Microarray analysis of novel genes involved in HSV-2 infection

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Research Article

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Abstract

Background: Herpes simplex virus type 2 infects the body and becomes an incurable and recurring disease. The pathogenesis of HSV-2 infection is not completely clear.

Methods: We analyze the GSE18527 dataset in the GEO database in this paper to obtain distinctively displayed genes(DDGs)in the total sequential RNA of the biopsies of normal and lesioned skin groups, healed skin and lesioned skin groups of genital herpes patients, respectively.The related data of 3 cases of normal skin group, 4 cases of lesioned group and 6 cases of healed group were analyzed.The histospecific gene analysis , functional enrichment and protein interaction network analysis of the differential genes were also performed, and the critical components were selected.

Results: 40 up-regulated genes and 43 down-regulated genes were isolated by differential performance assay.

Histospecific gene analysis of DDGs suggested that the most abundant system for gene expression was the skin, immune system and the nervous system.Through the construction of core gene combinations, protein interaction network analysis and selection of histospecific distribution genes, 17 associated genes were selected

CXCL10,MX1,ISG15,IFIT1,IFIT3,IFIT2,OASL,ISG20,RSAD2,GBP1,IFI44L,DDX58,USP18,CXCL11,GBP5,GBP4 and CXCL9.The above genes are mainly located in the skin, immune system, nervous system and reproductive system.

Conclusion:This paper elucidates an effective approach for a new mechanism of HSV-2 infection, and the molecular mechanism of the selected core genes in the process of HSV-2 infection requires future experimental studies.

Background

Herpes simplex virus type 2 (HSV-2) infection is a widespread infectious disease that is the primary cause of most clinical cases of genital ulcers worldwide; it can be activated repeatedly after infection. HSV-2 can cause meningitis, encephalitis, sacral radiculitis and myelitis.HSV-2 can cause multi-system diseases after infecting the body, and the etiology is complicated[1]. However, the pathogenesis of HSV-2 infection is not fully understood.

IFN- β plays a key role in antiviral activity in early-induced defenses to limit viral replication and transmission after HSV-2 enters the genital epithelium; previous studies confirm the inhibition of IFN β production during HSV-2 infection. Multiple viral components during HSV-2 infection produce immune escape by inhibiting type I interferon expression through inhibition of the DNA receptor pathway, resulting in lifelong infection of the organism[2].

There was a large infiltration of T cells, monocytes/macrophages, numerous myeloid cells and a small number of plasmacytoid dendritic cells, low expression of type I interferons (IFN α and IFN β), as well as detection of large amounts of HSV-2 antigen and HSV-2 RNA within skin lesion biopsy specimens in the acute phase of HSV-2 infection[3]. Data analysis and exploration of genes associated with low expression of type I interferon during HSV-2 infection are still insufficient. Genes associated with low expression of type I interferon after HSV-2 infection were selected in this paper by database analysis and data exploration.

Therefore, we used statistical analysis and data mining to reveal the genes responsible for the low expression of type I interferon associated with HSV-2 infection. We used the GSE18527 dataset created by whole genome analysis analysis of skin biopsies during HSV-2 activation in the skin mucosa by Peng T et al. to obtain DDGs between normal and lesioned skin groups, healed skin and lesioned skin group, and then the integration of the 2 groups is taken.

This study contributes to the understanding of the etiology of immune escape after HSV-2 infection in the organism and provide new thinking for the clinical management of HSV-2 infection.

Results

Distinctively displayed genes

The GSE18527 dataset was imported at the GEO database and differentially expressed mRNAs were analyzed in R software using the Limma package (version: 3.40.2). Adjusted P values were analyzed in GEO to correct for false positive results; "Adjusted -P < 0.05 and log₂ (fold change) > 4" was defined as the threshold to screen for DDGs. 49 upper-regulated genes and 192 lower-regulated genes were isolated in the normal and lesioned skin groups; 110 upper-regulated genes and 43 lower-regulated genes were isolated in the healed and lesioned skin groups. 40 upper-regulated genes and 43 lower-regulated genes were recognized after taking crossovers for the 2 subgroups. As **Table 1** shows, 36 of the 83 DDGs (43.4%, 31 up-regulated and 5 down-regulated) were mainly distributed in the skin, and most of the above genes were concerned with epidermal development, skin development, keratin-forming cell differentiation and other functions; 21 genes (fcer1a, cd1a, fpr1, fcn1, etc.) were relevant to the immune system. CXCL10 gene expression was significantly down-regulated with a significantly lower P value (P < 0.0001). Figure 1 displays the Venn diagram and heat map of the DDGs.

