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SHORT COMMUNICATION

IDENTIFICATION OF PROTEIN COMPONENTS OF THE TRANSFORMATION SYSTEM IN THE CELL LINE OF IMMORTALIZED HUMAN KERATINOCYTES HaCaT EXPOSED TO SURFACTANTS

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Using the method of shotgun mass spectrometry, we have evaluated changes in the proteomic profile of HaCat cells in response to the treatment with sodium dodecyl sulfate (anionic surfactant) and Triton-X100 (non-ionic surfactant) in two concentrations (12.5 μg/ml and 25.0 μg/ml). The study revealed induction of orphan CYP2S1 (biotransformation phase I) in response to Triton-X100. We have identified proteins of II (glutathione-S-transferases, GSTs) and III (solute carrier proteins, SLCs) biotransformation phases, as well as antioxidant proteins (peroxiredoxins, PRDXs; catalase, CAT; thioredoxin, TXN). Thus, proteins of all three xenobiotic detoxification phases were detected. The presented results suggest a new prospect of using HaCaT keratinocytes as a model of human epidermis for studying the metabolism of drugs/toxicants in human skin *in vitro*.

Key words: immobilized HaCaT keratinocytes; surfactants; Triton X-100; LC-MS/MS; cytochrome P450 2S1

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INTRODUCTION

This work is a continuation of our studies on applicability of the HaCaT cell line as a test system for chemical proteomics problems based on mass spectrometry. We have previously shown that HaCaT keratinocytes, as a model of the human epidermis, can be used for characterization of toxicity of chemical compounds, for example, surfactants of various structures [1–3].

One of the goals of chemical proteomics is the identification of interactions between small molecules and proteins forming the entire cellular proteome, including identification of particular proteins/metabolic pathways involved in transformation of xenobiotics [4]. The main organs of xenobiotic metabolism in the human body include the liver, kidneys, lungs, and intestine [5]. The skin has not been considered for a long time in the context of the metabolism of foreign substances, despite the fact that it is the largest organ of the human body exposed to many external aggressive factors [6]. However, in addition to its barrier function, the skin can also metabolize xenobiotics. For example, biotransformation enzymes such as cytochromes P450 (CYPs), flavin monooxygenases, glutathione-S-transferases, N-acetyltransferases and sulfotransferases have been found in the mammalian epidermis, although with relatively low specific activity [6, 7].

The risks of xenobiotics and mechanisms of their action are frequently evaluated using animal models. However, animal models are often poor predictors of human response due to differences in physiology and immunity. In addition, modern requirements for the pharmaceutical and cosmetics industry require the reduction and replacement of animals in experiments [8]. Therefore, various keratinocyte, fibroblast, or melanocyte-based models, including HaCaT (spontaneously immortalized human keratinocyte cell line), are currently used to evaluate the safety of various substances in relation to human health. A characteristic feature of HaCaT cells is their unlimited division capacity, which determines the possibility of obtaining a standardized research object [9].

The goal of this work was mass spectrometric identification of keratinocyte HaCaT proteins associated with the biotransformation of xenobiotics. As in our previous works, sodium dodecyl sulfate (anionic surfactant, AIS) and Triton X-100 (non-ionic surfactant, NIS) have been chosen as model toxicants, which are used in scientific research and

Abbreviations used: AIS – anionic surfactants; BSA – bovine serum albumin; CAT – catalase; CYPs – cytochromes P450; DTT – dithiothreitol; EDTA – ethylenediaminetetraacetic acid; GSTs – glutathione-S-transferases; HPLC – high performance liquid chromatography; NIS – non-ionic surfactants; NSAF – normalized spectral abundance factor; PMSF – phenylmethylsulfonyl fluoride; PRDXs – peroxiredoxins; TCEP – trichlorethyl phosphate; TEAB – triethylammonium bicarbonate; TFA – trifluoroacetic acid; Tris – tris(hydroxymethyl)aminomethane; SLCs – solute transport proteins; TXN – thioredoxin.

certification tests as a positive control in determining cytotoxicity [3]. In addition, these AIS and NIS are used in almost any type of liquid, paste and powder cleaning products, and are also included in pharmaceuticals and feed additives as stabilizers, solvents, and emulsifiers.

