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Phenotypic and lipidomic characterization of primary human epidermal keratinocytes exposed to simulated solar UV radiation

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ABSTRACT

Background: Ultraviolet (UV) radiation is known to be one of the most important environmental hazards acting on the skin. The most part of UV radiation is absorbed in the epidermis, where keratinocytes are the most abundant and exposed cell type. Lipids have an important role in skin biology, not only for their important contribution to the maintenance of the permeability barrier but also for the production and storage of energy, membrane organization and cell signalling functions. However, the effects on the lipid composition of keratinocytes under UV radiation are little explored.
Objective: The present work aims to explore the effects on the phenotype and lipid content of primary human keratinocytes exposed to simulated solar UV radiation.
Methods: Keratinocytes were exposed to a single (acute exposure) and repeated simulated solar UV irradiations for 4 weeks (chronic exposure). Cell viability and morphology were explored, as well as the production of reactive oxygen species. Then, lipid extracts were analysed through liquid chromatography coupled to mass spectrometry (LC–MS) and the data generated was processed using the ROIMCR chemometric methodology together with partial least squares discriminant analysis (PLS-DA), to finally reveal the most relevant lipid changes that occurred in keratinocytes upon UV irradiation. Also, the potential induction of keratinocyte differentiation was explored by measuring the increase of involucrin.
Results: Under acute irradiation, cell viability and morphology were not altered. However, a general increase of phosphatidylcholines (PC) phosphatidylethanolamines (PE) and phosphatidylglycerol (PG) together with a slight sphingomyelin (SM) decrease were found in UV irradiated cells, among other changes. In addition, keratinocyte cultures did not present any differentiation hallmark. Contrary to acute-irradiated cells, in chronic exposures, cell viability was reduced and keratinocytes presented an altered morphology. Also, hallmarks of differentiation, such as the increase of involucrin protein and the autophagy induction were detected. Among the main lipid changes that accompanied this phenotype, the increase of long-chain ceramides, lysoPC and glycerolipid species were found.
Conclusion: Important lipid changes were detected under acute and chronic UV irradiation. The lipid profile under chronic exposure may represent a lipid fingerprint of the keratinocyte differentiation phenotype.

1. Introduction

Sunlight is composed of a continuous spectrum of electromagnetic radiation which has three main wavelength regions: ultraviolet (UV), visible and infrared. UV radiation comprises UVA (320–400 nm), UVB (280–320 nm) and UVC (200–280 nm). Whereas UVA radiation represents 95% of the total solar UV that reaches the earth, UVC and most of the UVB radiation are absorbed by the stratospheric ozone layer [1,2]. Skin, the outermost tissue of the body, is exposed to various environmental aggressions, among which solar ultraviolet (UV) radiation is the most significant. UVA is responsible for indirect cell damage through the generation of reactive oxygen species (ROS), whereas UVB causes direct DNA damage and results in a variety of harmful effects on human skin, including epidermal thickening, collagen damage, skin wrinkling, and the development of skin tumors [3,4].

Most of the UV radiation is absorbed in the epidermis, the outermost layer of the skin, where keratinocytes are the most abundant and exposed cell type. The epidermis is continuously being regenerated through a process called differentiation, in

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which keratinocytes are gradually modified to become flat cells named corneocytes and migrate into the upper layer of the epidermis, where they are ultimately released from the epithelial surface [5]. During this process, keratinocytes lose progressively their nucleus and cytoplasmic organelles and provide a protective barrier to retain body fluids and to prevent environmental insults. Disruption of epidermal barrier formation and abnormal keratinocyte differentiation are involved in the pathophysiology of several skin diseases, such as psoriasis, atopic dermatitis and basal and squamous skin cancer [6]. Concerning UV radiation, a recent study has demonstrated that UVB-irradiated keratinocytes present altered differentiation and impaired barrier function leading to increased permeability [7].

Lipids have an important role in skin biology. During epidermal differentiation, lipids are synthesized in the keratinocytes and extruded into the extracellular domains, where they form extracellular lipid-enriched layers. These lipids, after enzymatic processing, are incorporated in lipid lamellar membranes, which together with the keratin-filled corneocyte envelope, form the permeability barrier [8]. The impairment of sphingomyelinase activity has been shown to be involved in the aberrant differentiation of keratinocytes in atopic dermatitis, correlating with reduced ceramide content in the stratum corneum. Also, recent studies pointed out increased ceramide levels upon UV radiation [9–11]. Besides the structural role in the permeability barrier, lipids also have other important functions in cells, such as energy production and storage, cell signalling, protein trafficking and membrane organization. However, few works are dedicated to the investigation of UV effects on skin cells lipid composition.