Histospecific Gene analysis

Histospecific representation of DDGs were investigated by genecards database. The most histospecific expressed systems were the skin system (43.4%, 36/83); the blood/immune system was next (25.3%, 21/83); the nervous system (7%, 6/83); the reproductive system (2%, 2/83); and the visceral and digestive systems were the least enriched (1%, 1/83) (**Table 2**).

Functional and pathway enrichment analysis of DDGs

GO and KEGG enrichment assay of underlying mRNAs was performed using the ClusterProfiler suite of programs in R software. Expression heat maps were presented by the R package pheatmap. 3 functional enrichment aliases, 328 enriched GO species and 21 KEGG pathways were isolated. **Table 3** lists the enrichment aliases with $P < 0.000005$; they include Viral protein interaction with cytokine and cytokine receptor ($P = 1.51065E-09$) and IL-17 signaling pathway ($P = 4.47045E-07$); the MF class of chemokine receptor binding ($P = 1.85516E-08$) and G protein-coupled receptor binding ($P = 5.19828E-07$). In addition, nine enriched keywords were included, including epidermis development ($1.19541E-17$); response to virus ($5.02351E-12$), etc. Figure 2 illustrates the P values and gene counts of the 12 enriched functional items.

PPI network analysis of DDGs

A PPI network having 82 nodes as well as 429 edges was computed from the STRING database; the interaction score of this network was > 0.4 (**Fig. 3A**). The dots stand for genes and the edges stand for the linkage between genes. Red means upper-regulated genes and blue means lower-regulated genes.

We used MCODE, cytoHubba in Cytoscape to carry out gene clustering to recognize critical PPI network segments. As displayed in **Fig. 3B,C,D**, 3 critical segments were isolated. The above 3 critical segments are mainly concerned with the response to the virus, the development of the epidermis, and the structural components of the cytoskeleton (**Table 3**).

Characterization of genes of interest

We used the MCODE plugin in cytoscape to identify the top 20 genes by the MCC method, of which 8 down-regulated genes (IFIT2, IFIT3, RSAD2, gbp1, ifi44l, gbp4, GBP5, cxcl8) were connected to the immune system.

PPI network analysis screened a critical combination of 21 genes (DDX58, CD163, EPSTI1, OASL, MX1, USP18, ISG15, GBP1, CCL8, IL6, GBP4, ISG20, IFIT3, IFIT1, CXCL9, IFIT2, RSAD2, CXCL11, CXCL10, GBP5, IFI44L). Type I interferon plays a key role in suppressing HSV-2 infection. Using the genecards database, we screened four genes (gbp1, gbp4, ifit3, rsad2) associated with the expression of type I interferon. In addition, a few other underlying relevant genes among the histospecific displayed genes were singled out using the genecards database. **Table 4** exhibits all genes of interest.

Discussion

In this research, we based on the GSE18527 dataset in the GEO database to recognize the distinctively displayed genes (DDGs) of the total RNA after sequencing of normal skin of controls and lesioned skin of genital herpes patients, healed skin biopsies and lesioned skin, respectively; and took the crossover of the 2 sets of samples to minimize and enhance the potential pathogenic genes in HSV-2, selecting a couple of novant genes to be correlated with this disease that have not been investigated yet.

Previous studies have shown that RNA sequencing of lesion biopsies from the same sites during healing of HSV-2-infected skin tissue and at 2 and 4 weeks after healing showed no HSV-2 nucleic acids or

antigens; however, IFN- γ was consistently expressed and IFN- β and IFN- α levels were very low; HSV-2 preventing the innate immune system from producing type I interferon may be a major factor in allowing the virus to break through the host mucosal defenses[3]. In our study, we found a significant decrease in the expression level of type I interferon-related genes, which proves the accuracy of the method of this study.

