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# Insight into the Mechanism of SDS Irritation on Human Skin Keratinocytes by Examination of Changes in Gene Expression

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## Abstract

Sodium dodecyl sulfate (SDS) is widely used as an irritant. Inflammatory and immune related cytokines/ chemokines are released by keratinocytes following SDS irritation. However, a specific effect of SDS on keratinocytes and the mechanism of skin irritation caused by SDS have not been investigated. To explore the irritant mechanism of SDS on keratinocytes, a gene microarray was used to detect the changes in gene expression after treating keratinocytes with SDS for different amounts of time. After 0.5 h and 1 h SDS exposure, there were changes mainly in genes in the rheumatoid arthritis pathway (CCL5, IL-6, FOS, CSF1, and HLA-DPB1) and TNF signaling pathway (TNF, CSF2, CXCL3, TNFAIP3, PTGS2, CXCL8, CCL20, and PIK3CA) to cause a pro-inflammatory reaction as well as autoimmune activation. After treating with SDS for 2 h and 4 h, there were changes in the expression of genes (LIF, PIM1, MAP3K8, CCNA1, RUNX1, and CYP1) that are related to cell apoptosis and cancerization. This was related to changes in transcriptional activity in the cancer and tryptophan metabolism pathway. Overall, SDS irritation caused different changes in gene expression over time, which altered the state of keratinocytes affecting processes of inflammation, autoimmune response, cell apoptosis, and cancerization. These results provide insight into the irritation process of SDS and provide reference for the future evaluation of skin irritation.

**Keywords:** SDS irritation, Keratinocytes, Gene microarray, Signaling pathway, Inflammation

  

## 1. Introduction

Sodium dodecyl sulfate (SDS) is an anionic surfactant that is frequently used in domestic cleaning products. However, SDS is often used as a positive control for study of the effects of skin irritation. In the human patch test, SDS caused an 84% positive response at 20% concentration [1]. Skin irritation is non-immunological and a local reversible inflammatory reaction. Once SDS comes into contact with the stratum corneum, it can disrupt the skin barrier to penetrate into the skin [2]. The structure of proteins will be damaged and new water binding sites become exposed which leads to hyper-hydration of the stratum corneum and disorganization of the lipid bilayers. The skin barrier damage triggers the activation of the innate immunity and the production of pro-inflammatory cytokines [3]. Keratinocytes constitute 95% of the epidermis cell matrix and once activated, keratinocytes play an important role in skin inflammatory and immunological reactions by the production of several cytokines and chemokines [4]. Many irritants can cause the expression of IL1A after stimulation, which subsequently induces the expression of other cytokines and chemokines such as TNF-α, IL-6, CXCL8, and others. The nature and intensity of irritation is closely related to the production of inflammatory cytokines. Mai Le reported that SDS influences keratinocytes directly by the over-expression of cell biological markers such as the up-regulation of involucrin and epidermal fatty acid binding protein [5].

However, the impact of SDS on gene expression and signaling pathway of keratinocytes remain poorly understood. Here we used a human genome expression profile microarray chip to analyze changes in gene expression to elucidate the mechanism of SDS irritation.

## 2. Materials and Methods

### 2.1 MTT assay

The primary human epidermal keratinocytes (HEKa) were obtained from the Beijing Forland Pharma Biological Technology Company and were extracted from adult foreskin and cultured to the third generation. The cell density of HEKa was 6000 cells/well with 90 μl culture medium (Epilife, Gibco, USA) per well. 10 μl of different concentration of SDS (Sigma-Aldrich Chemicals, reagent grade, USA) were prepared in PBS and added to the plate. The final concentrations of SDS were 100 μg/ml, 33.33 μg/ml, 11.11 μg/ml，3.70 μg/ml, 1.23 μg/ml, and 0.41 μg/ml. Cells were cultured in an incubator at 37 °C with 5% $CO_2$ for 24 h. Then, 10 μl of 5mg/ml MTT (J&K, reagent grade, CN) was added to each well and the cells were cultured for another 4 h. The supernatant was removed and 150 μl DMSO (J&K, reagent grade, CN) was added to each well. After the DMSO was mixed thoroughly and formation was formed, the plate was detected at 570 nm. Cell viability was calculated by $(OD_{sample}-OD_{control})/OD_{control}\times100\%$.