MATERIALS AND METHODS

Reagents

The following reagents have been used in this work: sodium dodecyl sulfate, Triton X-100, bovine serum albumin (Merck, Germany); modified lyophilized trypsin from porcine pancreas (Promega, USA); trifluoroacetic acid (Fluka, Germany); acetonitrile, dithiothreitol; deionized water (Acros, USA); 2,2-bicinchoninic acid (Pierce, USA), as well as chemically pure reagents from local suppliers.

Cultivation of HaCaT Cells

Cells of the immortalized cell line HaCaT (CLS Cell Lines Service, Germany, 300493) were seeded in 75 cm^2 flasks (Corning, USA). Their cultivation was carried out in DMEM/F12 1:1 medium (PanEco, Russia) containing 10% FBS and antibiotics (100 units/ml penicillin and 100 mg/ml streptomycin), in a CO_2 incubator (at 37±1°C, humidity 90±10%, CO_2 content of 5.0±1.0%). After reaching 60–70% confluency, the culture medium was removed, and the cells were exposed to solutions of AIS (25.0 μg/ml), NIS1 (12.5 μg/ml), or NIS2 (25.0 μg/ml) in a nutrient medium. Fresh nutrient medium was added to control samples. The cells were exposed to AIS or NIS1/NIS2 for 48 h.

After cultivation the cells were dispersed with 3 ml of trypsin-EDTA solution (PanEco) incubated for 3 min at 37°C to dissociate from the surface of the culture flasks; the cell sediment was washed 2-3 times with potassium phosphate buffer, pH 7.4, and centrifuged at 10,000 *g* for 20 min (4°C).

Preparation of Protein Extracts and Trypsinolysis in Solution

In order to obtain sufficient amounts of the HaCaT protein for proteomic analysis, we pooled cells from three culture flasks, representing three biological replicates for each group (control, AIS-exposed, or NIS-exposed) in one tube for further processing.

The HaCaT sediment was resuspended in 400 μl of cold H_2O containing the protease inhibitor PMSF (1%); the aqueous cell suspensions were then homogenized in an ice bath using ultrasound: two cycles of 25 s, 20 kHz with an interval between cycles of 25 s (Sonoplus HD2070, BANDELIN Electronic, Germany) and then centrifuged at 14,000 *g* for 20 min (4°C). The resulting supernatant was collected, and the supernatant protein content was determined using 2,2-bicinchoninic acid at 562 nm (8453 UV-visible spectrophotometer, Agilent, USA), using BSA as a standard.

HaCaT cell extracts (175 μg protein per sample) were subjected to tryptic digestion as described previously [10]. Denaturation of proteins and reduction of disulfide bonds was carried out using a reducing solution containing 87 mM DTT and 6.7 mM TCEP in denaturing buffer (12 mM sodium deoxycholate, 2 M thiourea, 2.5 mM EDTA and 75 mM Tris-HCl, pH 8.5); samples were incubated at 42°C for 60 min under constant stirring in a GFL Shaking Incubator 3032 (GFL, Germany). The ratio of the reducing solution to the protein sample was 1:1 (v/v). Then an alkylating buffer (100 μl of denaturing buffer, 10 μl of 4-vinylpyridine, and 90 μl of N,N-dimethylformamide, pH<9.0) was added to each sample (the ratio of alkylating solution volume to sample volume was 1:12). After thorough mixing the reaction mixture was incubated at 20°C for 60 min in the dark.

After the incubation, digestion buffer containing 100 mM $CaCl_2$ and 42 mM triethylammonium bicarbonate (TEAB, 42 μl) in H_2O (water for UV, HPLC, ACS) was added (up to 100 μl). Trypsin was added to the sample at a trypsin:protein ratio of 1:100 and then incubated in the dark at 44°C under stirring (50 rpm) for 120 min in a GFL Shaking Incubator 3032. Then another portion of trypsin (11.4 μl) was added and samples were incubated in the dark at 37°C under stirring (50 rpm) for 120 min in the same incubator. Enzymatic digestion was stopped by adding formic acid to a final concentration of 1%, and then the samples were centrifuged at room temperature (30 min, 10,000 *g*). Digested peptide mixtures were analyzed without further processing using liquid chromatography coupled to LC-MS/MS.