We identified 83 DEGs in HSV-2-infected patients, including 40 up-regulated and 43 down-regulated genes, with the most differentially expressed genes in the cutaneous system, followed by the immune, nervous and reproductive systems, and the differential tissue expression could explain the life history after HSV-2 infection of the organism. HSV-2 virus invades through exposure to supraepithelial cells to produce initial infection and duplicates within the supraepithelial cells, after which HSV-2 ascends through the periaxonal sheaths of sensory nerves to the sacral ganglia of the host nervous system, where it becomes a reservoir for future outbreaks and subclinical genital viral shedding[4].

The differential genes were most expressed in the cutaneous system, suggesting that the skin is the primary barrier against HSV-2 infection and exerts a crucial role in innate and acquired immunity. Although HSV-2 can infect skin epithelial cells, immune cells and neuronal cells, it first infects mucosal epithelial cells during sexual transmission; keratinocytes are the main cells involved in HSV-2 clinical damage. Keratin-forming cells in the skin epidermis are considered to be immunologically active cells that not only act as a physical barrier, but also recognize the source of infection and initiate the innate immune response, including secretion of various cytokines (e.g., interleukins, interferons, clone-stimulating factors, growth factors, etc.), expression of cytokine receptors, recruitment of immune cells, enhancement of acquired immunity, and indirect elimination of the initial infection and prevention of subsequent infections[5].

PPI network and critical components analysis selected four genes (gbp1, gbp4, ifit3, rsad2) associated with type I interferon expression. the GBPS family is induced by both type I and type II IFNs, and according to the uniprot database annotation gbp1 hydrolyzes GTP to GMP; gbp4 hydrolyzes GTP by binding GTP, GDP and GMP. The cGAS-STING pathway performs a core role in the generation of type I interferon after identification of double-stranded DNA virus; the cyclic GMP-AMP (CGAMP) synthase (CGAS) upstream of the pathway is a cytoplasmic DNA sensor that triggers downstream STING by catalyzing the synthesis of cyclic GMP-AMP (CGAMP) from ATP and GTP, which ultimately leads to transcriptional elicitation of type I interferon[6]. Studies have confirmed that endogenous GBPS levels can induce the release of DNA from exposed bacteria in the cytoplasm, which in turn is sensed by cGAS[7].

ifit3 is an IFN-inducible antiviral protein that enhances the MAVS-mediated host antiviral response by acting as an articulator linking TBK1 to MAVS, leading to TBK1 activation and IRF3 phosphorylation, with phosphorylated IRF3 translocating into the nucleus to promote antiviral gene transcription[8]. Also known as interferon-induced iron-sulfur (4FE-4S) cluster-binding antiviral protein, rsad2 functions predominantly in the type I and type II interferon-induced cellular anti-virus status and inhibits a broad diversity of DNA and RNA viruses[9]. These information indicate that disorders of gbp1, gbp4, ifit3, and rsad2 may

contribute to the suppression of type I interferon expression leading to the production of HSV-2 immune escape.

Myelitis and encephalitis caused by HSV-2 infection are common in HSV-2 infection. We identified four upregulated genes *ephb6*, *nfix*, *mlana*, *tyrp1* and two downregulated genes *wars1*, *mx1* by screening for genes specifically expressed in nervous system tissues. *ephb6* regulates cell adhesion and migration by binding to ephrin-B1 and ephrin-B2[10]. NFIX identifies and combines the palindromic sequence 5'-TTGGC>NNNGCCAA-3' present in viral and cytosolic promoters as with the adenoviral type 2 replication motif[11]. *Mlana* is engaged in melanin production and plays a crucial role in the production, maintenance, export and machining of the melanocyte protein PMEL, which is essential for the generation of phase II melanosomes[12]. *Tyrp1* plays a role in melanin biosynthesis and can regulate or influence the type of melanin that is synthesized[13]. Studies have confirmed that melanocytes are melanin-producing cells with emerging innate immune functions, including the expression of antiviral type I interferon cytokines[14]. Tryptophanyl-tRNA synthetase 1 (WARS1) is an endogenous ligand of mammalian Toll-like receptors (TLR2 and TLR4). Microarray data, using mRNA from WARS1-treated human peripheral blood mononuclear cells (PBMCs), had indicated WARS1 to mainly activate innate inflammatory responses[15]. *Mx1* synthesizes interferon-inducible kinesin-like GTPases with antiviral activity against a variety of RNA viruses and some DNA viruses[16]. Their imbalance may contribute significantly in the etiology of HSV-2-induced CNS infections.

