene 5; MAVS, mitochondrial antiviral signaling protein; TBK, TANK-binding kinase 1; IKKε, inhibitor of kappaB kinase epsilon; IRF3, interferon regulatory factor 3; AKT, v-akt murine thymoma viral oncogene homolog 1; mTOR, mechanistic target of rapamycin; FAS, fas cell surface death receptor; UBE2N, ubiquitin conjugating enzyme E2N; 3'UTR, 3′ untranslated region; MDM2, murine double minute 2; TP53, tumor protein p53.

* Corresponding author.
E-mail address: nsaini@igib.in (N. Saini).

http://dx.doi.org/10.1016/j.cellsig.2015.10.015
0898-6568/

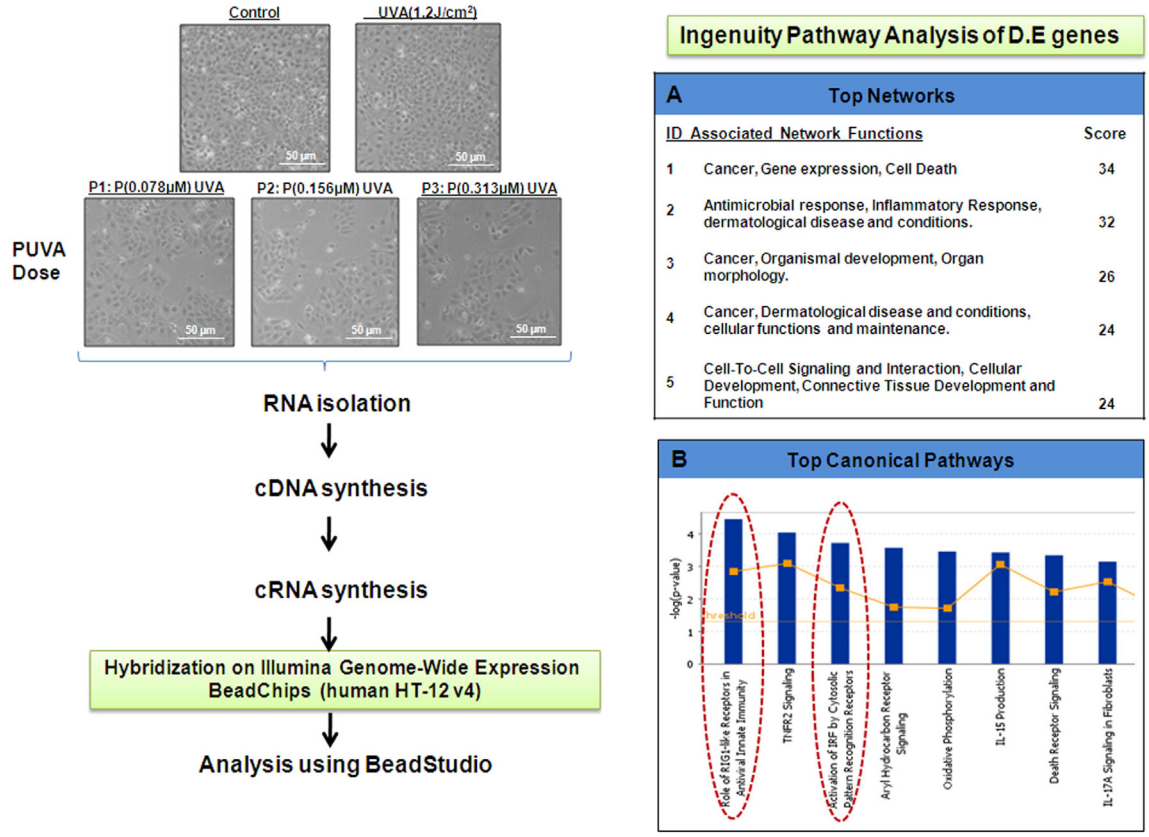

Fig. 1. Work flow for illumina microarray and ingenuity pathway analysis summary: Left panel shows the bright field images of HaCaT keratinocytes treated with vehicle (0.01% DMSO), UVA(1.2 J/cm2) alone, and PUVA after 24 h. at 10× magnification and the work flow of Illumina microarray experiment. Right panel shows enriched top networks and the top canonical pathways as generated by Ingenuity Pathways Analysis (IPA) along with their representative scores (A) The top networks (B) Top canonical pathways. PUVA treatment: P1 (0.078 μM 8MOP + UVA), P2(0.156 μM 8MOP + UVA), P3(0.313 μM 8MOP + UVA).

