CHANGES IN THE CELL MOTILITY PROTEINS PROFILE IN THE HaCaT KERATINOCYTES RESPONSE TO THE UVA EXPOSURE

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A comparative analysis of HaCaT keratinocyte proteins has been performed after cell exposure to subtoxic doses (5 J/cm^2 and 25 J/cm^2) of ultraviolet A (UVA) radiation. 930 proteins were identified by two or more unique peptides. More than half of all identified proteins (54.5%) demonstrated at least 2-fold increase in their relative content after HaCaT keratinocyte irradiation with a cumulative dose of 5 J/cm^2, while a decrease in the relative content was found only for 4 proteins. Irradiation of keratinocytes with a cumulative dose of 25 J/cm^2 resulted in a decrease in the proportion of up-regulated proteins (43.0%) and an increase in the number of down-regulated proteins (84). Among the proteins with increased relative content in HaCaT keratinocytes the most proteins were associated with "cell motility" (GO: 0048870), as well as regulation of cell shape and size, cell morphogenesis, and skin remodeling.

Keywords: keratinocytes HaCaT; UVA; 1DE-gel concentration; LC-MS/MS; MaxQuant; cell motility proteins

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INTRODUCTION

Human HaCaT keratinocytes are spontaneously immortalized cells from histologically normal human epithelium. They are capable of unlimited division and this determines the feasibility of their use as an *in vitro* epidermal cell model for cytotoxicity studies [1]. Keratinocytes are the main cells of human skin epidermis. They form a protective barrier against skin damage such as mechanical trauma, thermal injury, chemical exposure and ultraviolet (UV) radiation. UV radiation is one of the main initiators of cellular stress, causing responses such as inflammation induction and cell cycle dysregulation [2]. The ultraviolet spectrum is divided into three components: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). UVC is extremely harmful to the skin, but is completely absorbed by the stratospheric ozone layer and does not reach the ground. UVB, the main wavelength that causes skin cancer, has the widest range of negative effects. UVA (95% of UV radiation reaches the Earth's surface) is less harmful, but is associated with skin aging, particularly changes in proteins and dermal elasticity [2]. UVA-induced development of oxidative stress in the skin stimulates specific defense mechanisms, such as thickening of the stratum corneum of the epidermis, pigmentation, and keratinocyte remodeling due to intensification of intracellular regeneration [3]. UV therapy of the far long-wave range (wavelength 320–400 nm, UVA) is used in dermatologic practice. UVA dosimetry is classified into low (≤40 J/cm^2), medium (40-80 J/cm^2), and high (80–130 J/cm^2) dose regimens [4]. UV therapy has shown good results in the treatment of atopic dermatitis and sclerotic skin diseases [5].

In recent years, the mechanisms involved in UVA-induced damage to skin cells, including HaCaT keratinocytes, have been widely studied [6–10]. Since UV is known to induce oxidative and genotoxic stress [6], it was important for us to test whether a low UV dose (5 J/cm^2) could contribute to changes in stress protein levels. The results of other authors show that even a low dose (6 J/cm^2) of UVA radiation is able to induce mitochondrial fragmentation, nucleocytoplasmic translocation triggered by DNA damage [7]. Low-dose UV radiation has a significant impact on the proteomic architecture of skin cells, provoking reorganization of subcellular structures due to genotoxic and metabolic stress [7]. The expression of genes related to cell death and apoptosis was insignificantly altered in HaCaT cells exposed to UVA [8]. Although the effects of UVA on DNA modification [9], gene expression [10], protein expression [11], and post-translational modifications [12, 13] have already been studied, information on the UVA effects on the organization of motility proteins in epidermal cells is still lacking.

The development of the adaptive response of skin cells (mast cells, Langerhans cells, keratinocytes) is realized through activation of numerous mechanisms responsible for cell survival, one of which is cell motility. Intracellular motility is necessary to return cells to homeostasis in response to UVA exposure.

In addition, cell motility mediates tissue regeneration and plays an important role in many physiological and pathological processes such as wound healing, migration of tumor metastatic cells. It is known that the reorganization of actin filaments, which are involved in the maintenance of cell shape, transport of various molecules, cell division, and signal transduction, is crucial for enhancing the motility of epithelium-like cancer cells [14]. All forms of skin cancer develop from epidermal cells, mainly keratinocytes [15]. Cell motility is an extreme case of large-scale remodeling of the actin cytoskeleton. Cell motility is provided mainly by controlled assembly and disassembly of the actin cytoskeleton. In the field of cell motility, importance of studying molecular mechanisms *in vitro* or cell behavior in an environment far removed from the tissue is a subject of open debate. A better characterization of the proteins involved in these processes should contribute to a deeper understanding of the skin's response to UVA radiation. It is known that a low dose of UVA radiation equivalent to 20 min of midday sun exposure [16] leads to remodeling of the skin cell proteome.

The study of changes in the abundance of cell motility proteins in response to low-dose UVA irradiation of HaCaT keratinocytes may become a promising direction for further investigation of molecular mechanisms underlying UV-induced cell damage and skin-specific defense mechanisms.

An effective strategy to study cellular proteome changes is comparative proteomics, an informative method for relative label-free quantification of the protein composition of biological samples [17].

The aim of this work was to perform a comparative proteomic analysis of proteins associated with cell motility in HaCaT keratinocytes in response to exposure to subtoxic doses of UVA for skin.