### 2.2 SDS exposure and RNA isolation

According the results of MTT assay, 0.7 μg/ml, 0.55 μg/ml, and 0.4 μg/ml lead viability of keratinocytes to 75%, 80%, and 85%, respectively. Cells were cultured with SDS of 0.7 μg/ml, 0.55 μg/ml, and 0.4 μg/ml for 1 h to confirm for the best treatment concentration. Then cells were exposed to SDS for 0.5 h, 1 h, 1.5 h, 2 h and 4 h at the most appropriate concentration (0.55 μg/ml). Total RNA was isolated from the keratinocytes using EastepTM Total RNA Extraction Kit (Promega, LS1030, CN). The RNA was qualified as the ratio of $OD_{260}/OD_{280}$ and material with a ratio greater than 1.8 was used for gene expression microarray and RT-PCR.

### 2.3 DNA microarrays and data analysis

Total RNA samples were hybridized to a 8 x 60K Agilent human mRNA array. Total RNA samples were converted to cDNA using a CapitalBio cRNA Amplification and Labeling Kit (CapitalBio). cDNA labeling and hybridization were performed according to the manufacturer's instructions. The array data were analyzed for data summarization, normalization, and quality control by using the GeneSpring software V12 (Agilent). Significantly expressed genes were selected by comparison of signals. A ratio of sample/control greater than 2 was considered

  

"up-regulated," and a ratio less than 2 was considered "down-regulated".

### 2.4 Validation of oligomicroarray results using real-time PCR analysis

Gene expression microarray and pathway data were validated by quantitative real-time RT-PCR analysis. Total RNA was reverse transcribed with a cDNA synthesis kit (Takara, RR036A, CN). Real-time PCR was performed with cDNA and gene-specific primer pairs (Takara, RR820A, CN). Primers are listed in Table1.

## 3. Results

### 3.1. Cell viability

The cell cytotoxicity of SDS presented an inverse "S" curve (Figure 1). Cell viability was higher than 70% when the concentration of SDS was lower than 1.23 μg/ml. When the SDS concentration was higher than 11.11 μg/ml, the viability of keratinocytes was extremely close to zero. SDS concentrations of 0.7 μg/ml, 0.55 μg/ml, and 0.4 μg/ml showed keratinocyte viability of 75%, 80%, and 85%, respectively.

**Table 1: Primer sequences used in RT-PCR analysis**

| Gene symbol | | Primer sequence |
|---|---|---|
| ERK1 | Sense | CTACACGCAGTTGCAGTACAT |
| | Antisense | CAGCAGGATCTGGATCTCCC |
| CXCL8 | Sense | TCAGAGACAGCAGAGCACAC |
| | Antisense | GGCAAAACTGCACCTTCACA |
| IL6 | Sense | GGCAAAACTGCACCTTCACA |
| | Antisense | TTCACCAGGCAAGTCTCCTC |
| TNF | Sense | CCCTACGGGTCATTGAGAGA |
| | Antisense | GGTTGTGGACTGCCTTTTGT |
| TNFAIP3 | Sense | AGGACAGAAGAGCAACTGAGATCG |
| | Antisense | TTGGGATGCTGACACTCCATGCA |
| CSF2 | Sense | TCCTGAACCTGAGTAGAGACAC |
| | Antisense | TGCTGCTTGTAGTGGCTGG |
| IL1A | Sense | AGTAGCAACCAACGGGAAGG |
| | Antisense | AAGGTGCTGACCTAGGCTTG |

### 3.2. Cytokine release at different exposure concentration

CXCL8, IL-6, and TNF are key genes with functions that are closely related with skin inflammation and are up-regulated at inflammatory sites, so we determined the expression of these three genes by RT-PCR to determine the appropriate exposure concentration of SDS (Figure 2).

Gene expression changes with different concentrations of SDS were detected after the keratinocytes were exposed to SDS for 1 h. The relative expression of CXCL8, IL-6, and TNF were all increased in the treated cells relative to the untreated cells. The expression level was the highest at the concentration of 0.55 μg/mL, which allowed 80% viability of keratinocytes (cell viability 80, CV80).

