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Proteomic characterization of HaCaT keratinocytes provides new insights into changes associated with SDS exposure

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Abstract

Background: Using human keratinocyte HaCaT cell line model, we screened for proteins that changed their content due to SDS exposure in non-toxic dose (25 µg/ml, as determined by the MTT assay and microscopic examination) during 48 h.

Methods: The altered level of proteins from HaCaT keratinocytes exposed to SDS was analyzed by LC-MS/MS approach and quantified using Progenesis LC software.

Results: The Pathview map of 131 upregulated proteins was built, and enhancement of glycolysis/gluconeogenesis was found.

Conclusions: The results of our study admit the possibility of promotion of the cutaneous neoplasia and/or the peculiarity of the response of immortalized keratinocytes to the SDS treatment and provide new insights into possible role of SDS as integrator of diverse signaling that influence cell fate decisions.

Keywords: HaCaT keratinocytes, Sodium dodecyl sulphate, Exposure, LC-MS/MS, Pathview map, Glycolysis/gluconeogenesis

Introduction

Skin diseases are one of the most common human illnesses. The International Classification list of human disease contains more than 1000 skin or skin-related disorders. Widespread of skin diseases is caused with the variety of skin functions, the features of its structure, and exposure with a large number of external factors including dermatologic, cosmetic, and pharmaceutical products (Sprengrer et al. 2010).

Contact dermatitis is one of the most common skin pathologies. The definition of contact dermatitis refers to any skin inflammation that occurs because of exposure to irritants (chemicals, mechanical, and physical) or allergens (Chiang et al. 2012). Among the well-known irritants of the skin are detergents, i.e., sodium dodecyl sulphate (SDS), which is used as a component in

domestic goods such as toothpaste and shampoos, cleansing agent in cosmetics, dispersing agent in creams, and an ingredient in food additives. As have been reported, SDS is toxic to humans and the environment (Banipal et al. 2016; Bondi et al. 2015). Exposure of SDS to mammals results in physical and biochemical effects: skin irritation, hyperplasia, alteration of serum lipid composition, damage of cells, and decrease in cell proliferation (Lindberg et al. 1992; Miura et al. 1989; van de Sandt et al. 1995). In amounts of 2 to 5%, SDS can cause sensitizing reactions, increase the transepidermal water loss of the stratum corneum, and result in mild skin inflammation affecting keratinocytes (Törmä et al. 2008; Dahl and Trancik 1977).

In addition, SDS is also used as a positive control when studying the cytotoxic effect of various substances (Sakai et al. 1998). Cell lines and their cultivation systems are of great importance for modern biomedical and clinical research (Sprengrer et al. 2010). The most suitable target cells for evaluating the mechanisms of the

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cytotoxic and pharmacological action of substances on the skin are human keratinocytes (Hoh et al., 1987).

It was shown that immortalized, non-tumor-derived cell lines appear to behave very similarly to primary cells, keeping major specific characteristics, at least in early passages (Sprenger et al. 2010). The spontaneously immortalized human keratinocyte cell line HaCaT is often used as a model to study keratinocyte functions (Gibbs 2009). HaCaT cells have proved to be helpful to determine skin toxicity of various agents and to investigate the mechanisms of cutaneous allergic reactions, inflammatory, or neoplastic processes, as well as effects of UV irradiation and reactive oxygen species (Dickson et al. 1993; Bae et al. 2001; Ermolli et al. 2001; Fukunaga et al. 2001; Phillipson et al., 2002). Immortal cells may be classified as preneoplastic cells and as such provide the starting material for the search for changes involved in neoplastic and malignant transformation (Hedrick et al. 1993). Immortalized human keratinocyte lines such as HaCaT may spontaneously acquire the tumorigenic potential during continued growth in culture or after induction of additional genetic alterations by carcinogenic agents (Fusenig and Boukamp 1998).

Usually, effects of the SDS in cultured keratinocytes were studied using the MTT assay and mRNA expression of inflammatory mediators, markers of keratinocyte differentiation, and enzymes synthesizing barrier lipids by PCR (Törmä et al. 2006). Biomarkers such as interleukins 1 alpha, 6, and 8, prostaglandin E2 receptor EP2 subtype (PGE2), elafin (SKALP), heat shock 70 kDa protein (HSP70), and kinases are described along with changes in metabolic activity (MTT assay) and cytosolic leakage (lactate dehydrogenase assay) (Le et al. 1996; Törmä et al. 2006).

Noticeable is the limited number of genomic and proteomic studies (Gibbs 2009). At the same time, proteomics provides an effective approach for the analysis of protein changes in cells under certain drug treatment and for the study of protein-protein interactions (Tyagi et al. 2011). It should be noted that proteomics analysis of SDS toxicity is mainly conducted using normal human epidermal keratinocytes. For example, the analysis of normal keratinocytes treated with SDS by two-dimensional electrophoresis and MALDI-TOF (or MALDI-TOF/TOF) mass spectrometry allowed identification of a number of proteins (i.e., involucrin, peroxiredoxin 1, serine protease inhibitor, small heat shock protein 27, and some others) that could be used as general markers for skin irritation (Fletcher and Basketter 2006; Zhang et al. 2011). In particular, a proteomic study of HaCaT keratinocytes through liquid chromatography-tandem mass spectrometry and bioinformatics analysis was performed to identify proteins of circadian rhythms (Avitabile et al. 2014). Recently, HaCaT cells were used

to profiling proteins of inflammation and malignant transformation in nonmelanoma skin cancer (Paulitschke et al. 2015) and the altered abundance of proteins after treatment with arsenic (Udensi et al. 2014) by a label-free LC-MS/MS.

In this paper, we describe a survey of the HaCaT proteome together with an analysis of cellular protein changes induced by relatively long (48 h) exposure to the SDS in non-cytotoxic dose.

Materials and methods

Cell culture and treatments

HaCaT cells were seeded in T75 tissue culture flasks (Corning, USA) and cultured in DMEM supplemented with 10% fetal bovine serum (FBS), Glutamax™ Supplement, and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Media components were purchased from Gibco, USA. Cells were maintained at 37 °C and in a humidified 5% CO₂ atmosphere. The medium was changed every other day.