LC-MS/MS Analysis

One microgram of peptides (1–4 μl) was applied to an Acclaim μ-Precolumn (0.5 mm × 3 mm, particle size 5 μm, Thermo Fisher Scientific, USA) at the loading rate 10 μl/min for 4 min. Elution was carried out in the isocratic mode using the mobile phase C (2% acetonitrile, 0.1% formic acid). The peptide mixture was then separated using high performance liquid chromatography (Ultimate 3000 Nano LC System, Thermo Fisher Scientific) on a 15 cm long C_{18} column (Acclaim PepMap RSLC 75 μm i.d., Thermo Fisher Scientific). Next, the peptides were eluted with a gradient of buffer B (80% acetonitrile, 0.1% formic acid) at a flow rate of 0.3 μl/min. The total analysis time was 90 min. This included initial equilibration of the column with buffer A (0.1% formic acid) for 4 min, then the concentration of buffer B was linearly increased from 5% to 35% (60 min) and within 6 min to 99%. After a 10-minute wash with 99% buffer B, its concentration was linearly reduced to the initial 2% (6 min). Finally, the analytical column was equilibrated with buffer A for 5 min.

Mass spectrometry analysis was performed using a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific) as previously described [10].

For proteins of the HaCaT keratinocytes (control and after exposure to surfactants), 21 MS/MS spectra were obtained in the ".raw" format, which were then converted into MGF files using the ProteoWizard MSConvert program [11]. The files were imported into the SearchGUI platform (v. 3.3.17) [12] and analyzed using the X!Tandem and MS-GF+ search algorithms against the SwissProt database (v. 1.4.2019, FASTA format) for the species *Homo sapiens*. The search was carried out using a database of inverted and random amino acid sequences (decoy), the percentage of false positive results (false discovery rate, FDR) ≤1%. The search parameters included: digestive enzyme — trypsin; the maximum number of possible missed trypsin cleavage sites was 1; fixed modification — pyridylethylation of cysteine; variable modification — oxidation of methionine; accuracy of measuring the theoretical and experimental mass of the peptide ±5 ppm; accuracy of measurement of theoretical and experimental mass of fragment ions ±0.01 Da; the value of the charge state of the peptide ions was "2+, 3+, and 4+". The PeptideShaker integrator [13] was used to produce an Excel spreadsheet file with protein identification results. The dataset is available at Mendeley Data, DOI: 10.17632/45w5hbhpg6.1.

Data Processing

The statistical significance of the differences between the compared parameters was evaluated using the Student's *t*-test for independent sets. For control HaCaT cells, the set consisted of 6 technical replicates (mass spectra), in the case of HaCaT keratinocytes treated with AIS the set consisted of 9 technical replicates, and 3 technical replicates each for NIS1 and NIS2, respectively. Data are presented as mean (M) ± standard deviation (SD). The results were considered as statistically significant at a threshold level of statistical significance (alpha level) of 0.05.

To quantify protein content, the NSAF (normalized spectral abundance factor) algorithm was chosen because of its high reproducibility. Using NSAF values it possible to compare protein content within a sample and/or between samples [14].

RESULTS AND DISCUSSION

To identify specific proteins/metabolic pathways involved in metabolism of various types of xenobiotics in the human epidermis, a comparative analysis of the proteomes of control HaCaT cells and cells exposed to surfactants was carried out. A total of four lists of identified proteins were obtained. These included control, AIS, NIS1, and NIS2. Lists of proteins identified in the work are presented in Supplementary Materials (Tables S1-S4).