MATERIALS AND METHODS

Reagents

The following reagents were used in this work: sodium dodecyl sulfate (SDS), bovine serum albumin (BSA; Merck, Germany); trypsin from pig pancreas, modified lyophilized (Promega, USA); trifluoroacetic acid (TFA; Fluka, Germany); acetonitrile, dithiothreitol (DTT), deionized water (Acros, USA); methanol, 2,2-bicinchoninic acid (Pierce, USA), 0.25% trypsin-EDTA solution (PanEco, Russia) as well as reagents of the chemically pure grade from local suppliers.

Cell Cultivation and Processing

Cells of the immortalized HaCaT cell line (CLS Cell Lines Service, Germany) were seeded in 75 cm^2 flasks (Corning, USA) and cultured in DMEM/F12 1:1 medium (PanEco) containing 10% FBS (fetal bovine serum; Dia-M, Russia) and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) in a CO_2 incubator (at 37±1°C, humidity 90±10%, and CO_2 content 5.0±1.0%). The incubation medium was changed every other day. After reaching 60-70% confluency, the culture medium was removed and the cells were divided into three groups: control and experimental (three Petri dishes for each group). Keratinocytes (3×10^5 cells per dish) were seeded in 100 mm Petri dishes 48 h before irradiation and cultured under standard conditions. The cells were washed with DPBS solution (Dulbecco's phosphate-buffered saline, PanEco) and placed in the chamber of the Vilber BIO-LINK dosed irradiation system (5×8 W, 365 nm; Vilber, Germany) under a thin layer of DPBS solution. The exposure time to the UVA source was selected to achieve the cumulative doses of absorbed radiation of 5 J/cm^2 and 25 J/cm^2. After the UV exposure, the DPBS solution was replaced with the culture medium and the cells were incubated for 24 h in a CO_2 incubator (at 37±1°C, humidity 90±10%, and CO_2 content 5.0±1.0%). The medium was then removed, the cells were washed with PBS, detached with 0.25% trypsin-EDTA (3 ml per Petri dish), and incubated for 2–3 min at 37°C. Before transferring the cells to a centrifuge tube, the Petri dishes were placed under a microscope to ensure that the cells were completely detached from the adherent surface of the Petri dishes. Keratinocytes were centrifuged at 10,000 *g* for 5 min and then washed three times with ice-cold potassium phosphate buffer (pH 7.4). The buffer was removed from the tubes, and the cells from three culture flasks for each group were pooled into one tube (3 biological replicates/culture flasks). As a result, 3 samples/tubes with control (Control-HaCaT) and UVA-treated (UVA1-HaCaT and UVA2-HaCaT) cells were obtained. The keratinocyte pellet was dissolved in the lysis buffer (500 µl 0.2% SDS in 100 mM Tris-HCl (pH 7.4), 120 mM NaCl, 5 mM EDTA, and 1% PMSF) and the lysates were homogenized as described previously [18]. The protein content of the homogenates was determined using 2,2-bicinchoninic acid at 562 nm using BSA as a standard [19].

The MTT assay

The cells were plated in wells of a 96-well plate (6 wells/technical replicate for each sample) at a density of 2500 cells/well. After 24 h, the culture medium was replaced with 100 µl DPBS solution and cells were irradiated with UVA (365 nm). After irradiation, the DPBS solution was replaced with fresh culture medium. After 24 h, the medium was replaced with a medium containing 1 mg/ml MTT (tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and incubated under standard conditions (37°C in a humidified atmosphere with 5% CO_2) for 4 h. Upon completion of the incubation period, the medium was removed from the plate wells and cells were lysed in DMSO. Optical density was measured using an iMark plate reader (Bio-rad, USA) at 560 nm [20].

Statistical processing of differences in the relative increase in the number of cells during cultivation was performed in GraphPad Prism 7.0 using one-way ANOVA. Differences between groups were determined using the Student's *t*-test with the Benjamini-Hochberg correction for multiple comparisons. The level of statistical significance was set at $p<0.05$.

In-Gel Electrophoresis Procedure Followed by In-Gel Trypsin Digestion

HaCaT cell extracts were subjected to the 1DE-gel concentration procedure in a polyacrylamide gel as described previously [18]. A single protein band (a piece of gel about 2 mm wide) was then excised with a hand microtome, transferred to an Eppendorf tube and subjected to in-gel trypsin digestion as described by Shevchenko et al. [21]. The over-gel solution of the tryptic peptide mixture from the gel band was used for liquid chromatography with tandem mass spectrometry (LC-MS/MS).

LC-MS/MS Proteomic Profiling

Proteomic profiling was performed using an Ultimate 3000 nano-flow HPLC chromatography system (Dionex, USA) integrated with an Orbitrap Q Exactive HF mass spectrometer (Thermo Scientific, USA) with a Nanospray Flex Ion Source electrostatic ionization source (Thermo Scientific) of the Orbitrap Q Exactive HF mass spectrometer (Thermo Scientific), as described previously [18].