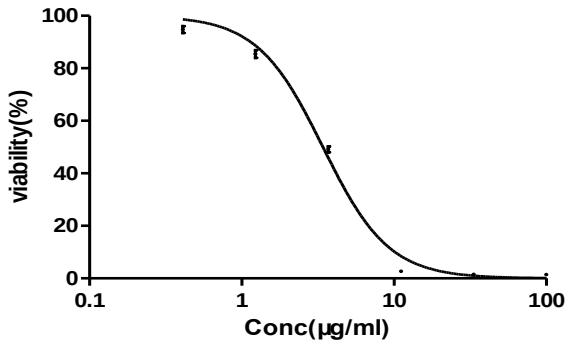


**Figure 1: Cytotoxicity of SDS at different concentrations on HEKa.** Keratinocytes were cultured in the presence of SDS (100, 33.3, 11.1, 3.70, 1.23, and 0.41 μg/mL) for 24 h. Cell viability was measured by MTT assay (n=3).

  

### 3.3. Significant signaling pathway involved in SDS irritation

Next, keratinocytes were treated with SDS at the concentration that allowed 80% viability of keratinocytes, CV80, for 0.5 h, 1 h, 1.5 h, 2 h, and 4 h. The gene expression varied with exposure time. The numbers of genes that showed over-expression after 0.5 h, 1 h, 1.5 h, 2 h, and 4 h of treatment were 930, 605, 788, 528, and 353, respectively, and the numbers of genes showing down-regulation were 337, 368, 336, 389, and 375, respectively. Pathway analysis was then performed for the significant genes and the KEGG pathways were used for reference. The pathways that were significantly affected by SDS are listed in Table 2 ($P$<0.01).

Gene expression patterns changed in the different SDS irritation stages, which led to changes in the signaling pathway. Rheumatoid arthritis was the main signaling pathway affected after 0.5 h exposure, and the TNF signaling pathway was the most significant after 1 h and 1.5 h irritation. The rheumatoid arthritis pathway also participated in gene regulation during this time stage. After 2 h and 4 h of SDS irritation, there were significant changes in the transcriptional levels in cancer and tryptophan metabolism pathways. The TNF signaling pathway and tryptophan metabolism showed changes after 2 h irritation, and the tryptophan metabolism pathway was predominant at the later irritation stage. The TNF signaling pathway and rheumatoid arthritis showed changes in gene expression at three time stages, suggesting these are the main signaling pathways during the initial and metaphase of the SDS irritation process. As the NF-kappa B and MAPK signaling pathways are within the TNF signaling pathway, activation of the TNF signaling pathway may lead to activation of these two pathways. The NF-kappa B pathway plays a role in immune regulation, inflammation, and cell survival. The MAPK pathway participates in cell proliferation, differentiation, and migration and leads to cell apoptosis and necroptosis. Another affected pathway is the rheumatoid arthritis pathway that shows strong activity in the disease of rheumatoid arthritis (RA). RA is a chronic autoimmune disorder disease characterized by increased inflammation. During the initial stage of SDS stimulation, the significant pathways are closely related to the inflammation response, and in the later stage, the pathways are mainly related with cancer and tryptophan metabolism.

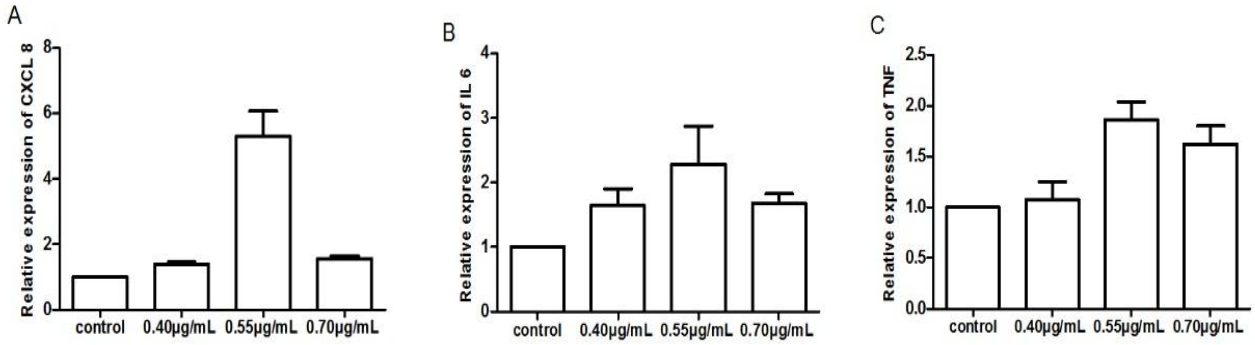


**Figure 2: RT-PCR analysis of gene expression.** A: CXCL8; B: IL-6; C: TNF. RT-PCR was performed to measure the expression of CXCL8, IL-6, and TNF in control un-treated keratinocytes and keratinocytes treated with SDS (0.4, 0.55, and 0.7 μg/ml).