According to the recommendations of the HUPO Committee (Human Proteome Organization), protein identification is considered reliable when at least two proteotypic peptides are detected [15]. As a result, in total we have identified 803±129 proteins. At the same time, 460±154 proteins were identified in control HaCaT keratinocytes. After exposure to AIS, a slight increase in the number of identifications was observed — 553±150. However, the comparison did not reveal statistical differences between the number of proteins identified in control and AIS-treated cells (p>0.20). After exposure to NIS1 and NIS2, a small and statistically insignificant (p>0.05) decrease in the number of identified proteins was found as compared to the control (410±111 and 406±110 proteins, respectively).

For identification of proteins involved in cellular detoxification and metabolism of drugs and xenobiotics in the HaCaT cells the corresponding lists of human proteins were extracted from the Gene Ontology database [16]. Comparison of the obtained lists with the HaCaT cell proteomes before and after exposure to surfactants resulted in identification of 18 proteins involved in cellular detoxification and metabolism of xenobiotics (Table 1). At the same time, in the control cells and cells exposed to NIS, almost the same number of proteins was identified: 13 and 14 proteins, respectively. Exposure to AIS and NIS1 led to a slight increase in the number of identified proteins responsible for the biotransformation of xenobiotics (15 and 17, respectively).

Only eleven common proteins (61%) involved in the biotransformation of xenobiotics were found for control HaCaT cells and the cells exposed to AIS, NIS1, and NIS2. These included: SLC7A5, GSTK1, GSTP1, GSTO1, PRDX3, CAT, TXN, PRDX6, PRXL2A, PRDX5, PRDX1 (Table 1). The content of five proteins (GSTK1, GSTP1, PRDX3, CAT, TXN), assessed by the values of the normalized spectral abundance factor NSAF [14], increased in response to AIS as compared to the control (Table 1). Among these proteins, PRDX6 attracts much interest because its activation protects keratinocytes from cell death under stress conditions induced by reactive oxygen species *in vitro* and *in vivo* [17]. In contrast to AIS, the effect of NIS1 and NIS2 on HaCaT keratinocytes had virtually no effect on the NSAF values of the proteins GSTK1, GSTP1, PRDX3, CAT, and TXN as compared to the control.

Biotransformation of xenobiotics is a complex process involving a large number of enzymes; this process consists of three phases: activation (phase I), detoxification (phase II), and excretion (phase III). A key role in the detoxification of numerous compounds,

Table 1. The list of identified proteins in HaCaT keratinocytes involved in the xenobiotic metabolism and cellular detoxification

##	Identifier	Gene	Protein name	Number of validated peptides/spectra				Normalized spectral abundance factor (NSAF)			
				Control	AIS*	NIS1**	NIS2***	Control	AIS	NIS1	NIS2
1	Q01650	*SLC7A5*	Large neutral amino acids transporter small subunit 1	3/16	2/14	2/10	2/8	0.061	0.050	0.038	0.031
2	P16152	*CBR1*	Carbonyl reductase [NADPH] 1	4/10	ND	6/14	5/11	0.041	ND	0.058	0.045
3	Q9Y2Q3	*GSTK1*	Glutathione S-transferase kappa 1	4/14	6/26	3/9	5/15	0.064	0.128	0.053	0.074
4	P78417	*GSTO1*	Glutathione S-transferase omega 1	3/17	4/21	2/9	2/6	0.096	0.119	0.051	0.034
5	P00390	*GSR*	Glutathione reductase. mitochondrial	ND	1/1	1/1	ND	ND	0.003	0.003	ND
6	P09211	*GSTP1*	Glutathione S-transferase pi 1	11/60	14/154	11/89	11/57	0.397	1.020	0.589	0.378
7	P04179	*SOD2*	Superoxide dismutase [Mn]. mitochondrial	ND	1/7	1/3	1/1	ND	0.042	0.018	0.006
8	Q96SQ9	*CYP2S1*	Cytochrome P450 2S1	ND	ND	ND	3/5	ND	ND	ND	0.006
9	P14550	*AKR1A1*	Aldo-keto reductase family 1 member 1A1	ND	6/15	4/8	ND	ND	0.055	0.028	ND
10	P53985	*SLC16A1*	Monocarboxylate transporter 1	ND	ND	1/1	ND	ND	ND	0.005	ND
11	A0A0A0MRQ5	*PRDX1*	Peroxiredoxin 1	5/44	7/55	7/30	4/16	0.271	0.349	0.182	0.099
12	P32119	*PRDX2*	Peroxiredoxin 2	4/23	7/39	5/15	ND	0.113	0.229	0.081	ND
13	P30048	*PRDX3*	Peroxiredoxin 3	1/8	2/10	3/11	4/12	0.009	0.049	0.054	0.059
14	P30044	*PRDX5*	Peroxiredoxin 5	7/30	10/52	6/8	5/10	0.158	0.274	0.042	0.053
15	P30041	*PRDX6*	Peroxiredoxin 6	7/27	10/97	10/44	8/19	0.139	0.500	0.227	0.098
16	Q9BRX8	*PRXL2A*	Peroxiredoxin-like 2A	3/5	2/3	5/7	6/13	0.031	0.019	0.044	0.082
17	P04040	*CAT*	Catalase	2/4	6/10	2/5	5/9	0.009	0.023	0.012	0.021
18	P10599	*TXN*	Thioredoxin	5/49	8/156	6/61	5/20	0.511	1.660	0.645	0.213