After growing to ~ 60–70% confluence, the cultured cells were divided into two groups, one is control (fresh medium was added) and another was exposed to SDS (25 µg/ml) diluted in complete DMEM. Forty-eight hours later, the medium was aspirated and cells were washed with PBS, trypsinized with 3 ml of 0.25% trypsin-EDTA per flask, and incubated briefly at 37 °C. The cells were examined microscopically to ensure that cells were completely detached and then transferred to a centrifuge tube. The cells were centrifuged at 1200 rpm for 5 min and washed in ice-cold PBS three times. Finally, PBS was aspirated and the pellet was resuspended in 200 µl of ice-cold deionized water.

Cytotoxicity assays (MTT and NRU assays)

Cells were treated with SDS dissolved in complete DMEM medium within a range of concentrations from 0 to 256 µg/ml SDS for 48 h.

A total of 5000 cells in 200 µl complete medium was added to each well of a 96-well plate and incubated at 37 °C in 5% CO₂ to a maximum confluence of 60% after which the culture medium was replaced by SDS dilutions in three replicates of each concentration. They were incubated in a humidified incubator at 37 °C in 5% CO₂ for a period of 48 h. Cytotoxicity in HaCaT cells following exposure to SDS was determined by measuring the viability of cells using MTT (tetrazolium salts) assay (Mossmann 1983) or NR assay (Borenfreund and Puerner 1984).

For MTT assay, 20 µl of the yellow MTT reagent (10 mg dissolved in 2 ml PBS) was added to each of the wells and incubated at 37 °C for 3 h until purple formazan precipitate was visible. After that, MTT solution was aspirated and cells were washed in the wells by warm (37 °C) PBS

solution (250 μ l). Then, 200 μ l of the DMSO was added to each of the wells and incubated for 5 min at 150–200 rpm/min. The absorbance was measured at 520 nm with a reference filter of 655 nm using the microplate spectrophotometer system (iMark, BioRad).

In parallel, the same experiment was performed and the cell viability (metabolic activity) was determined by vital staining with neutral red dye (NR). Working solution of NR (33 μ g/ml) in DMEM medium was prepared and filtered through 0.22 μ m to remove undissolved crystals NR. Two hundred fifty microliters of the NR working solution was added to each well and incubated at 37 °C for 3 h. After that, NR solution was aspirated and cells were washed in the wells by warm (37 °C) PBS solution (250 μ l). A mixture of 1% acetic acid, 49% H₂O, and 50% ethanol (100 μ l) was added to each well and incubated in the dark at 150–200 rpm/min during 20 min. The absorbance was measured at 520 nm.

Preparation of the cell homogenates

The cell homogenates (control and after SDS treatment) were prepared by sonication of the suspension at + 4 °C following the program for the ultrasonic BANDELIN Sonopuls HD 2070 instrument: 2 cycles for 50 s with a 5-s interval. The resultant ultrasonic treatment included 20 active seconds.

Total protein concentration of HaCaT cell samples was determined with 2,2-bicinchoninic acid (Walker 1994), using bovine serum albumin (BSA) as a standard.

In-solution tryptic digestion

The pair of HaCaT samples (175 μ g of protein) for each cell type (untreated and SDS-exposed) was processed in-solution digestion as described previously (Petushkova et al. 2017). In brief, alkylation was performed with vinylpyridine. A 9.9- μ l aliquot of 200 ng/ μ l trypsin solution was added to the sample and incubated at 44 °C for 2 h, after which another 11.4 μ l of trypsin was added, and the solution was incubated at 37 °C for an additional 2 h, then the enzymatic digestion was stopped by the addition of 9.6 μ l of formic acid. Peptides were collected by centrifugation (15 min). The samples were immediately analyzed in triplicate by LC-MS/MS.

LC-MS/MS analysis

Separation and identification of the peptides were performed on Ultimate 3000 nano-flow HPLC (Dionex, USA) connected to Orbitrap Exactive (Thermo Scientific) mass spectrometer equipped with a Nanospray Flex NG ion source (Thermo Scientific). One microliters of peptide solution was carried out on a RP-HPLC column Zorbax 300SB-C18 (C18 particle size of 3.5 μ m, inner diameter of 75 μ m, and length of 150 mm) using a linear gradient from 95% solvent A (water, 0.1% formic acid)

and 5% solvent B (water, 0.1% formic acid, and 80% acetonitrile) to 60% solvent B over 90 min at a flow rate of 0.3 μ l/min.

Mass spectra were measured in the positive ion mode. Data was acquired in the Orbitrap Q-Exactive analyzer with resolution of 70,000 (at m/z 400) for MS and 15,000 (m/z 400) for MS/MS scans. Survey MS scan was followed by MS/MS spectra acquisition for the ten most abundant precursors. For peptide fragmentation, higher energy collisional dissociation (HCD) was used, the signal threshold was set to 17,500 for an isolation window of 1 m/z, and the first mass of HCD spectra was set to 100 m/z. The collision energy was set to 35%. Fragmented precursors were dynamically excluded from targeting for 10 s. Singly charged ions and ion with not defined charge state were excluded from triggering MS/MS scans. Three independent LC-MS/MS runs were performed for each sample.

Mass spectrometry measurements were performed using the equipment of “Human Proteome” Core Facilities of the Institute of Biomedical Chemistry (Russia).

Data processing

LC-MS/MS runs in raw format were carried out for untreated and SDS-exposed HaCaT cells and processed separately or jointly (control vs SDS-exposed) using Progenesis LC-MS software (Nonlinear Dynamics Ltd.), where raw files were converted into Mascot generic format (mgf) containing feature list for protein identification by using Mascot software (www.matrixscience.com). Mascot data in xml formats were re-imported into Progenesis LC-MS for convenient work with identified protein list.