For protein identification raw files from the mass spectrometer were analyzed using the Andromeda search algorithm [22] integrated into the MaxQuant software platform (v1.6.3.4) [23]. The following search parameters were used: SwissProt database (v.1.4.2019, FASTA format) for the *Homo sapiens* species, cleaving enzyme — trypsin/P; the permissible error in measuring the monoisotopic mass of the peptide was ±0.01 Da, the permissible error in measuring the fragment ion was ±0.05 Da, the number of possible missed trypsin cleavage sites was 1; fixed modification was set to cysteine carbamidomethylation; variable modification was set to methionine oxidation. Label-free quantification (LFQ) was used as the quantitative analysis mode. The search was performed in a database of inverted and random (decoy) amino acid sequences; the percentage of false positive results (false discovery rate, FDR) was set to 0.01. Proteomic analysis was performed in three technical replicates. The values of the LFQ intensity for the identified proteins were further used as quantitative parameters of protein abundance in the samples. Proteins identified by two or more unique peptides with fold change (FC) ≥2 in intensity values were considered as differentially altered in response to UVA. Statistical significance of differences between compared parameters was determined using the Student's *t*-test for independent samples. For control HaCaT cells, the sample consisted of 6 technical replicates (mass spectra), and for HaCaT keratinocytes exposed to UVA1 and UVA2, 12 technical replicates. The results were assessed as statistically significant at a threshold significance level (alpha level) of 0.05. The Gene Ontology (GO) Database [24] and the PANTHER tool [25] were used to analyze the biological pathways (species — *Homo sapiens*, test type — binomial, ontology — GO-Slim Biological Process, *p*-value <0.05). Construction of Venn diagram and GO analysis of stress proteins was performed using the Functional Enrichment analysis tool (FunRich v. 3.1.3) [26]. Proteome enrichment analysis of protein-protein interactions was performed using the online resource STRING (v. 12.0), the results for proteins with a minimum interaction reliability of 0.7 were considered. The dataset is available in Mendeley Data [27].

RESULTS AND DISCUSSION

The results of the MTT test have shown that the HaCaT cells exposed to UVA l are characterized by a dose-dependent decrease in viability. Figure 1 shows, that a UVA dose of 5 J/cm^2 did not cause a decrease in the viability of keratinocytes, while a dose of 25 J/cm^2 resulted in 50% death of keratinocytes. Thus, according to the results of the MTT test, in accordance with ISO 10993-5, the UVA dose of 5 J/cm^2 was considered non-toxic, and the dose 25 J/cm^2 was considered toxic [28].

In order to identify specific proteins/pathways involved in cell motility processes in human epidermis, a comparative analysis of HaCaT cell proteomes was performed before and after UVA irradiation of the cells with two cumulative doses of absorbed radiation: 5 J/cm^2 (UVA1) and 25 J/cm^2 (UVA2).

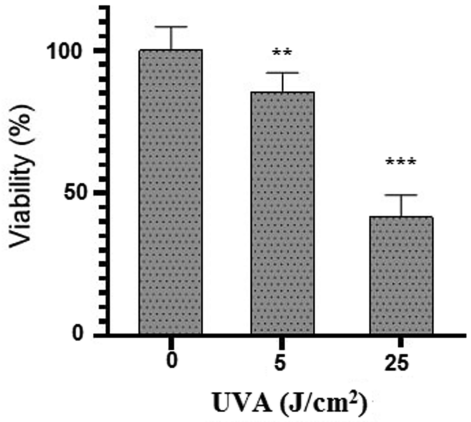

Figure 1. Dose-dependent decrease in HaCaT keratinocyte viability in response to ultraviolet A (UVA) exposure. Cell viability was measured as a percentage of untreated control cultures (n=6). Statistical significance: $^{**}p<0.0015$ for 5 J/cm^2 and $^{***}p<0.0001$ for 25 J/cm^2.

A total of 930 proteins were identified in immortalized HaCaT keratinocytes by two or more unique peptides; these included 662 proteins in the control samples, 873 proteins in UVA1 and 602 in UVA2 samples. The list of identified proteins in HaCaT cells is given in Table S1 (Supplementary Materials). Figure 2 shows the Venn diagram reflecting the number of identified proteins and their distribution in the control and experimental samples of HaCaT cells.

514 common proteins found for all three cell groups, indicate similarity of the proteomes. Nevertheless, relative proteome quantification revealed changes in LFQ intensity values for more than half (54.5%) of all identified proteins, despite the low absorbed doses investigated in this study. In UVA1-HaCaT, 476 proteins were up-regulated and only 4 proteins were down-regulated compared to the control. In UVA2-HaCaT, 260 proteins were up-regulated and 84 proteins were down-regulated compared to the control.

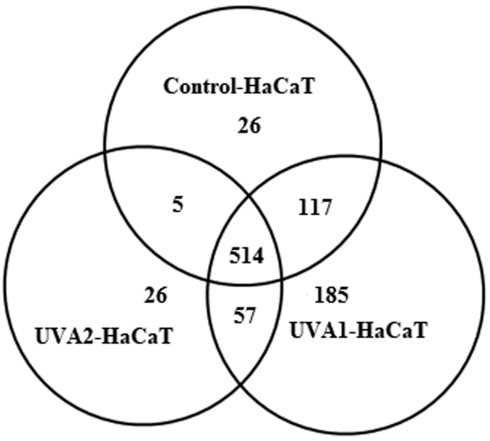

Figure 2. Venn diagram showing the number of identified proteins and their distribution in control and experimental samples of HaCaT keratinocyte cells. Control-HaCaT: untreated HaCaT cells, UVA1-HaCaT: cells irradiated with the dose 5 J/cm^2, UVA2-HaCaT: cells irradiated with the dose 25 J/cm^2.