HSV-2 infection is mainly transmitted through sexual contact, and we identified the *pla2g2a* and *mamdc2* gene to be distributed mainly in the genital system. *pla2g2a* synthesizes secreted calcium-dependent phospholipases that primarily target extracellular phospholipids and are associated with host antimicrobial defense, inflammatory response and tissue regeneration; contributing to lipid remodeling of cell membranes and production of lipid mediators involved in pathogen clearance[17]. Also, by disrupting the integrity of the mitochondrial membrane, it promotes the release of effective damage-related molecular pattern molecules in the circulation, thereby activating the innate immune response[18]. Single-cell RNA sequencing analysis confirms host-length non-coding RNA MAMDC2-AS1 as a cofactor for HSV-1 nuclear transport[19]. The role of *pla2g2a* and *mamdc2* in the pathogenesis of HSV-2 infection needs to be confirmed by further experiments.

Genes associated with type I interferon expression were identified from GO enrichment analysis, including IFIT2, IFIT3, ISG20, MMP12, MX1, ISG15, and RSAD2. RSAD2 was significantly downregulated 3.246-fold and 3.103-fold in the control-lesioned and healed-lesioned groups, respectively. RSAD2 is an ancient mechanism of protection against viral infection and serves an essential and many-sided role in the innate autoimmune reaction to viral infection; RSAD2 protein interacts with viral proteins and RSAD2 protein fosters the antiviral response by directly challenging viral proteins for decay and also contributes to the ubiquitin-dependent proteasomal decay of some of the antiviral proteins with which it interacts[20]. In one study, immunohistochemical staining of vaginal tissue after systemic injection of 2'3'-cGAMP in mice revealed strong expression of RSAD2 protein in cells in the induced mesenchyme; epithelial cells close to the basement membrane were positive for RSAD2 protein, while epithelial cells located on the luminal

side of the canal expressed less. Vaginal tissues of mice locally infected with HSV2 had strong RSAD2 protein expression in cells at the site of infection. A few cells co-expressed RSAD2 protein and HSV-2 antigen, but cells surrounding HSV-2 infected cells expressed high levels of RSAD2 protein suggesting that RSAD2 protein is involved in the body's immune response to HSV2 infection[21]. ISG15 was significantly downregulated 4.401-fold and 2.203-fold in control-lesioned and healed-lesioned, respectively. ISG15 can inhibit viral translation, replication and release. ISG15 was found to preferentially co-translationally bind to newly synthesized proteins, suggesting that ISGylation may be a general, non-specific host defense mechanism[22]. A study found that *isg15*^{-/-} mice were prone to infection with influenza A and B, HSV-1 and Sindbis virus[23].

In this study, we employed finding-driven analysis to identify DDGs and selected four genes (*gbp1*, *gbp4*, *ifit3*, *rsad2*) associated with type I interferon expression by building a PPI network and recognizing critical components. The association of these genes with HSV-2 infection needs to be further investigated.

Conclusion

This research used the GSE18527 dataset of the GEO database to screen distinctively displayed genes in the overall RNA of skin biopsy sequences from genital herpes patients at different stages, followed by histospecific Gene analysis, feature enrichment and protein-protein interaction network analysis to define critical components. The most enriched system for histospecific Gene expression was the skin, and then the immune system and the nervous system. Functional and pathway enrichment analysis of differential genes was performed for nine enrichment keywords, including epidermal development; response to viruses, etc. We used Cytoscape for gene clustering to pinpoint critical PPI network components and defined three key components. We used the MCODE plugin in Cytoscape to identify the top 20 genes, and on basis of the genecards database, we artificially pinpointed four genes associated with type I interferon expression.

Materials And Methods

Microarray data

The GSE18527 dataset created from Peng T et al. was downloaded at GEO database. The dataset was derived from the GPL6255 Illumina humanRef-8 v2.0 expression beadchip platform, the experiment contained 19 samples, including 3 pre-treatment normal skin, 4 pre-treatment lesioned skin, 6 post-healing group lesioned skin biopsies and 6 post-healing group normal skin biopsies. Since this is a public dataset, age, health status and medication use of these individuals are not available. Annotated files for GPL6255 were also downloaded from GEO.