ND – not determined; * AIS – anionic surfactant (sodium dodecyl sulfate, 25.0 μg/ml); ** NIS1– nonionic surfactant (Triton X-100, 12.5 μg/ml); *** NIS2 – nonionic surfactant (Triton X-100, 25.0 μg/ml).

both of endogenous and exogenous origin, belongs to enzymes of the CYP superfamily. Most drugs used in dermatology are either CYP substrates, or inducers, or inhibitors. The main site of metabolism of these compounds in the skin is keratinocytes, which are characterized by a low level of CYP mRNA expression. In the skin of all animal species and *in vitro* skin systems, the content of CYPs is very low or they are not even detectable [18].

Using mass spectrometry analysis we were able to detect a CYP belonging to family 2, subfamily S (CYP2S1) in the immortalized human keratinocyte cell line HaCaT exposed to NIS2. According to the GeneCards database, CYP2S1 is a participant in the interconnected metabolic pathways "Oxidation by cytochrome P450" and "Metapathway biotransformation Phase I and II". Using the SearchGUI platform, we have identified three corresponding CYP2S1 peptides with a high degree of confidence (the scoring confidence index was 100%, Table 2). Table 3 shows the theoretically possible and identified (marked in bold) fragment ions of the peptide ^{251}QVQQHQGNLDASGPAR specific for cytochrome 2S1 (CYP2S1).

Previously, induction of CYP2S1 in the HaCaT cells was detected only as expression of the protein-coding gene based on an increase in the mRNA level in response to exposure to ultraviolet radiation [19]. The NeXtProt human proteome project database also does not contain information about the presence of CYP2S1 in keratinocytes at the protein level [20].

CYP2S1 belongs to the so called orphan CYPs, which are known to be expressed predominantly in extrahepatic tissues but have no defined function with endogenous or exogenous substrates [21]. Maximum expression of CYP2S1 is observed in the epithelium of tissues exposed to the external environment, such as the respiratory and digestive tracts and skin [22].

From the data in Table 1 it follows that in HaCaT cells four proteins of phase II of xenobiotic metabolism belonging to the glutathione S-transferase (GST) family were identified, including the main skin glutathione S-transferase pi 1 (GST P09211, GSTP1).

In our samples of HaCaT keratinocytes, two transporter proteins of phase III of xenobiotic biotransformation were detected (Table 1). SLC7A5 was identified in all four studied samples

Table 2. The list of peptides identified in HaCaT keratinocytes using the SearchGUI platform and belonging to the orphan cytochrome P450 2S1 (CYP2S1)

##	Amino acid sequence	*m/z* exper.	charge	*m/z error (mln^{-1})*	Significance
1	^{251}QVQQHQGNLDASGPAR	569,62	3+	2,53	100
2	^{144}EGEELIQAEAR	622,81	2+	1,20	100
3	^{89}EALGGQAEEFSGR	675,82	2+	2,96	99