Among the proteins showing the greatest up-regulation (average FC = 4.4) in UVA1-HaCaT, 30 proteins belonged to the "cell response to stress" biological process (GO: 0033554) according to the GO classification [29]. Increase in the cumulative UVA dose to 25 J/cm^2 resulted in a decrease in the number of stress-induced proteins. In UVA2-HaCaT samples, only 15 such proteins were detected, characterized by an average 3.7-fold increase in relative abundance. We performed a comparative analysis of the functional enrichment of stress proteins with increased regulation in HaCaT keratinocytes after exposure to UVA1 and UVA2 using FunRich (Fig. 3).

The comparative analysis has shown that in UVA2-HaCaT samples, compared to UVA1-HaCaT, there was an increase in the proportion of proteins associated with such biological processes as cellular response to UV, cellular response to oxidative stress, and inflammatory response. At the same time, the proportion of proteins associated with stabilization decreased in UVA2-HaCaT cells compared to UVA1-HaCaT (Fig. 3). The obtained results may indicate that the UVA dose can have a significant impact of the effect of stress (enhance or suppress a particular biological process); this is consistent with the literature data [7, 30, 31].

The proteins identified in UVA-HaCaT cells involved in the stress response and changes in their regulation levels compared to the control are listed in Table S2 (Supplementary Materials). In accordance with previously reported results by Valerio et al. [7], we detected up-regulation of stress proteins such as XRCC6, PRDX1, and heat shock proteins. However, in the case of the PRDX3 protein, which

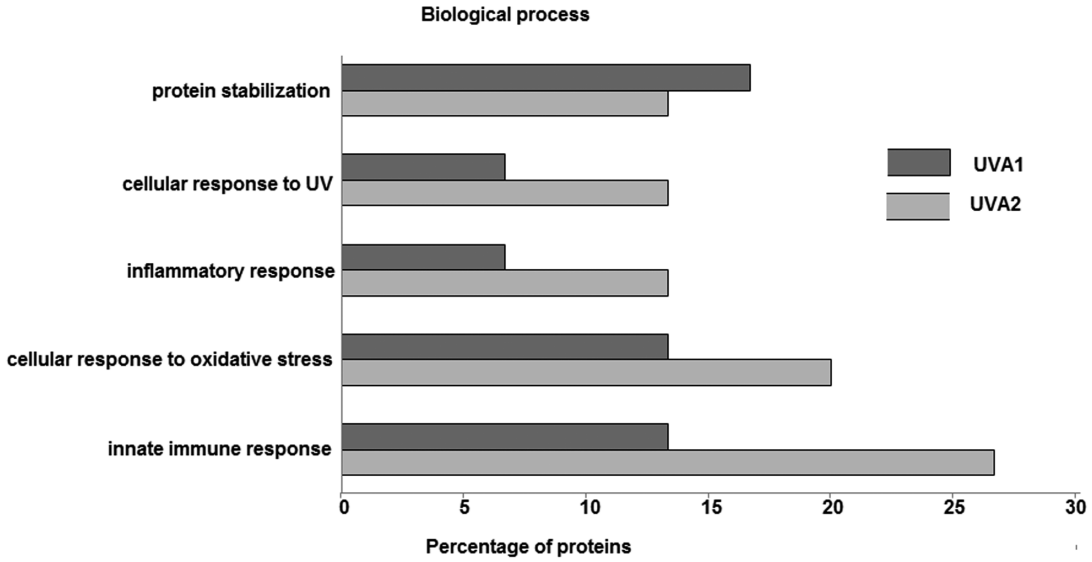

Figure 3. GO enrichment analysis of stress proteins with increased relative abundance in HaCaT keratinocytes in response to UVA exposure (UVA1 – 5 J/cm^2, UVA2 – 25 J/cm^2). The total number of proteins for this process from the UniProt database was defined as 100%.

was down-regulated in the cited work, we recorded its 4- and 3-fold increase in the UVA1 and UVA2 samples, respectively (Table S2). The difference in the results of the low dose UVA on HaCaT found in our study and in [7] may be due to the fact that the shotgun proteomics method has a number of limitations related to the fact that the molecular ion is not always registered in the mass spectrum and with different fragmentation of the sample. In our work, we used trypsinolysis in gel, while Valerio et al. used trypsinolysis in solution; in addition, the difference in the results could also be due to different conditions of cell lysis [7].

We used the STRING database (v.12.0) [32] to generate a protein-protein interaction (PPI) network for proteins up-regulated in UVA-HaCaT (442 proteins). The PPI network (PPI enrichment *p*-value: $<1.0\times10^{-16}$) was constructed with the required (high confidence) interaction score of 0.7. As a result, two main clusters were identified, each with a major functional enrichment according to GO. In terms of biological function, proteins of the first cluster are involved in the cellular response to stress (358 proteins); proteins of the second cluster are associated with the process of binding to cadherin (39 proteins). Proteins of the cadherin family are an important component of adhesive junctions that control the beginning and end of cell migration and participate in the formation of the epithelial layer. Analysis of the PPI network proteins related to cadherin binding (GO: 0045296, cluster 2, Fig. 4) revealed 11 proteins associated with the biological process of cell migration (GO: 0016477). This has not been previously described for UVA-HaCaT keratinocytes at the proteome level.