Protein identification in Mascot software was performed with decoy (Elias and Gygi, 2007) against SwissProt (SP, 2012_11 version, .fasta format) for *Homo sapiens*. Trypsin was specified as the proteolytic enzyme, and up to one missing cleavages were allowed. Pyridylethylation (C) was used as a static modification. Oxidation of methionine was set as a variable. Charge states of + 2, + 3, and + 4 were taken into account for parent ions. Mass tolerance was set to \pm 15 ppm for parent ion masses and \pm 0.01 Da for fragment ion masses. Maximum false discovery rate (FDR) = 1%. The peptides identified in Mascot software with significance index (SI) > 13 were considered significant.

Overrepresentation enrichment analysis was performed using WebGestalt resource (Wang et al. 2013). The analysis was performed separately for upregulated and downregulated proteins with a fold change greater than 1.5 against biological processes of Gene Ontology (Ashburner et al. 2000) and KEGG pathways (Kanehisa et al., 2017). Multiple correction adjustment was done using Benjamini-Hochberg procedure with FDR < 0.05. Resulting lists of enriched Gene Ontology categories

were visualized with the help of REViGO (Supek et al. 2011). Enriched KEGG (Kyoto Encyclopedia of Genes and Genomes, <http://www.genome.ad.jp/kegg/>) pathways were colorized according to protein fold change using Pathview package (Luo and Brouwer, 2013).

Results

Cytotoxicity of SDS in HaCaT cells as determined by MTT and NRU assays

Changes in viability of immortalized HaCaT keratinocytes when exposed to different doses of SDS for 48 h were studied using light microscopy, MTT assay, and NR uptake test. It was shown that HaCaT cell line is characterized by a dose-dependent decrease in viability when exposed to sodium dodecyl sulphate. The values of these parameters, measured by different methods (using the MTT and the NRU assays), are similar (Fig. 1) and were in accordance with microscopic observations (Fig. 2).

Considering the results presented in Fig. 1 for following proteomic analysis, the 25 µg/ml SDS concentration was selected. Morphological changes of HaCaT cells after incubation with SDS at concentration 25 µg/ml are illustrated at Fig. 2. This concentration did not cause any essential effect on cell morphology (Fig. 2b) in comparison with control (Fig. 2a).

Analysis of HaCaT cell proteome

Here, we employed human HaCaT cells as a model system to identify cellular proteins that accompany SDS-induced changes due to subtoxic concentration based on a proteomic approach. Protein extracts of the control HaCaT cells and keratinocytes exposed to SDS were obtained and analyzed by LC-MS/MS. The examination of HaCaT keratinocytes proteome was performed by

Progenesis LC-MS software (Nonlinear Dynamics Ltd.), where two peak lists (separately for unexposed and exposed to SDS cells) were generated and exported for a Mascot MS/MS search. A total of 4183 peptides were revealed in control cells, which represented 771 proteins (Additional file 1: Table S1; 0.73% false discovery rate using a decoy database from 78859 spectra). The SDS-exposed group yielded 4383 peptides and 795 proteins (Additional file 2: Table S2; 1.0% FDR using a decoy database from 56030 spectra). 62.5% of all protein identifications in control and 58% after treated with SDS were made by two or more high-quality peptides (i.e., peptides automatically selected for identification and quantification by Progenesis LC software), and others were made by one peptide or did not have some high-quality peptides. A total of 568 proteins were common; 203 and 227 proteins were detected only in control cells and after treatment with SDS, respectively.

Proteomic analysis showed that proteins identified in SDS-exposed keratinocytes are predominantly localized in mitochondrion (Fig. 3) such as isocitrate dehydrogenase, cytochrome c1, ATP-specific succinyl-CoA ligase, and carnitine O-acetyltransferase. Meanwhile, proteins, which were found in control cells, are mainly located in a small region on the cell surface, which anchors the cell to the extracellular matrix (Fig. 3), e.g., testin, desmoglein-3, Na/K-transporting ATPase beta-1, and tenascin-X.

Identification of proteins with differential abundance in HaCaT keratinocytes exposed to SDS

For the comparison of the protein pattern of control and SDS-treated HaCaT cells, 131659 MS/MS spectra were collected. Raw data obtained by LC-MS/MS were analyzed by Progenesis LC-MS software where a single mgf file containing feature list for protein identifications of both control and SDS-treated cells was generated. Proteins with > 2-fold changes were regarded as being differentially altered in response to SDS exposure. As a result, a total of 380 changed proteins (Additional file 3: Table S3) were detected between the control and SDS-treated cells, of which 349 and 41 were found to be up-regulated and downregulated, respectively. The most important up- and downregulated proteins observed after SDS treatment are listed in Table 1.

We detected significant growing of keratin 14 (5.4-fold) and cell division control protein 42 homolog (4.2-fold) in 48 h SDS-treated cells that indicated the alterations in the proliferation and differentiation status. Among proteins with upregulated abundance due to SDS were proteins, which plays a role in the activation of procarcinogens (alcohol dehydrogenase), in regulation of apoptotic process (glutathione S-transferase P, calreticulin, cytochrome c, and ADP/ATP translocase 3). Besides, our analysis revealed increased abundances of fructose-bisphosphate

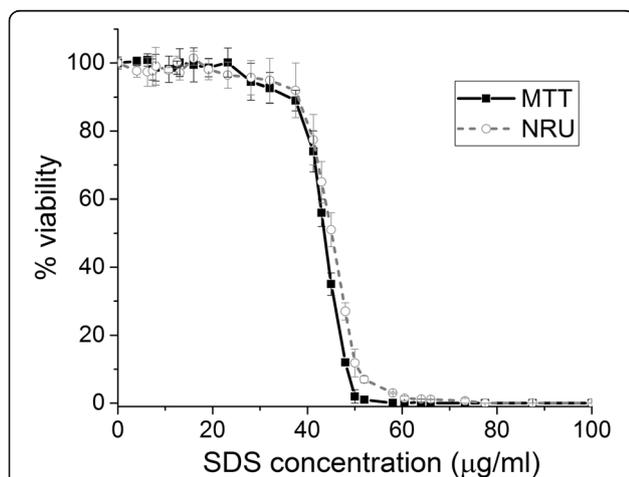


Fig. 1 Comparison of MTT and neutral red (NR) assays in HaCaT cells after exposure to SDS for 48 h; data presented as percentage of control ($n = 3$) \pm standard error mean