### 3.4. Effect of SDS irritation on gene expression

There were 19 genes that showed significant changes in expression for at least two time points and these were selected as key genes for additional analysis. The genes were classified by the initial expression time. There were 5, 8, 5 and 1 genes initially expressed at 0.5 h, 1 h, 1.5 h, and 2 h, respectively. In addition to these 19 genes, we also examined the expression of TNF, which is the initiator of the TNF signaling pathway. Thus, we analyzed the expression of 20 key genes including TNF. Of these 20 genes, 13 genes are part of the

  

TNF signaling pathway, CSF1, CCL5, IL6, FOS, CSF2, CXCL3, PIK3CA, PTGS2, TNFAIP3, TNF, CCL20, LIF, and MAP3K8. An additional 11 genes participated in the RA pathway including CSF1, CCL5, IL-6, FOS, CSF2, TNF, CCL20, HLA-DPB1, HLA-DQB1, and CXCL8. There were also two genes that participate in tryptophan metabolism, CYP1B1 and CYP1A1. Two expressed genes, CCNA1 and PIM1, are in the acute myeloid leukemia pathway. As a gene may participate in multiple signaling pathways, only the primary pathways in SDS irritation were selected.

**Table 2: Signal pathways in different exposure times**

| Exposure time | Signal pathways | Differential up expressed genes | Differential down expressed genes | P-value |
|---|---|---|---|---|
| 0.5 h | Rheumatoid arthritis | CCL5, HLA-DPB1, IL6, CD86, FOS, ATP6V0E2 | CSF1 | 0.0038 |
| 1 h | TNF signaling pathway | CSF2, CCL5, CXCL3, IL6, PTGS2, TNFAIP3, TNF, CCL20 | CSF1, PIK3CA | 0.0002 |
| | Hematopoietic cell lineage | GP9, CSF2, IL6, CD1A, MS4A1,TNF, CD1B | CSF1 | 0.001 |
| | Cocaine addiction | GRIN2A,GRIN1,BDNF, DRD2, GPSM1, FOSB | | 0.0012 |
| | Rheumatoid arthritis | CSF2 ,CCL5, HLA, IL6,CXCL8 , TNF , CCL20 | CSF1 | 0.0013 |
| | NOD-like receptor signaling pathway | NLRC4,IL6,CCL5,CXCL8,TNFAIP3,TNF | | 0.0023 |
| | Legionellosis | NLRC4,CXCL3,TNF, CXCL8, IL6 | | 0.0094 |
| 1.5 h | TNF signaling pathway | CSF2,CSF1,CCL5,CXCL3,FOS,PTGS2, TNFAIP3, LIF , MAP3K8 , MAPK12, CCL20 | PIK3CA | 0.0001 |
| | Rheumatoid arthritis | CSF2,CSF1,CCL5, HLA-DPB1, CXCL8, FOS, CD80, CCL20 | HLA-DQB, HLA-DRB3 | 0.0004 |
| | Acute myeloid leukemia | RUNX1, PIM1, TCF7L2 ,MYC, CCNA1 | PIK3CA | 0.0078 |
| | NOD-like receptor signaling pathway | CCL5, NLRP3, CXCL8, TNFAIP3, MAPK12 | CARD8 | 0.0078 |
| 2 h | Transcriptional misregulation in cancer | CEBPE, RUNX1,CXCL8, DDIT3 , EYA1, GRIA3 ,IGFBP3, NR4A3,CCNA1 | ETV1, PAX7, NFKBIZ, NFKBIZ | 0.0004 |
| | Tryptophan metabolism | DDC ,CYP1A1, CYP1B1 ,HAAO | INMT | 0.0031 |
| | Jak-STAT signaling pathway | CLCF1 ,IL11,IL24 ,PIM1, IL23A, LIF | IL15RA, AKT3, PIK3CA, SPRY1 | 0.0042 |
| | TNF signaling pathway | FOS , PTGS2 ,LIF ,MAP3K8,CCL20 | AKT3, CXCL2, PIK3CA | 0.005 |
| | MAPK signaling pathway | MAP3K8, DDIT3, MAP3K13, EGF, FOS, NR4A1, FGF1, DUSP10 | RASGRF, STMN1, ELK1, RASGRP3, AKT3 | 0.008 |
| 4 h | Tryptophan metabolism | CYP1B1, CYP1A1 | AANAT, INMT | 0.0007 |