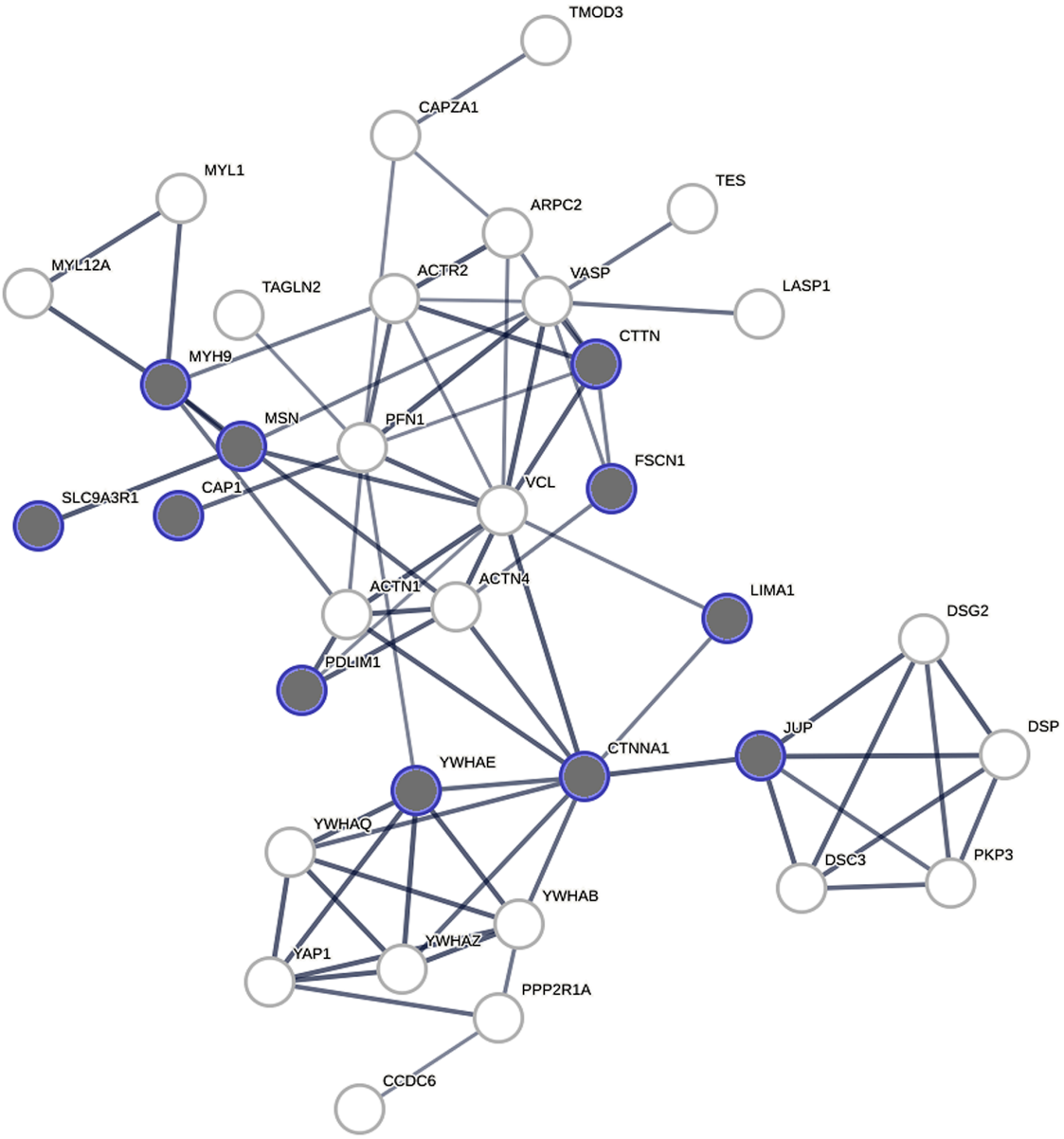

Figure 4. The protein-protein interaction network for proteins (PPI enrichment p-value: $<1.0\times10^{-16}$) with elevated levels in UVA-HaCaT obtained using the STRING tool (version 12.0, minimum required interaction score 0.7). Proteins associated with the biological process "cell migration" (cell migration, GO: 0016477) are indicated in gray.

Cell survival in response to toxic effects (UVA radiation) is realized through the activation of numerous mechanisms, one of which is cell motility (including cell migration). According to the GO hierarchy, cell migration is a child termin relation to the broader GO term "cell motility"(GO: 0048870).

In order to identify proteins involved in the cell motility of HaCaT keratinocytes before and after UVA exposure, a corresponding list of human proteins containing 355 annotations was retrieved from the Human Protein Atlas (HPA) database [33]. For human keratinocytes this list included 31 annotations. Comparison of these two lists of HPA-specific cell motility proteins with the proteomes of HaCaT cells before and after UVA exposure revealed 23 proteins (Table 1). This number of identifications corresponded to 6.5% of the total number of HPA proteins regulating cell motility and 74.2% of the number of human keratinocyte-specific motility proteins.

The data in Table 1 show that we have registered significant changes in the relative level of proteins even after irradiation with a very low dose of UV (5 J/cm^2). The LFQ intensity value significantly increased for 17 proteins in UVA1-HaCaT compared to the control keratinocyte samples; the intensity of 6 proteins remained virtually unchanged. At the same time, in UVA2-HaCaT samples, only 8 proteins were characterized by higher LFQ intensity values, the relative content of 9 proteins did not change, and 6 decreased compared to the control samples.