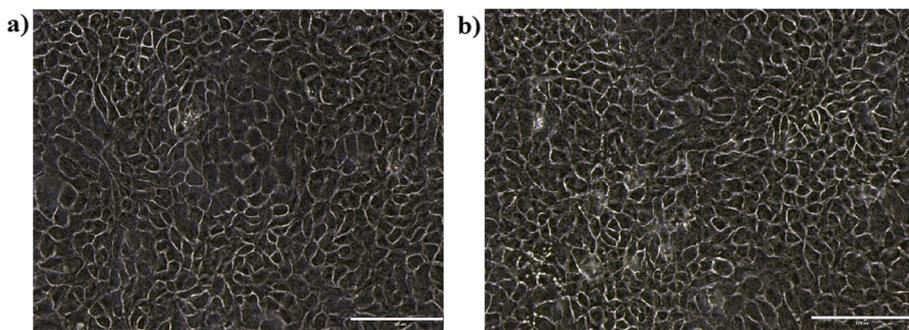


Fig. 2 Microscopic images of HaCaT cells in control (a) and after 48 h exposure with 25 µg/ml SDS (b)

aldolase C (6.4-fold) and alpha-enolase (3.0-fold) that are involved in glycolysis/gluconeogenesis. As followed from Additional file 1: Table S1, SDS-exposed HaCaT cells also demonstrate the higher level of glucose-6-phosphate isomerase and phosphoglycerate mutase 1.

In order to characterize the effects of SDS in terms of biological processes, we conducted GO enrichment analysis of up- and downregulated proteins using the Web-Gestalt website [http://www.webgestalt.org]. We found that upregulated proteins (Fig. 4) were overrepresented in such categories as glucose 6-phosphate and pyruvate metabolism (GO:0051156 and GO:0006090), cell junction organization (GO:0034330), skin development (GO:0043588), response to fibroblast growth factor (GO:0071774), and regulation of apoptotic signaling pathway (GO:2001233). These data suggest the main processes affected in HaCaT cells during the treatment of SDS are upregulation of cellular metabolism.

Pathway analysis of proteins with differential altered abundance upon SDS treatment

We also performed overrepresentation analysis against KEGG pathways. We found that glycolysis/gluconeogenesis KEGG pathway (hsa00010) was noticeably enriched with upregulated proteins (FDR = 4E-5, see Fig. 5). This suggests that these processes are perturbed in HaCaT cells during the treatment of SDS. Within the glycolytic pathway, we detected that the average normalized abundances of fructose-bisphosphate aldolase (EC 4.1.2.13), glyceraldehyde -3-phosphate dehydrogenase (EC 1.2.1.12), glucose-6-phosphate isomerase (EC 5.3.1.9), alpha-enolase (EC 4.2.1.11), and pyruvate kinase PKM (EC 2.7.1.40) were ≥ 1.5-fold higher in SDS-exposed keratinocytes compared to control cells (Table 1). Despite the fact that in the current study we did not consider the increase of proteins < 1.5-fold, it should be noted that there is a slight rise (1.4-fold) of triose-phosphate isomerase (EC 5.3.1.1) content in HaCaT cells

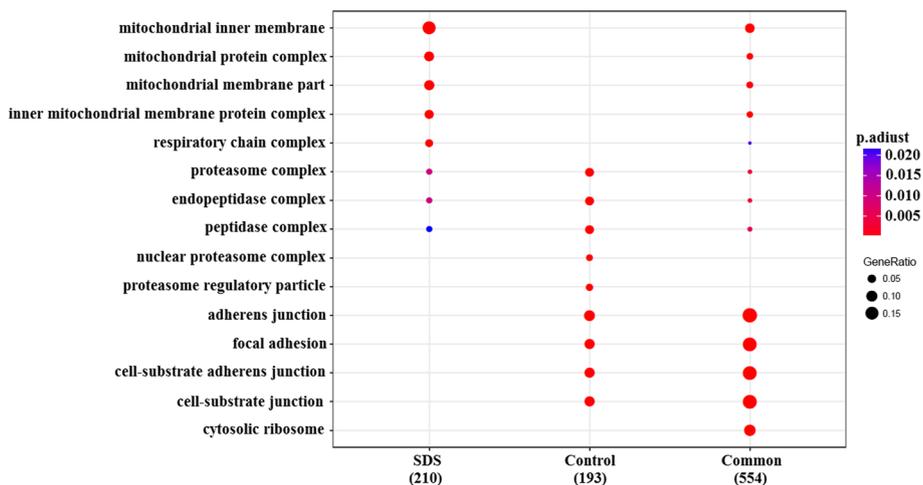


Fig. 3 Gene Ontology (GO) overrepresentation analysis of proteins identified during SDS treatment, in control samples and in both lists. Each significantly enriched GO term (cellular component) is represented as a circle. Circle size and color encode the number of genes associated with the specific term and the *p* value of enrichment significance

Table 1 The list of top upregulated and downregulated proteins after SDS treatment (25 µg/ml)