In the recent years, the development of high-throughput analytical platforms and data analysis tools has allowed the emergence of lipidomics studies, in which the exploration of the lipid composition in a variety of biological matrices and conditions has enabled a better understanding about the lipid roles in the biological systems. The huge amounts of data obtained from these developing technologies have favoured the popularity of untargeted omic approaches, in which all data are simultaneously analysed by chemometric data analysis methodologies to extract information about the relevant molecules in the process studied.

In this work, a solar simulation unit has been used to expose primary cultures of keratinocytes to a single dose (acute exposure) and repeated doses (chronic exposure) of UV radiation. First, cell viability and morphology changes were examined and reported. Second, an untargeted LC–MS lipidomic study was performed and the obtained data were analysed by the ROIMCR (Regions of Interest Multivariate Curve Resolution) procedure. The main lipid changes that occur under acute and chronic exposures were investigated. Since differentiation is an important process in keratinocytes, its possible induction was also investigated under UV radiation. Finally, phenotype changes observed in these keratinocytes are discussed together with the observed lipid changes to hypothesize the functional role of lipids in keratinocyte differentiation.

2. Materials and methods

2.1. Cell culture

Primary cultures of human epidermal keratinocytes were obtained from Dr. P. Descargues (Genoskin, Toulouse). These cells were maintained in DermaLife K Complete Medium (CellSystems Biotechnologie) protected from sunlight and supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, at 37 °C in a humidified atmosphere containing 5% of CO_2.

2.2. UV irradiation

Cultured keratinocytes were irradiated in a Suntest CPS (Atlas, USA), solar simulation unit. This simulator is equipped with a xenon arc lamp that provides irradiance that simulates sunlight. The UVA/UVB radiation was controlled by a radiometer VLX-3 W (Vilber Lourmat). The simulated UV daylight applied in this work presented a UVA/UVB irradiance ratio of 29 (it has been estimated that a UVA/UVB ratio comprised between 23 and 32 is representative of UV daylight spectrum) [12]. The irradiation was selected taking into account the UVB irradiation intensities used in literature for studies in skin cells related to sunlight exposure [10,13–15] and the results of a previous keratinocyte viability test carried out after UV exposure at different irradiation intensities. The chosen UV dose (728 mJ/cm^2 UVA and 25 mJ/cm^2 UVB) was the highest dose that did not produce any change in cell viability after 72 h post-irradiation (data not shown).

Keratinocytes were seeded in 6-well plates (Nunclon surface, Thermo Scientific) and cultured under standard conditions. Some of the wells contained glass coverslips to perform the immunofluorescence detection of involucrin. Just before irradiation, an acetate plastic sheet was placed above the plate to adapt the UVA/UVB ratio to 29:1. Cells were placed inside the Suntest CPS and were irradiated for five min, which represented the final UV radiation dose of 728 mJ/cm^2 UVA and 25 mJ/cm^2 UVB. After light irradiation, cells were rinsed with PBS 1X and incubated again under standard conditions. Following this procedure, irradiations were applied twice a week to the keratinocyte cultures. In this work, effects of acute and chronic irradiations were investigated: 0.5 week, which is equivalent to one irradiation (acute irradiation), and 4 weeks, which represents a total of 8 exposures to UV radiation (chronic irradiation). For the evaluation of sphingomyelinase activity, involucrin increase and autophagy activation progress over time, an additional intermediate irradiation was performed for 1.5 weeks (1.5 W), equivalent to 3 UV irradiations. At each endpoint, cells exposed to sham light (regular laboratory light) were used as control references.

2.3. Cell viability

Cell viability was estimated by using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) assay on 96-well plates, according to the manufacturer instructions.

2.4. Autophagy assessment by western blot

Cells were harvested by trypsinization, centrifuged and washed twice with PBS 1 ×. Then, cells were lysed using mammalian lysis buffer 1X (ab179835, Abcam) and cocktail protein inhibitor 1X (Thermo Scientific). Protein in cell lysates was quantified using the BCA assay (Thermo Scientific) and 90 μg of protein per sample were resolved by 10% SDS-PAGE. Proteins were transferred to PVDF membranes (Roche). Membranes were blocked with TBS 1X containing 0.1% Tween 20 and probed with the LC3 polyclonal antibody (ab150917, Abcam). Membranes were developed using the chemiluminescent signal detection kit ECL™ Prime Western Blotting detection reagent (GE Healthcare) and visualized using LICOR C-DiGit blot scanner. Relative quantification of Western Blot band intensities was carried out using Image Studio Lite version. 5.0 software; values were normalized to those of β-actin, and the differences between UV-irradiated and control samples were calculated.

2.5. Reactive oxygen species (ROS) assay

To evaluate the formation of ROS after UV acute irradiation, keratinocytes were seeded in 96-well plates at 10^5 cells/ml and

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