  

The 20 genes are listed in Table 3. Most of the genes showed significant increased expression when measured after 1 h, 1.5 h, and 2 h of SDS treatment. The 1.5 h time point corresponded to a "stage of high expression" as 17 genes showed significantly increased expression at this time point.

**Table 3: The key genes in significant signaling pathways**

| Entrez Gene ID | Gene symbol | Expression state | | | | |
|---|---|---|---|---|---|---|
| | | SDS 0.5 | SDS 1 | SDS 1.5 | SDS 2 | SDS 4 |
| 3569 | interleukin 6 (IL6) | up | up | / | / | / |
| 6352 | chemokine (C-C motif) ligand 5 (CCL5) | up | up | up | / | / |
| 1435 | colony stimulating factor 1 (CSF1) | down | down | up | / | / |
| 3115 | major histocompatibility complex, class II, DP beta 1 (HLA-DPB1) | up | / | up | / | / |
| 3119 | major histocompatibility complex, class II, DQ beta 1 (HLA-DQB1) | / | up | down | / | / |
| 7124 | tumor necrosis factor (TNF) | / | up | / | / | / |
| 1437 | colony stimulating factor 2 (CSF2) | / | up | up | / | / |
| 2921 | chemokine (C-X-C motif) ligand 3 (CXCL3) | / | up | up | / | / |
| 7128 | tumor necrosis factor, alpha-induced protein 3 (TNFAIP3) | / | up | up | / | / |
| 2353 | FBJ murine osteosarcoma viral oncogene homolog (FOS) | up | / | up | up | / |
| 5743 | prostaglandin-endoperoxide synthase 2 (PTGS2) | / | up | up | up | / |
| 3576 | chemokine (C-X-C motif) ligand 8 (CXCL8) | / | up | up | up | / |
| 6364 | chemokine (C-C motif) ligand 20 (CCL20) | / | up | up | up | / |
| 5290 | phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CA) | / | down | down | down | / |
| 3976 | leukemia inhibitory factor (LIF) | / | / | up | up | / |
| 5292 | Homo sapiens Pim-1 proto-oncogene, serine/threonine kinase , transcript variant 1 (PIM1) | / | / | up | up | / |
| 1326 | mitogen-activated protein kinase kinasekinase 8 (MAP3K8) | / | / | up | up | / |
| 8900 | Homo sapiens cyclin A1, transcript variant 1(CCNA1) | / | / | up | up | up |
| 1545 | Homo sapiens cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1) | / | / | / | up | up |
| 1543 | cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) | / | / | / | up | up |

### 3.5. Validation by RT-PCR

We confirmed the microarray results with RT-PCR. The RT-PCR results showed (Figure 3) that expression of CXCL8 was up-regulated at1 h and 1.5 h, the same trend that was showed by the microarray data. The relative expression of IL1A

  

determined by RT-PCR was also in agreement with the microarray data as the expression was relatively unchanged with treatment, similar to a housekeeping gene. However, a variety of reports have showed that IL1A is highly expressed in response to irritation. The explanation for these differences in results is not clear, and future investigation of IL1A expression under different conditions is warranted. The relative expression of CXCL8, TNFAIP3, CSF2, and IL1A coincided with the results from the gene microarray.