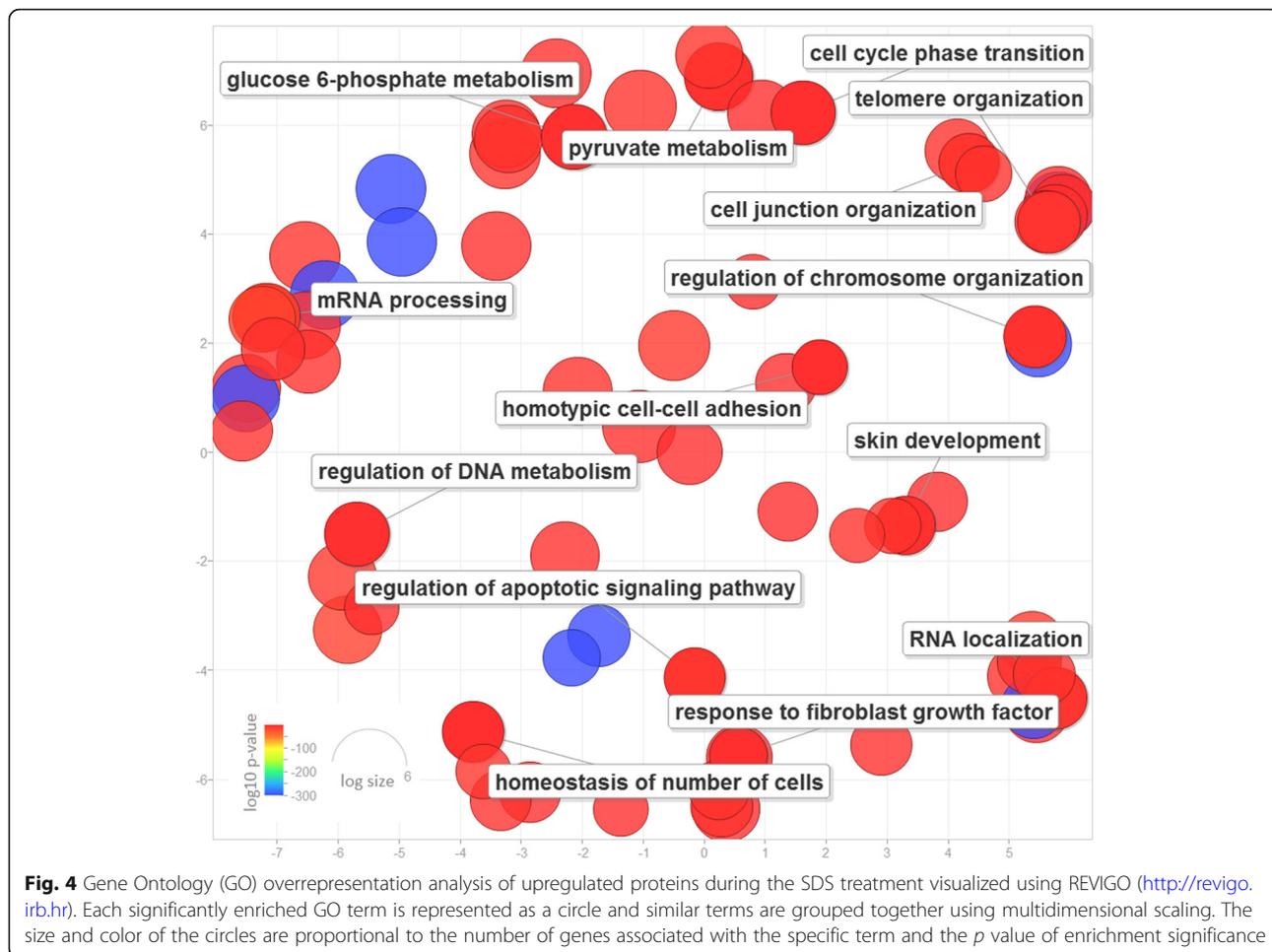
##	Accession	Description	Gene symbol	Peptides*	Fold change	GO—molecular function/biological process**
1	P09972	Fructose-bisphosphate aldolase C (ALDOC_HUMAN)	ALDOC	4	6.4	Glycolysis/gluconeogenesis
2	P04406	Glyceraldehyde-3-phosphate dehydrogenase (G3P_HUMAN)	GAPDH	18	5.9	Apoptosis, glycolysis
3	P07954	Fumarate hydratase, mitochondrial (FUMH_HUMAN)	FH	5	14.3	Tricarboxylic acid cycle
4	P06748	Nucleophosmin (NPM_HUMAN)	NPM1	5	5.7	Positive regulation of cell proliferation, Response to stress
5	P02533	Keratin, type I cytoskeletal 14 (K1C14_HUMAN)	KRT14	8	5.4	Keratinization, epidermis development
6	P09211	Glutathione S-transferase P (GSTP1_HUMAN)	GSTP1	10	5.2	Negative regulation of apoptotic process, detoxification of reactive oxygen species
7	P61981	14-3-3 protein gamma (1433G_HUMAN)	YWHA3	3	5.2	Regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway
8	O75367	Core histone macro-H2A.1 (H2AY_HUMAN)	H2AFY	5	4.8	Regulation of response to oxidative stress, keratinocyte differentiation
9	P60953	Cell division control protein 42 homolog (CDC42_HUMAN)	CDC42	5	4.2	Differentiation
10	P13646	Keratin, type I cytoskeletal 13 (K1C13_HUMAN)	KRT13	17	3.0	Keratinization
11	P06733	Alpha-enolase (ENOA_HUMAN)	ENO1	21	3.0	Glycolysis/gluconeogenesis
12	P31930	Cytochrome b-c1 complex subunit 1, mitochondrial (QCRI_HUMAN)	UQCRC1	8	2.9	Electron transport, respiratory chain
13	P25705	ATP synthase subunit alpha, mitochondrial (ATPA_HUMAN)	ATP5A1	18	2.8	ATP synthesis, hydrogen ion transport, response to oxidative stress
14	P27797	Calreticulin (CALR_HUMAN)	CALR	7	2.6	Regulation of apoptotic process
15	P09758	Tumor-associated calcium signal transducer 2 (TACSD2_HUMAN)	TACSD2	8	2.2	Response to stimulus
16	P29590	Protein PML (PML_HUMAN)	PML	4	2.0	Negative regulation of cell growth and translation in response to oxidative stress
17	P99999	Cytochrome c (CYC_HUMAN)	CYCS	4	2.0	Apoptosis, electron transport, respiratory chain, transport
18	P31947	14-3-3 protein sigma (1433S_HUMAN)	SFN	14	1.9	Keratinocyte development
19	P00558	Phosphoglycerate kinase 1	PGK1	17	1.84	Glycolysis
20	P14550	Alcohol dehydrogenase [NADP(+)] (AK1A1_HUMAN)	AKR1A1	2	9.7	Plays a role in the activation of procarcinogens and in the metabolism of various xenobiotics and drugs
21	P30048	Thioredoxin-dependent peroxide reductase, mitochondrial (PRDX3_HUMAN)	PRDX3	2	3.4	Detoxification of reactive oxygen species
22	P09758	ADP/ATP translocase 3 (ADT3_HUMAN)	SLC25A6	2	1564	Apoptosis
23	P06744	Glucose-6-phosphate isomerase (G6PI_HUMAN)	GPI	11	1.7	Glycolysis/gluconeogenesis
24	P18669	Phosphoglycerate mutase 1 (PGAM1_HUMAN)	PGAM1	8	1.6	Glycolysis
25	O9P258	Protein RCC2 (RCC2_HUMAN)	RCC2	4	- 1.8	Cell cycle, cell division, mitosis
26	O60437	Periplakin (PEPL_HUMAN)	PPL	5	- 2.0	Keratinization
27	P41252	Isoleucine-tRNA ligase, cytoplasmic (SYIC_HUMAN)	IARS	3	- 2.0	Protein biosynthesis
28	O60716	Catenin delta-1(CTND1_HUMAN)	CTNND1	11	- 2.3	Cell adhesion