Table 3. Theoretically possible and identified (shown in bold) fragment ions of the peptide ^{215}QVQQHQGNLDASGPAR specific for cytochrome 2S1 (CYP2S1)

	b+	b++	b-H_2O	b++-H_2O	b-NH_3	b++-NH_3	Seq	y	y++	y-H_2O	y++-H_2O	y-NH_3	y++-NH_3	
1	*129.066*	*65.037*	*129.066*	*65.037*	*129.066*	*65.037*	***Q***							16
2	*228.134*	*114.571*	*228.134*	*114.571*	*228.134*	*114.571*	***V***	*1577.778*	*789.393*	*1577.778*	*789.393*	*1577.778*	*789.393*	15
3	*356.193*	*178.600*	*356.193*	*178.600*	*356.193*	*178.600*	***Q***	*1478.709*	*739.858*	*1478.709*	*739.858*	*1478.709*	*739.858*	14
4	*484.251*	*242.629*	*484.251*	*242.629*	*484.251*	*242.629*	***Q***	*1350.651*	*675.829*	*1350.651*	*675.829*	*1350.651*	*675.829*	13
5	*621.310*	*311.159*	*621.310*	*311.159*	*621.310*	*311.159*	***H***	*1222.592*	*611.800*	*1222.592*	*611.800*	*1222.592*	*611.800*	12
6	*749.369*	*375.188*	*749.369*	*375.188*	*749.369*	*375.188*	***Q***	*1085.533*	*543.270*	*1085.533*	*543.270*	*1085.533*	*543.270*	11
7	*806.390*	*403.699*	*806.390*	*403.699*	*806.390*	*403.699*	***G***	*957.475*	*479.241*	*957.475*	*479.241*	*957.475*	*479.241*	10
8	*920.433*	*460.720*	*920.433*	*460.720*	*920.433*	*460.720*	***N***	*900.453*	*450.730*	*900.453*	*450.730*	*900.453*	*450.730*	9
9	*1033.517*	*517.262*	*1033.517*	*517.262*	*1033.517*	*517.262*	***L***	*786.410*	*393.709*	*786.410*	*393.709*	*786.410*	*393.709*	8
10	*1148.544*	*574.776*	*1148.544*	*574.776*	*1148.544*	*574.776*	***D***	**673.326**	*337.167*	*673.326*	*337.167*	*673.326*	*337.167*	7
11	*1219.581*	*610.294*	*1219.581*	*610.294*	*1219.581*	*610.294*	***A***	**558.299**	*279.653*	*558.299*	*279.653*	*558.299*	*279.653*	6
12	*1306.613*	*653.810*	*1306.613*	*653.810*	*1306.613*	*653.810*	***S***	**487.262**	*244.135*	*487.262*	*244.135*	*487.262*	*244.135*	5
13	*1363.635*	*682.321*	*1363.635*	**682.321**	*1363.635*	*682.321*	***G***	**400.230**	*200.619*	**400.230**	*200.619*	*400.230*	*200.619*	4
14	*1460.688*	*730.847*	*1460.688*	*730.847*	*1460.688*	*730.847*	***P***	*343.209*	*172.108*	**343.209**	*172.108*	*343.209*	*172.108*	3
15	*1531.725*	*766.366*	*1531.725*	*766.366*	*1531.725*	*766.366*	***A***	*246.156*	*123.582*	*246.156*	*123.582*	*246.156*	*123.582*	2
16							***R***	*175.119*	*88.063*	*175.119*	*88.063*	*175.119*	*88.063*	1

of HaCaT cells, while SLC16A1 was detected only in HaCaT keratinocytes treated with NIS1 (by 1 peptide). In addition, it turned out that while the effect of AIS had virtually no effect on the protein content, both NIS1 and NIS2 led to a some decrease in the NSAF SLC7A5 value (approximately 2 times, Table 1).

Comparison of the content of proteins involved in phase I (CYP2S1) and phase II (GSTK1 and GSTP1) biotransformation reactions in NaCaT cells treated with NIS2 showed that the NSAF values for these GSTs significantly exceeded the NSAF value for CYP2S1 (Table 1). This is consistent with the literature data that the activity/expression of phase II biotransformation enzymes such as GST and glucuronosyltransferases is much higher than the activity/expression of CYP. It is believed that the high content of GST conjugating enzymes, primarily responsible for detoxification, means that the skin may be protected from reactive metabolites generated by CYP [23].