100 Units/ml penicillin, 100 mg/ml streptomycin, 0.25 mg/ml amphotericin at 37 °C in humidified atmosphere at 5% CO_2. Experimental groups: *vehicle* (0.1% DMSO in HBSS); PUVA doses: P1 = (0.078 μM 8MOP + UVA), P2 = (0.156 μM 8MOP + UVA), P3 = (0.313 μM 8MOP + UVA), P4 = (0.626 μM 8MOP + UVA) are used in the study as described earlier [16]. miR-4516 mimic and miR-4516 inhibitor from ambion (Ambion, Inc., Austin, TX) and UBE2N inhibitor NSC697923 from SIGMA (Sigma, St. Luis, MO, USA) were procured. Tp53 shRNA was a kind gift from Dr. Uttam Pati's lab, School of Biotechnology, Jawaharlal Nehru University, Delhi.

2.1.1. Illumina Microarray, data analysis and pathway analysis

Microarray was performed using human HT-12 v4 expression bead chip as described earlier [16]. The data was average normalized and FDR calculation was done using illumina (Beadstudio 2.0) custom test. The genes which crossed the threshold of detection p-value ≤ 0.05 and differential score p-value ≤ 0.05 were considered to be differentially expressed [17]. Ingenuity Pathway Analysis software (IPA Tool; Ingenuity Systems, Redwood City, CA USA; http://www.ingenuity.com) [18] was used to explore possible biological interactions of differentially expressed genes (DEG). The list of DEG was also imported into the GeneCodis software. We used the default settings of GeneCodis, which employs hypergeometric test for calculating p-values and false-discovery rate for p-values correction [18].

2.2. Antibodies and immunoblotting

All the antibodies used for this work were obtained from commercial sources. TP53/NFκB(p65)/pNFκB(p65) from Santa Cruz Biotechnology (Santa Cruz, CA, USA); Akt/pAkt/RIG-1/MDA5/MAVS/TBK1/pTBK1/IKKε/IRF3/pIRF3/mTOR/pmTOR(Ser2448)/AMPK/pAMPK(T172) from Cell Signaling Technology (Denvers, MA, USA), anti-GAPDH from Abcam. Western blotting was performed as described earlier [19] and wherever indicated fractionation was done using mitochondrial/Cytosol fractionation kit (Biovision, USA).

2.3. Detection of IRF3/pIRF3/NFκB/pNFκB/TP53 by immunofluorescence

Nuclear translocation was checked in presence or absence of PUVA/miR-4516/antimiR4516 after 24 h as described previously. DAPI staining was done for 15 min and fluorescent images were captured through an inverted microscope Nikon ECLIPSE Ti (Nikon Corporation, Tokyo, Japan) at 40X magnification, using a DS-Qi1MC camera equipped with NIS Element AR 3.0 software.

2.4. Plasmid constructs

The sequence of the 3'UTR of UBE2N (ubiquitin conjugating enzyme E2N) gene was retrieved from Ensembl Genome Browser and for primer design, Primer3 (http://frondo.wt.mit.edu) software was used. To generate a 3'UTR reporter construct, a 616 kb region of 3'UTR of UBE2N gene harboring the predicted binding sites for hsa-miR-4516, was cloned in pMIR REPORT Luciferase Vector (Ambion Inc., Austin, TX) between Spe1 and Mlu1 restriction sites using the forward primer 5′ ACGATCATCAAGTGTGCATCA 3′ and reverse primer 5′ GGGGAATC TACACTTGACAGC 3′ and designated as UBE2N 3'UTR. The resulting plasmid was sequenced to ensure accuracy. To generate mutant constructs site-directed mutagenesis was performed using the QuickChange