Table 1 The list of top upregulated and downregulated proteins after SDS treatment (25 µg/ml) (*Continued*)

#	Accession	Description	Gene symbol	Peptides*	Fold change	GO—molecular function/biological process**
29	O14896	Interferon regulatory factor 6 (IRF6_HUMAN)	IRF6	3	- 2.3	Keratinocyte differentiation
30	Q9UIV8	Serpin B13 (SPB13_HUMAN)	SERPINB13	5	- 2.4	Regulates transcription of genes involved in differentiation of keratinocytes
31	Q9BS26	Endoplasmic reticulum resident protein 44 (ERP44_HUMAN)	ERP44	5	- 4.2	Stress response
32	O43809	Cleavage and polyadenylation specificity factor subunit 5 (CPSF5_HUMAN)	NUDT21	2	- 4.8	Differentiation, mRNA processing

*peptide number automatically selected for identification and quantification by Progenesis LC software

**Adapted from UniProt Knowledgebase



after exposure to SDS that may indicate the upregulation of this enzyme upon such toxicant.

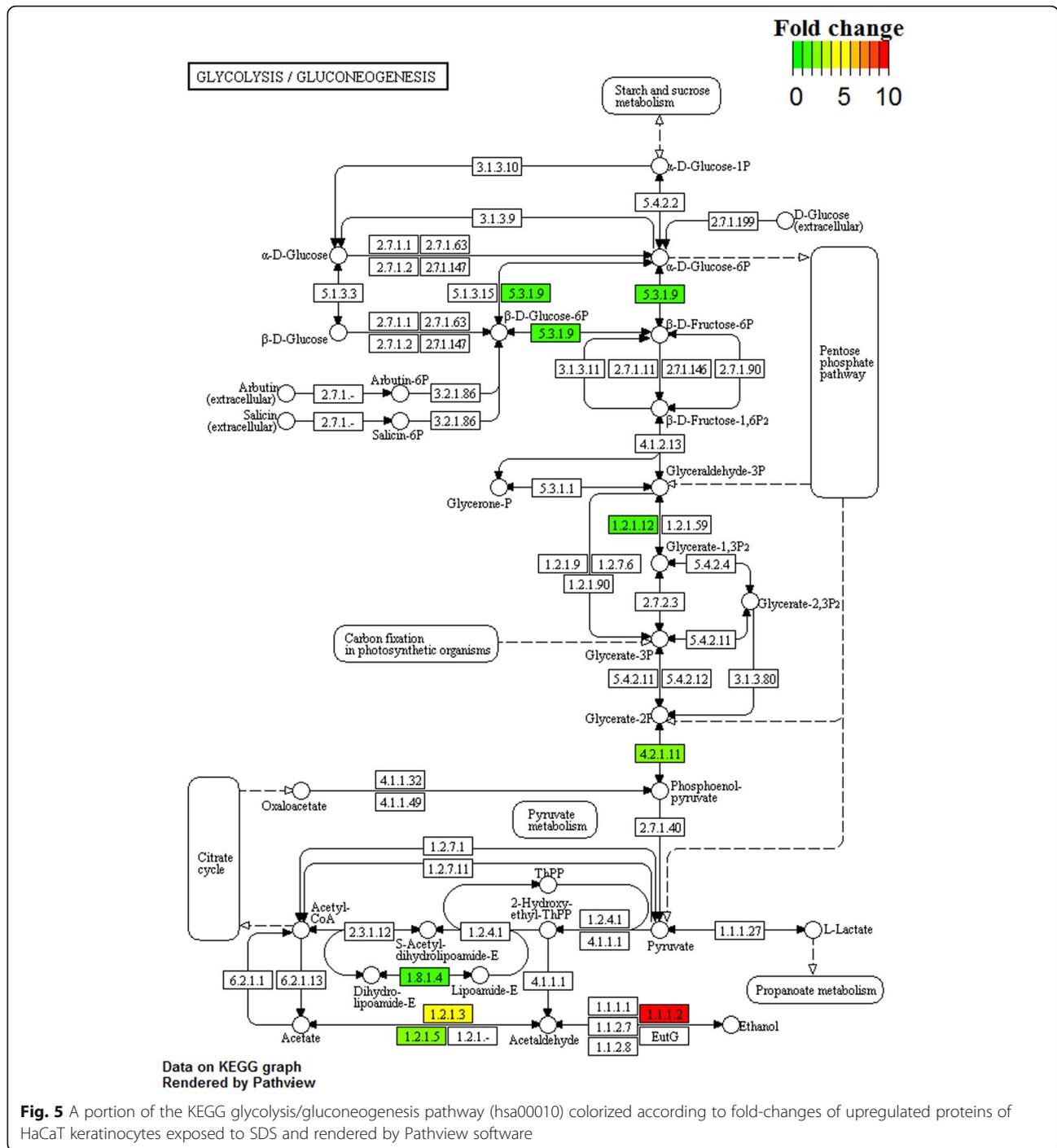
Discussion

Skin diseases are one of the most common human illnesses. The International Classification list of human disease contains more than 1000 skin or skin-related disorders. Widespread of skin diseases is caused with the variety of skin functions, the features of its structure, and exposure with a large number of external factors including dermatologic, cosmetic, and pharmaceutical products (Sprengrer et al. 2010).