Most of the cell motility proteins are involved in the formation of actin microfilaments. We registered a 2-fold up-regulation of the Fascin protein (FSCN1_HUMAN), important for cell motility and migration, in the experimental cells compared to the control ones. This actin-binding protein plays a role in the organization of actin filament bundles. Another identified protein is transforming protein RhoA (RHOA_HUMAN); it promotes reorganization of the actin cytoskeleton and regulates cell motility by stimulating Rho-associated kinase (ROCK); the latter activates LIM kinase thus leading to inactivation of cofilin, thereby stabilizing actin filaments [34, 35]. In our study, the level of RHOA remained virtually unchanged in UVA-HaCaT, indicating minor to keratinocyte damage after irradiation. This is consistent with the literature data that UVA radiation does not cause significant disturbances in the directionality of HaCaT cells [36]. However, according to our data, out of 31 proteins that were annotated as involved in the process of cell motility, the level of 20 proteins changed significantly (Table 1). These included cytoskeletal actin-binding proteins, the main group regulating UVA-stimulated keratinocyte cell motility. Comparison with data obtained at higher radiation doses is not revealing, since the expression of genes associated with cell death and apoptosis did not undergo significant changes in HaCaT cells exposed to UVA [8].

It is known that in motile cells the RAC1 protein controls formation of the actin polymerization network [37]. The relative level of this protein insignificantly increased in HaCat cells exposed to UVA1 conditions, while in the case of cells treated with UVA2 the relative level of its content was 3-fold higher compared to the control. This may indicate a more significant development of the adaptive response to UVA exposure in UVA2-HaCaT cells to return the cells to a state of homeostasis. In this regard, it should be noted that HaCaT cells only slightly reduce proliferation upon irradiation, restoring their proliferative capacity in the long term [38].

From the data in Table 1 it is evident that the change in the level of some of HaCaT keratinocyte proteins (increase or decrease) depended on the radiation dose. For example, in UVA1-HaCaT the relative level of PLEC, MYH9, SRC8, PHP14, BICD2, and KTN1 proteins increased, while in UVA2-HaCaT it decreased.

Multidirectional regulation has been also observed for the Plectin protein (PLEC_HUMAN), which is involved in keratinocyte development, cell morphogenesis, and cell response to stimuli [39]. In UVA1-HaCaT cells, we found a 2-fold increase, while in UVA2-HaCaT cells, a 2-fold decrease in the relative content of this protein. For the Src substrate cortactin protein (SRC8_HUMAN), we also found a 2-fold increase in UVA1-HaCaT and a 3-fold decrease in UVA2-HaCaT. The SRC8 protein is involved in the actin cytoskeleton organization and cell shape formation; it plays a role in intracellular protein transport and cell motility [40]. A decrease in the content of this protein upon exposure of HaCaT keratinocytes to a cumulative UVA dose of 25 J/cm^2 may indicate a decrease in microtubule-associated motility of UVA2-HaCaT. At the same time, it is known that SRC8 overexpression is associated with a poor prognosis in various types of cancer and promotes migration and invasion of SGC-7901 gastric cancer cells *in vitro* [41].

Four proteins demonstrated a significant decrease in LFQ intensity values in response to UVA exposure of 25 J/cm^2. The most pronounced decrease (30-fold) in the relative level in UVA2-HaCaT has been found in the case of the Kinectin protein (KTN1_HUMAN), which is involved in vesicle movement and protein transport [42]. The relative level of MYH9 and BICD2 proteins, involved in the regulation of cell shape and restoration of the plasma membrane and promoting cell motility [43, 44], demonstrated a 8-fold decrease in UVA2-HaCaT. The level of phosphatase PHP14, involved in the regulation of cell motility in UVA2-HaCaT decreased 6-fold [45].

Using comparative proteome analysis of HaCaT keratinocytes, we have identified proteins whose relative levels (versus control samples) increased in UVA1-HaCaT (4.4-fold on average) and changed

Table 1. Proteins categorized as cell motility proteins in HaCaT cells exposed to UVA radiation