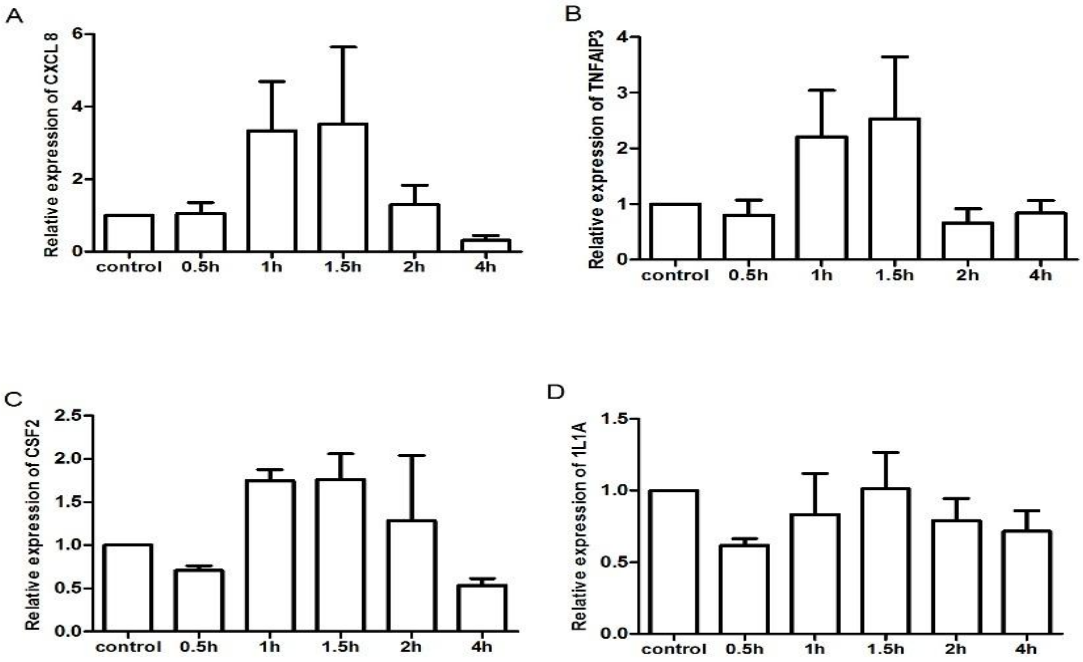


**Figure 3: Validation analysis by RT-PCR.** A: CXCL8; B: TNFAIP3; C: CSF2; D: IL 1A. Expressions of CXCL8, TNFAIP3, CSF2 and IL1A in the keratinocytes treated with SDS (0.5, 1, 1.5, 2, 4 h) were validated by RT-PCR.

## 4. Discussion

The genes that were initially expressed at 0.5 h included CCL5, IL-6, FOS, CSF1, and HLA-DPB1. These genes were all up-regulated except for CSF1, which was down-regulated at 0.5 h and1 h, and then up-regulated at 1.5 h. These genes involved in pro-inflammation, leukocyte recruitment, and apoptosis promotion. CCL5 is an important pro-inflammatory chemotactic cytokine that may drive the recruitment of leukocytes to inflammatory sites. The inhibition of CCL5 and its receptors may decrease the level of inflammation [6]. IL-6 is correlated with a variety of acute and chronic inflammatory disease and is elevated in many inflammatory states, as are TNF and IL1A. In the synovial fluids and serum of rheumatoid arthritis patients, IL-6 and IL-6 receptors levels were found to be elevated and the IL-6 level was associated with disease severity [7]. SDS irritation caused increased IL-6 at 0.5 h and 1 h. TNF was also increased when it was measured at 1 h, so IL-6 is likely playing a pro-inflammatory role in the SDS irritation process. FOS also plays a key role in the regulation of cell apoptosis. Recent research showed that FOS promotes apoptosis by inhibiting the expression of anti-apoptotic cellular-FLIP expression and regulating the endogenous apoptotic pathway [8]. ValeÂrie Mils reported that FOS increased the sensitivity of keratinocytes to apoptosis [9]. In the rheumatoid arthritis pathway, TNF and IL-6 may stimulate FOS up-regulation thus leading to inflammatory cell infiltration, inflammation. FOS was up-regulated after 0.5 h, 1.5 h, and 2 h SDS exposure. CSF1 plays a role in the etiology of inflammation through binding to the tyrosine kinase receptor CSF1R, the principal regulator of