Application of standard irritants such as SDS has been usually used as a model to study cytotoxic damage to epidermal cell populations due to detergents (Le et al. 1996). SDS is an anionic surfactant which is widely used as an additive to cleaning products and cosmetics. Concentration of SDS varies in the ranges from 0.01 to 50% in cosmetic products and 1 to 30% in cleaning products (Bondi et al. 2015). In the present research, we used an SDS concentration of 25 µg/ml—approximately 0.0025%—which is lower

than typically used in cosmetics. We used this concentration, because as determined by the MTT assay and microscopic examination during 48 h, it is a non-toxic dose even though this concentration causes the alteration of protein levels. It should also be noted that when applied to the skin, SDS first interacts with the stratified layer of the epidermis, which prevents its penetration to the underlying nucleated keratinocytes. And in this way, in order to have a toxic effect on the cells in the skin, a significantly higher concentration of SDS is required than in the case of exposure to cell cultures. In this study, we worked with a HaCaT cell monolayer, and this model does not reproduce the 3D structure of the normal skin.

The spontaneously immortalized HaCaT cell line has been a widely employed keratinocyte model for biological and medical research due to its ease of handling and unlimited growth and near normal phenotype (Deyrieux and Wilson 2007; Sprengrer et al. 2010). There are a lot of evidences that keratinocytes are important regulatory and effector cells in skin diseases involving the epidermis as a particular feature (Werfel 2009).



Increasing keratinocyte proliferation in the basal cell layer and in the immediate suprabasal layers and changes in epidermal differentiation is often observed in response to damage (inflammation) (Fuchs and Raghavan 2002). The imbalance between keratinocyte proliferation, differentiation, and death can result in pathological changes. For example, in epidermal acanthosis, there was

hyperproliferation inducing and reducing the differentiative process (Luzgina et al. 2009). Recently, SDS induction of an inflammatory response in keratinocytes and alteration of the mRNA expression of important barrier lipid enzymes and markers of keratinocyte differentiation have been reported (Törmä et al. 2006). By NR uptake, it was established that there is a strong correlation between in vitro cytotoxicity in a

human keratinocyte line (HaCaT) and in vivo human data for a homologous series of anionic surfactants (Wilhelm et al. 2001).

Here, we studied the reaction pattern of human skin to 48 h exposure of 2.5% SDS used as an in vitro model HaCaT keratinocytes. By MTT assay and NR uptake test, we detected that SDS at concentration 25 µg/ml did not cause any essential effect on cell morphology in comparison with control (Fig. 1). Both methods showed that exposure of HaCaT cells to SDS at this concentration corresponded to > 90% cell viability. The data obtained is comparable with similar studies of HaCaT sensitivity to SDS in a neutral red uptake cytotoxicity assay (Olschläger et al. 2009).

The examination of the HaCaT cell proteome changes induced by relatively long (during 48 h) exposure to the skin irritant SDS was performed by Progenesis LC-MS software (Nonlinear Dynamics Ltd.). The pooling method was used for increasing the reproducibility of different sample preparation steps of the three biological replicates for each type of keratinocyte samples (control and after SDS-treatment). In addition, pooling of biological replicates has the potential to reduce biological variability by measuring the average change instead of individual change (Kennedy and Xiangqin 2011). Two peak lists (unexposed and exposed to SDS cells) were generated and exported for a Mascot MS/MS search. In total, 962 proteins were detected in HaCaT cells, and this number of identified proteins is comparable with similar studies using shotgun proteomics (Udensi et al. 2014).

For HaCaT keratinocytes proteome analysis, we selected only identifications, which were made by multiple peptides (≥ 2) with Mascot score ≥ 30 . Proteins matched by one unique peptide were also considered when could be identified in all replicates. For example, TRAP1 was identified by one high-quality peptide both in all control and in all SDS-treated samples; the score value was about 100 in both types of HaCaT cells (Additional file 1: Table S1 and Additional file 2: Table S2). However, this protein plays an important role in negative regulation of mitochondrial respiration and modulating the balance between oxidative phosphorylation and aerobic glycolysis (Sciavovelli et al. 2013). As shown in Additional file 3: Table S3, TRAP1 demonstrates the tendency to increasing its content due to SDS treatment (Additional file 3: Table S3).

Among all identified proteins, 41 proteins including desmoplakin, keratins 7 and 10, epiplakin, envoplakin, and involucrin were assumed specific to keratinocytes. The majority of them were quantified, but some proteins (for example, keratins 3, 12, 22, 23, 73, 75, and 79) did not contain high-quality peptides.

LC-MS/MS analyses showed that HaCaT cells (Additional file 1: Table S1 and Additional file 2: Table S2) contain several members of the 14-3-3 family of proteins including

1433S, which regulates protein synthesis and epithelial cell growth. Proteins involved in anti-oxidant defense such as peroxiredoxin and thioredoxin or specifically expressed during cornification (for example, periplakin, plakophilin) also were observed.

Immortalized cell lines (especially the skin keratinocyte lines) have been successfully used to elucidate further stages of the transformation process and considered more relevant as model systems for human skin carcinogenesis than rodent in vivo or in vitro systems (Deyrieux and Wilson 2007). This model is based on the spontaneously immortalized keratinocyte cell line HaCaT, which escaped cellular senescence in vitro but remained non-tumorigenic over extended culture periods (Deyrieux and Wilson 2007).

SDS is not a known carcinogen, according to the American Cancer Society, but some studies show that it may cause the molecular changes involved in epithelial pathologies (Bondi et al. 2015). However, repeated exposure of the skin to irritants such as SDS may lead to an inflammatory response (Le et al. 1996; Fletcher and Basketter 2006). The inflammation in turn can promote tumorigenesis (Darido et al. 2016).

The hallmarks of cancerous cells are their ability to escape apoptosis, sustain an active cell proliferation, and eventually invade other tissues. Induction of apoptosis and/or reducing the cell proliferation in cancer cells could retard the cancerous tissue from developing and cure the patient (Kalvik and Arnesen 2013). GO enrichment analysis (Fig. 4) demonstrated that regulation of apoptotic signaling pathways and activation of mRNA processing were prevailed in HaCaT keratinocytes in response to SDS exposure.