#	Accession number	Protein name	Molecular mass, kDa	LFQ intensity			Fold change UV1/control	Fold change UV2/control	Biological process (disease)
				Control	UVA1	UVA2			
1	P61586	Transforming protein RhoA (RHOA_HUMAN)	21.77	4.26E8±1.07E8	5.39E8±1.29E8	3.68E8±3.31E7	1.27±0.02	0.86±0.14	Cytoplasmic microtubule organization, cell migration, cell adhesion to matrix, regulation of actin cytoskeleton organization, Wnt signaling pathway, planar cell polarity pathway (ectodermal dysplasia)
2	P63000	Ras-related C3 botulinum toxin substrate 1 (RAC1_HUMAN)	21.45	7.28E7±1.60E7	1.15E8±4.70E7	2.23E8±7.58E7	1.58±0.30	3.07±0.40	Regulation of actin cytoskeleton organization, cell migration, cell motility, wounding response, regulation of lamellipodia assembly, cell adhesion
3	Q16658	Fascin (FSCN1_HUMAN)	54.53	1.37E9±2.74E8	3.45E9±4.10E8	2.85E9±3.70E8	2.51±0.20	2.08±0.15	Regulation of actin cytoskeleton organization, cell motility, establishment or maintenance of cell polarity, and positive regulation of lamellipodia assembly
4	Q15149	Plectin (PLEC_HUMAN)	531.78	2.72E7±6.80E6	5.70E7±1.03E7	1.19E7±1.78E6	2.10±0.20	0.44±0.05	Keratinocyte development, cell response to extracellular stimulus, cellular morphogenesis, wound healing (epidermolysis bullosa)
5	O14745	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 (NHRF1_HUMAN)	38.87	7.15E8±1.36E8	1.88E9±1.50E8	4.15E8±3.73E7	2.63±0.30	0.58±0.06	Epithelial morphogenesis, negative regulation of cell population proliferation, regulation of cell shape and size, plasma membrane organization
6	P16144	Integrin beta-4 (ITB4_HUMAN)	202.16	4.53E8±8.60E7	8.58E8±8.50E7	2.42E8±3.15E7	1.89±0.18	0.53±0.03	Cell adhesion, cell motility, response to wounding, skin morphogenesis (form of epidermolysis bullosa, genodermatosis)
7	P35221	Catenin alpha-1 (CTNA1_HUMAN)	100.07	7.42E8±1.70E8	3.52E9±3.17E8	1.39E9±4.50E8	4.74±0.70	1.87±0.20	Intercellular adhesion, negative regulation of cell motility, establishment or maintenance of cell polarity
8	P35579	Myosin-9 (MYH9_HUMAN)	226.53	2.77E8±5.80E7	6.03E8±2.40E7	3.26E7±1.14E7	2.18±0.30	0.12±0.02	Promotes cell motility in complex with S100A4, actin cytoskeleton organization, cell shape regulation, meiotic spindle organization
9	Q14247	Src substrate cortactin (SRC8_HUMAN)	61.59	2.93E9±3.80E8	5.83E9±7.58E8	8.83E8±1.50E8	1.99±0.05	0.30±0.02	Actin cytoskeleton organization, cell motility, focal adhesion assembly, lamellipodium organization, positive regulation of actin filament polymerization
10	P61160	Actin-related protein 2 (ARP2_HUMAN)	44.76	6.83E7±2.32E7	1.81E8±2.17E7	2.13E8±2.10E7	2.65±0.60	3.12±0.80	Arp2/3 complex-mediated actin synthesis, cytosolic transport, cell polarity, and positive regulation of lamellipodia assembly
11	P31949	Protein S100-A11 (S10AB_HUMAN)	11.74	2.06E9±4.12E8	9.92E9±1.19E9	4.32E9±1.72E9	4.81±0.40	2.09±0.40	Negative regulation of cell population proliferation, signal transduction, promotes keratinocyte differentiation and keratinization

Table 1. Proteins categorized as cell motility proteins in HaCaT cells exposed to UVA radiation (continued)

#	Accession number	Protein name	Molecular mass, kDa	LFQ intensity			Fold change UV1/control	Fold change UV2/control	Biological process (disease)
				Control	UVA1	UVA2			
12	P09758	Tumor-associated calcium signal transducer 2 (TACD2_HUMAN)	35.71	5.22E8±5.10E7	2.42E9±4.84E8	4.02E8±6.83E7	4.64±0.50	0.77±0.06	Negative regulation of cell motility, regulation of epithelial cell proliferation
13	O15144	Actin-related protein 2/3 complex subunit 2 (ARPC2_HUMAN)	34.33	5.06E7±1.77E7	3.30E8±9.24E7	1.70E8±1.20E7	6.52±0.50	3.36±1.00	Actin polymerization-dependent cell motility, Arp2/3 complex-mediated actin nucleation, positive regulation of lamellipodia assembly
14	P50552	Vasodilator-stimulated phosphoprotein (VASP_HUMAN)	39.83	5.23E7±1.62E7	1.37E8±4.52E7	5.52E7±1.15E7	2.62±0.08	1.06±0.12	Actin cytoskeleton organization, positive regulation of actin filament polymerization
15	P40121	Macrophage-capping protein (CAPG_HUMAN)	38.50	2.32E8±1.86E7	7.45E8±1.34E8	4.03E8±1.05E8	3.21±0.32	1.74±0.40	Rupture of actin filaments, actin polymerization or depolymerization
16	P06744	Glucose-6-phosphateisomerase (G6PI_HUMAN)	63.15	6.28E8±1.63E8	9.13E8±1.82E8	1.23E9±2.09E8	1.45±0.10	1.96±0.20	Glucose homeostasis, positive regulation of endothelial cell migration, humoral immune response
17	Q9P0L0	Vesicle-associated membrane protein-associated protein A (VAPA_HUMAN)	27.89	1.08E8±3.13E7	3.48E8±9.04E7	1.23E8±3.40E7	3.22±0.10	1.14±0.020	Sphingolipid biosynthesis, cell death, regulation of lipid transport and membrane synthesis in the endoplasmic reticulum
18	P29966	Myristoylated alanine-rich C-kinase substrate (MARCS_HUMAN)	31.55	3.45E7±1.10E7	4.56E7±1.87E7	nd	1.32±0.13	—	Mitochondrial organization, endoplasmic reticulum stress response, actin filament organization, apoptotic process
19	Q9NRX4	14 kDa phosphohistidine-phosphatase (PHP14_HUMAN)	13.83	7.51E7±2.48E7	1.65E8±4.78E7	1.14E7±1.03E6	2.20±0.10	0.15±0.04	Actin cytoskeleton organization, lamellipodia organization, and positive regulation of cell motility
20	Q6NZI2	Caveolae-associated protein 1 (CAVN1_HUMAN)	43.48	5.35E8±1.60E7	2.63E9±7.36E8	8.88E8±1.95E8	4.92±1.20	1.66±0.30	Positive regulation of cell motility, protein secretion, rRNA transcription
21	Q8TD16	Protein bicaudal D homolog 2 (BICD2_HUMAN)	93.53	3.95E8±6.32E7	8.92E8±2.23E8	4.80E7±1.10E7	2.26±0.20	0.12±0.01	Movement along microtubules, protein transport, regulation of microtubule cytoskeleton organization, protein localization in the Golgi apparatus
22	Q86UP2	Kinectin (KTN1_HUMAN)	156.27	1.13E9±1.24E8	1.96E9±5.50E8	3.27E7±1.28E7	1.73±0.30	0.03±0.01	Movement along microtubules, protein transport
23	Q9GZT3	SRA stem-loop-interacting RNA-binding protein, mitochondrial (SLIRP_HUMAN)	12.35	5.73E7±1.90E7	4.25E8±1.44E8	4.41E7±6.61E6	7.40±0.08	0.77±0.15	Mitochondrial organization, an RNA-binding protein that acts as a corepressor of nuclear receptors