  

the survival, proliferation, differentiation, and function of macrophages and their precursors. Samuel found that Anti-CSF1R Ab significantly reduced the production of IL-6 and other inflammatory mediator in RA synovial explants, and paw swelling and joint destruction in collagen-induced arthritis (CIA) [10]. In vitro data indicate that synovial fibroblasts and chondrocytes stimulated with TNF or IL1B can produce CSF1 [10]. SDS stimulation inhibited CSF1 expression after 0.5 h and 1 h, but expression was increased at 1.5 h. That might be caused by TNF up-regulation through the TNF signaling pathway. The up-regulation of CSF1 might exacerbate the severity of inflammation. The over-expression of HLA (HLA-DPB1 and HLA-DQB1) at 0.5 h and 1 h may activate the autoimmune system. As HLA is located in the upstream part of the rheumatoid arthritis pathways, expression changes in HLA will trigger changes in expression in downstream genes in this pathway. HLA molecules play a crucial role in the detection and elimination of virally infected cells [11], and HLA is estimated to contribute 37% to the genetic variability of rheumatoid arthritis [12]. To summarize, all of CCL5, IL-6, FOS, CSF1, and HLA showed significant changes in expression after 0.5 h of SDS stimulation and participate in the rheumatoid arthritis pathway to cause pro-inflammatory reaction and autoimmune activation.

The genes that showed changes in expression by the 1 h time point included TNF, CSF2, CXCL3, TNFAIP3, PTGS2, CXCL8, CCL20, and PIK3CA. These genes are components of the TNF signaling pathway. As a pro-inflammatory cytokine, TNF can induce the expression of cutaneous and endothelial adhesion molecules to influence the development of inflammation. TNF is the initial gene in the TNF signaling pathway, so the up -regulation of TNF leads to altered expression of several downstream genes [13]. CSF2 showed increased expression 1 h and 1.5 h after SDS exposure, and may promote inflammation and cause leukocyte activation [14]. CSF2 promotes the differentiation of macrophages and granulocytes from hematopoietic precursors, which is important for sustaining the recruitment of immature monocytes to affected tissues during inflammation. By inducing macrophages into a pro-inflammatory phenotype, CSF2 may contribute to inflammation. The expression of CSF2 is regulated by TNF and MIP-1A [15]. Exogenous CSF2 was reported to exacerbate collagen-induced arthritis and deficiency of CSF2 decreased the level of arthritis disease. CSF2 participates in the TNF signaling pathway as well as the rheumatoid arthritis pathway. CXCL3 expression was increased at 1 h and 1.5 h, which may allow leukocyte recruitment and indicate that the cell is in an inflammatory state. CXCL3 belongs to the CXC chemokine family that attracts inflammatory and structural cells to injury sites. CXCL3 is powerful neutrophil chemo attractant, and participates in angiogenesis [16]. CXCL1-3 promotes inflammatory and reparative processes [17]. The intracellular signaling molecule and core inflammatory regulator TNFAIP3 was up-regulated at 1 h and 1.5 h, likely due to induction by TNF. The up-regulation of TNFAIP3 can block activation of the NF-kappa B pathway and inhibit TNF-induced apoptosis and inflammation. A gene knockout experiment showed that a TNFAIP3-deficient mouse exhibits a spontaneous inflammation response in multiple organs [18]. Increased TNFAIP3 expression at 1 h and 1.5 h might inhibit the activation of the NF-kappa B pathway to weaken the inflammation response caused by SDS. PTGS2 plays an important role in prostaglandin synthesis, and the inhibition of PTGS2 and non steroidal anti-inflammatory drugs will block the biosynthesis of prostaglandin. Prostaglandins are closely related with the generation of the inflammatory response and contribute to the development of acute inflammation [19]. High expression of PTGS2 promotes acute inflammation by increasing the synthesis of prostaglandin. CXCL8 induces migration of neutrophils and granulocytes to the infection site and activates phagocytosis. Increased expression of CXCL8 is closely related with tissue damage and irritation. TNF and IL1A can stimulate keratinocytes to produce CXCL8. In the RA pathway, CXCL8 plays a role in promoting leukocyte migration and causes

  

inflammatory cell infiltration. Enhanced expression of CXCL8 in the 1 h, 1.5 h, and 2 h time points suggested that the cell was in the stage of inflammation and activated the autoimmune system to regulate SDS irritation [13]. CCL20, also known as Macrophage Inflammatory Protein-3, is highly expressed in the synovial fluid of RA and involved in the recruitment of leukocytes to inflamed sites. Inflammatory cytokines TNF and INF-γ can induce CCL20 expression [20]. IL-6 is induced by CCL20 with stimulation of IL17. As a result, augmented expression of CCL20 after 1 h, 1.5 h, and 2 h could promote RA inflammation. In the TNF signaling pathway, TNF can bind to TNFR2 and induce the P13K-Akt signaling pathway, which is important in regulation of the cell cycle. Activation of PIK3CA is associated with cell proliferation. PIK3CA mutants have been identified in a variety of tumor cells and are a target for tumor therapy. The decreased expression of PIK3CA reduced cell viability and promoted apoptosis. Most genes that showed changes after 1 h were members of the TNF signaling pathway. Based on the previous characterization of the functions of these genes, SDS stimulation for 1 h caused the over-expression of pro-inflammatory cytokines and reduced cell viability. The abnormal expression of chemotactic factors alters the migration of leukocytes to inflammatory sites and contributes to the pathogenesis of the synovitis in RA. However, the activation of the autoimmune response caused TNFAIP3 up-regulation and induced the anti-inflammatory effect.