In order to identify differentially abundant proteins, the same MS/MS spectra (raw files) obtained for control and SDS-exposed HaCaT were merged into a single mgf file using the software Progenesis LC-MS. We identified 131 upregulated proteins and 86 downregulated proteins for SDS treatment of HaCaT keratinocytes cells.

Recently, it was shown that down-modulation of interferon regulatory factor 6 (IRF6) counteracts differentiation of primary human keratinocytes in vitro and in vivo, promoting ras-induced tumor formation (Restivo et al. 2011). Furthermore, the manifestation of hyperplastic features in epidermis surrounding nodular melanoma due to the loss of keratin 10 was detected (Kodet et al. 2015). According to the data in Additional file 3: Table S3, average normalized abundance of keratinocyte-specific protein IRF6 had decreased in SDS-exposed cells compared to its content in control HaCaT.

In this study, we observed slight upregulation (1.88 times) of 14-3-3 protein beta/alpha (1433B) (Additional file 3: Table S3). This protein belongs to the highly conserved 14-3-3 protein family, which has been tightly

integrated into the core phospho-regulatory pathways that are crucial for normal growth and development and that often become dysregulated in human disease states such as cancer (Morrison 2009). For example, Minamida et al. (2011) observed overexpression of this protein in cyst fluid from cyst-associated with renal cell carcinoma (RCC) and suggested that its differential expression makes it a potential urinary biomarker for RCC. Udensi et al. (2014) found dysregulation of the isoform Long of 14-3-3 protein beta/alpha in HaCaT cells treated with arsenic trioxide (8 passages). Based on this data, the authors supposed involvement of this protein in HaCaT cells carcinogenesis.

It is well known that alterations in cell metabolism may accompany tumorigenesis. The characteristic metabolic sign of tumor metabolism is enhanced aerobic glycolysis in the cytosol or the Warburg effect (Warburg 1956). Moreover, the correlation between glycolytic ATP production and tumor malignancy has been reported (Simonnet et al. 2002). Thus, the increasing level of the glycolysis/gluconeogenesis pathway in HaCaT keratinocytes may indicate that SDS was associated with tumor progression. Besides, recently, we identified about 40 proteins that were associated with oxidative stress according classification of biological processes using the GO and the concentration of nine proteins increased ≥ 2 times (Petushkova et al., 2017). Shi et al. (2009) found that tumor cells activate glycolysis through cellular oxidative stress and this specific mechanism could be exploited to selectively kill tumor cells.

The current strategy in biomarker discovery by MS-based proteomics involves analysis of a small number of patients and controls in great depth with low-throughput methods. This approach can provide candidate protein biomarkers related to the diseases. However, resulting potential biomarkers must be then validated with targeted MS-based methods or classical immunoassays in much larger cohorts (Anderson 2014). Thus, biomarker discovery remains a difficult task also related to the complexity of the samples and the dynamic concentration of proteins. Nowadays, “what could happen”, as written in the genome, is replaced by “what is happening” in the world of the proteins and means which of the identified proteins are mostly involved in pathways and activities related to disease including cancer (Anderson 2014; Rezaei-Tavirani et al. 2017). For example, Rezaei-Tavirani et al. (2017) offers the use of a protein panel, resulting due to protein-protein interaction analysis. The authors proposed that for each disease, there are specific related pathways, and analysis for altered proteins can provide essential evidences that confirm the crucial roles of this protein panel or biomarker panel in cancer, for example gastric adenocarcinoma.

A pathway analysis of upregulated proteins revealed that the most significant feature of SDS treatment was

the glycolysis/gluconeogenesis pathway (Fig. 5). Within the glycolytic pathway, we found that the average normalized abundances of glyceraldehyde-3-phosphate dehydrogenase (G3P, EC 1.2.1.12), glucose-6-phosphate isomerase (EC 5.3.1.9), phosphoglycerate kinase (EC 2.7.2.3), and alpha-enolase (ENOA, EC 4.2.1.11) were two and more times higher in SDS-exposed keratinocytes compared to control cells. Liu et al. (2017) recently showed the upregulation of G3P in colon cancer and suggested its important role in promoting cancer metastasis. ENOA is upregulated at the mRNA and/or protein level in several tumors including breast, lung, prostate, and pancreas and also expressed on the cell surface of several tumors, where it acts as a plasminogen receptor and contributes to cell invasion, metastasis, and inflammatory responses (Capello et al. 2016).

Conclusions

Regulatory changes of protein contents or enzyme activities are one of the biochemical features of diseases. Analysis of the ups and downs of determined protein set may be a useful tool to reveal the important role of these proteins in advances of the diseases. The findings of the present study showed a significant alteration of abundance of most targeted proteins in HaCaT cells exposed with SDS compared with control. Among proteins with altered abundance were keratinocyte-specific proteins, namely interferon regulatory factor 6, 1433S. The upregulation of five proteins controlled glycolysis also was observed. The results of this study lead us to speculate about the increasing of oncogenic potential of immortalized HaCaT cell line due to 48 h SDS treatment, and such could serve as a basis for using HaCaT cells for various applications, for example using revealed biomarker panel for the detection the important aspects of cancer development.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s41702-019-0054-y>.

Additional file 1: Table S1. The list of proteins identified in control HaCaT cells

Additional file 2: Table S2. The list of proteins identified in keratinocytes HaCaT exposed to SDS

Additional file 3: Table S3. Identification of proteins with differential abundance in HaCaT keratinocytes exposed to SDS

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Authors' contributions

NAP analyzed and interpreted the proteomic data and was a one of two major contributors in writing the manuscript. ALR did cell culture experiments, interpreted proteomic data, and was a one of two major contributors in writing the manuscript. MAP interpreted the proteomic data. OVL made sample preparation for MS experiments and analyzed the

proteomic data. VGG performed MS experiments. AVL interpreted the proteomic data. NGL did the cell culture experiments and interpreted the proteomic data. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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