It is known that the induction of some CYPs can be accompanied by an increase in the content of antioxidant enzymes, including such known antioxidant catalysts as: catalase, SOD, and peroxidases [24]. As can be seen from Table 1, an increase in the content of catalase (CAT), which, along with peroxiredoxins, is classified as a phase II protein of xenobiotic metabolism [25], was registered not only after exposure to NIS2, but also to AIS.

Thus, analysis of the proteomes of HaCaT cells (a model of human skin epidermis) has shown that HaCaT keratinocytes contain proteins involved in all three phases of xenobiotic detoxification.

CONCLUSIONS

Currently, *in vitro* toxicology methods and approaches using cell cultures are widely used to study the effects of drugs and toxic compounds as an alternative to animal experiments. The human keratinocyte cell line HaCaT (a model of human skin epidermis) is often used to assess the safety of various substances in relation to human health. A characteristic feature of HaCaT cells is their unlimited division capacity, which determines the possibility of obtaining a standardized research object.

An assessment of the possibility of using human keratinocytes of the HaCaT cell line as a test system for studying the processes of biotransformation of xenobiotics was carried out using surfactants — sodium dodecyl sulfate (AIS) and Triton X100 (NIS), which are used in the practice of certification tests as a positive control in the method for determining cytotoxicity. There were no noticeable differences in the amount of identified proteins in HaCaT keratinocytes before and after exposure to AIS and NIS. The response of the HaCaT keratinocyte proteome depended on the nature of the effect. In particular, induction of orphan cytochrome P450 2S1 was detected in response of HaCaT cells to treatment with 25.0 μg/ml NIS (Triton X100). In addition, phase II (glutathione-S-transferases, GSTs) and phase III (transporter proteins, SLCs) proteins of biotransformation reactions, as well as the antioxidant proteins (peroxiredoxins, PRDXs; catalase, CAT; thioredoxin, TXN), were identified. Thus, the immortalized human keratinocyte cell line HaCaT can be an effective test model for *in vitro* toxicology experiments.

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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.

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

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ИДЕНТИФИКАЦИЯ БЕЛКОВ-КОМПОНЕНТОВ СИСТЕМЫ ТРАНСФОРМАЦИИ В КЛЕТОЧНОЙ ЛИНИИ ИММОРТАЛИЗОВАННЫХ КЕРАТИНОЦИТОВ ЧЕЛОВЕКА HaCaT, ПОДВЕРГНУТЫХ ВОЗДЕЙСТВИЮ ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ

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Методом панорамной масс-спектрометрии проведена оценка изменений белкового профиля клеток HaCaT в ответ на воздействие поверхностно активных веществ (ПАВ) разной природы — додецилсульфата натрия (ПАВ анионного типа — АПАВ) и Тритона X-100 (неионный ПАВ — НПАВ) в двух концентрациях — 12,5 мкг/мл (НПАВ 1) и 25,0 мкг/мл (НПАВ 2). Была зарегистрирована индукция орфанного CYP2S1 (I фаза биотрансформации) в ответ на воздействие НПАВ2. Среди белков II и III фаз биотрансформации, были идентифицированы глутатион-S-трансферазы (GSTs) и белки-транспортёры растворённых веществ (SLCs) соответственно, а также белки-антиоксиданты (пероксиредоксины, PRDXs; каталаза, CAT; тиоредоксин, TXN). Таким образом, мы обнаружили белки всех трёх фаз детоксикации ксенобиотиков. Представленные результаты указывают на возможность использования иммортализованной клеточной линии кератиноцитов человека HaCaT в качестве модели эпидермиса кожи для оценки уровня индукции белков, участвующих в процессах биотрансформации токсикантов в коже человека *in vitro*.

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

Ключевые слова: иммобилизованные кератиноциты человека линии HaCaT; поверхностно-активные вещества; Тритон X-100; LC-MS/MS; цитохром P450 2S1

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