Genes initially expressed after1.5h treatment included LIF, PIM1, MAP3K8, CCNA1, RUNX1, and CYP1, and most of these genes are related tocancer.LIF is an inflammatory cytokine that is expressed in the TNF signaling pathway. Many studies have showed that LIF acts in many inflammatory disorders as a pro-inflammatory factor. Increased expression of LIF was found in synovial tissues and interstitial fluid in RA patients. LIF can be expressed in human articular cartilage and synovial cells. Pro-inflammatory cytokines such as IL1A, IL-6, IL-17 and TNF can promote LIF expression and LIF is down-regulated in the presence of the anti-inflammatory cytokine IL-4. In turn, LIF can promote the production of pro-inflammatory cytokines in the synovial cell culture medium [7]. The inflammatory response was inhibiting after injection of LIF binding protein into joints of goats that were previously injected with LIF. SDS stimulation up-regulated LIF expression after 1 h and 1.5 h, which might be caused by IL-6 and TNF as their expression was increased after 1h stimulation. Increased expression of LIF might exacerbate inflammation. PIM1 is in the Jak-STAT signaling pathway, and was up-regulated after stimulation by SDS for 1.5 h and 2 h. PIM1 is a proto-oncogene that encodes serine and threonine kinases, and plays a role in anti-apoptosis. MAP3K8belongs to the serine and threonine protein kinases family, and is involved in the TNF signaling pathway. Expression of MAP3K8 may be regulated by TNF through binding to TNFR1. Several previous studies showed that MAP3K8 exhibits predominantly pro-inflammatory effects. As a tumor-promoting oncogene, altered expression ofMAP3K8 can promote tumorigenesis depending on the cancer type [21]. CCNA1, cyclin A1, is part of the acute myeloid leukemia signaling pathway, and plays a critical role in meiosis regulation. Increased CCNA1 expression was observed in prostate cancer. Metastatic growth was found in the lymph node, lung, and liver in subcutaneous and orthotropic xenograft mouse models after the induction of CCNA1 over-expression in PC3 cells. Regina reported that CCNA1 interacts with Erαto promote breast cancer progression in xenograft mouse models [22]. SDS stimulation for 1.5 h caused a high level of CCNA1 expression, which is closely related with the development of cancers. CYP1B1 and CYP1A1 belong to the cytochromeP450 super-family of enzymes and participate in the tryptophan metabolism signaling pathway. CYP1A1 and CYP1B1 are regulated by the aryl hydrocarbon (AHR) receptor, which is commonly activated after toxic exposure to increase the expression of cytochrome P450 enzymes. Thus, cytochrome P450 enzymes can be used to assess the contamination levels of various environments [23]. Additionally, the over-expression of CYP1A1 and CYP1B1 induce carcinogenicity in

  

the metabolism of exogenous xenobiotics or endogenous hormones, and these enzymes may be critical makers in diverse types of cancers. SDS stimulation for 2 h and 4 h increased the expression of CYP1A1 andCYP1B1, which might induce keratinocyte cancerization [24]. Genes that were initially expressed at1.5h and 2h were all related with cancer, and the dys-regulation of these genes increases the risk of cancerization.

## 5. Conclusions

SDS stimulation caused the up-regulation of pro-inflammatory cytokines and abnormal expression of inflammatory related chemokines through the TNF signaling pathway and RA pathway in the initial stage. Autoimmune related genes were activated in this period to modulate the inflammatory state. At the later stage of SDS stimulation, expression of inflammatory genes decreased, but genes involved in cell apoptosis and cancer showed altered expression, indicating that the keratinocytes were in a state of disorder.


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