insignificantly in UVA2-HaCaT: CTNA1, TACD2, VASP, CAPG, VAPA, CAVN1, and SLIRP (Table 1). CTNA1 and TACD2 proteins are involved in the negative regulation of cell motility [46]. According to the UniProt database, CTNA1 provides intercellular adhesion and maintains cell polarity, and TACD2 regulates proliferation in response to a stimulus. CAPG protein, a component of the actin cytoskeleton, plays a role in actin polymerization or depolymerization, while VASP promotes the elongation of actin filaments and the organization of the actin cytoskeleton [47].

CAVN1 plays an important role in the formation and organization of caveolae and is involved in the positive regulation of cell motility [48]. We have registered a significant increase in the level of regulation of the ARP2 and ARPC2 proteins in both UVA1-HaCaT and UVA2-HaCaT. These proteins are components of the Arp2/3 complex, which mediates actin polymerization and provides cell motility [49]. The relative levels of some HaCaT proteins changed in opposite directions after cell exposure to the non-toxic and toxic dose of UVA. A decrease in the protein content in HaCaT keratinocytes exposed to the cumulative UVA dose of 25 J/cm^2 may indicate a decrease in cellular motility along microtubules in UVA2-HaCaT

Thus, our results have shown that UVA stimulates cell motility of human keratinocytes, and we recorded the proteins involved in this process. Taken together, the data obtained data indicate that the change in the relative level of proteins involved in intracellular motility (RAC1, CTNA1, PLEC, TACD2, SRC8, MYH9, PHP14, BICD2, KTN1) is obviously associated with the adaptive response of cells to the effect of subtoxic doses of UVA to return the cells to a state of homeostasis [50–52].

CONCLUSIONS

Using shotgun proteomic profiling of HaCaT keratinocytes exposed to UVA irradiation with cumulative doses of absorbed radiation of 5 J/cm^2 (UVA1-HaCaT) and 25 J/cm^2 (UVA2-HaCaT), proteins associated with the biological process "cell motility" (GO: 0048870) were identified. The main group of proteins regulating keratinocyte cell motility in response to UVA radiation included cytoskeletal actin-binding proteins (RAC1, CTNA1, PLEC, TACD2, SRC8, MYH9, PHP14, BICD2, KTN1). It is suggested that changes in the content of these proteins are associated with manifestation of the adaptive response of cells to the effect of subtoxic doses of UVA to return the cells to homeostasis. The changes in the level of regulation of cell motility proteins that we registered have not been previously described for HaCaT keratinocytes in response to UVA. Thus, the study of changes in the content of these proteins in the HaCaT cells can apparently become a promising direction for further study of the molecular mechanisms of UVA-induced cell damage and can be used to study physiological and pathological processes, in particular, in wounds and wound healing.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or the use of animals as objects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Supplementary materials are available in the electronic version at the journal site (pbmc.ibmc.msk.ru).

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ИЗМЕНЕНИЕ ПРОФИЛЯ БЕЛКОВ КЛЕТОЧНОЙ ПОДВИЖНОСТИ В ОТВЕТ НА ЭКСПОЗИЦИЮ UVA КЕРАТИНОЦИТОВ HaCaT

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Проведён сравнительный анализ белков кератиноцитов HaCaT в ответ на воздействие субтоксических доз (5 Дж/см^2 и 25 Дж/см^2) ультрафиолетового излучения типа А (UVA). По двум и более уникальным пептидам было идентифицировано 930 белков. На долю белков, относительное содержание которых увеличивалось в кератиноцитах HaCaT в ответ на облучение кумулятивной дозой 5 Дж/см^2 не менее, чем в 2 раза, пришлось более половины (54,5%) всех идентифицированных белков. Снижение относительного содержания отмечено всего для 4 белков. Облучение кератиноцитов кумулятивной дозой 25 Дж/см^2 привело к снижению доли белков (43,0%) с повышенным уровнем регуляции и увеличению количества белков (84) с пониженным уровнем регуляции. Среди белков, относительное содержание которых в кератиноцитах HaCaT увеличивалось наиболее сильно, были белки, ассоциированные с процессом “клеточной подвижности” (GO: 0048870 — Cell motility), вовлечённые в процесс регуляции формы и размеров клеток, морфогенеза клеток и ремоделирования кожи.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: кератиноциты HaCaT; UVA; 1DE-гель концентрирование; LC-MS/MS; MaxQuant; белки клеточной подвижности

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