Differential expression analysis

The data were downloaded in MINiML format. Differentially expressed mRNAs were studied using the Limma software package (version: 3.40.2) of R. Adjusted P values were analyzed in GEO to correct for

false positive results, and differentially expressed mRNAs were screened at a threshold defined by Adjusted $P < 0.05$ and $\log FC > 4$. We divided the samples into 2 groups, normal skin biopsy and lesioned skin group, and healed skin and lesioned skin group, and gain the DDGs after taking the crossovers for the two subgroups. Venn diagrams of DDGs were produced with web-based facility Venny (<https://www.xiantao.love>), and expression heat maps were presented by the R package pheatmap.

Histospecific Gene analysis

Histospecific expression of differential genes were analyzed by genecards database. Transcripts from individual tissues that meet the following quasi-assays are considered extremely histospecific: based on RNA-seq from GTEx reads to obtain a list of tissues with positive differential gene expression, ploidy change values were calculated for each sample using DESeq software, and each sample read was compared to all GTEx sample reads, and genes with ploidy change values > 4 in the tissue were defined as being positively differentially expressed in that tissue. Genes with maximum read counts below 5 across tissues were excluded from the calculation.

Functional enrichment Analysis of DDGs

Latent functions of Latent targets were identified through functional enrichment analysis of the data. Gene ontology (GO) containing molecular functions (MF), biological pathways (BP) and cellular components (CC) is widely used to provide indications of genes with defined functions. KEGG enrichment analysis is practical available for analyzing gene functions as well as related high-level genomic functional information. The ClusterProfiler in R was utilized to profile the GO function of latent mRNAs and enrich the KEGG pathway. Box line plots were performed by the R package ggplot2; PCA plots were performed by the R package ggord.

Protein interaction (PPI) network profiling

PPI network profiling was performed via the STRING database. The DDGs were uploaded to the STRING database and the threshold of the score of the correlation was adjusted to > 0.4 .

In addition, PPI networks were visualized and constructed using Cytoscape(v 3.6.0) software, and the node with the highest number of interactions with neighboring nodes was called the central node.

To identify critical PPI network components, gene network clustering analysis was performed using cytohubba and MCODE in the Cytoscape software package. Critical components were defined by sensitivity thresholds of $P < 0.05$.

Statistical analysis

All statistical analyses were performed with R statistical software. For all assays, two-sided P value of less than 0.05 indicated a statistically significant difference.

Declarations

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Authors' contributions

This research was conducted in collaboration with all authors. Zhang Hao performed the data curation and analysis. Zhang Hao analyzed and interpreted the results. Zhang Hao and Liu Tao drafted and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data of this study are derived from the GEO database ([https:// www. ncbi. nlm. nih. gov/ geo/](https://www.ncbi.nlm.nih.gov/geo/)), which are publicly available databases.

Ethics approval and consent to participate

Not necessary.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

TABLE 1 Differentially expressed genes in HSV-2 infection. The genes marked with an asterisk are mainly located in the skin.

gene symbol		adjusted p-value		Fold change		Gene title	Location
		control-lesioned	healed-lesioned	control-lesioned	healed-lesioned		
up regulated genes							
AXIN2*	2.40E-04	6.21E-05	2.555	2.334	Axin2		Chr17
TUFT1*	5.99E-04	2.03E-05		2.364	2.154	Tuftelin 1	Chr1
WFDC5*	6.55E-04	4.45E-05	2.121	2.247	WAP four-disulfide core domain 5		Chr20
KRT15*	1.10E-03	1.32E-04		3.336	3.286	keratin 15	Chr17
APCDD1*	1.24E-03	3.12E-05		2.768	2.547	APC down-regulated 1	Chr18
EXPH5*	1.35E-03	2.57E-04	2.587	2.065	Exophilin 5		Chr11
NMU*	1.36E-03	4.83E-05	2.389	2.661	Neuromedin U		Chr4
CDHR1*	1.44E-03	4.30E-05	2.787	2.360	Cadherin related family member 1		Chr10
COL7A1	1.66E-03	4.38E-05	2.573	2.358	Collagen type VII alpha 1 chain		Chr3
RAPGEFL1*	1.66E-03	2.03E-05	2.456	2.695	Rap guanine nucleotide exchange factor like 1		Chr17
EPHB6	1.74E-03	3.12E-05	2.531	2.508	EPH receptor B6		Chr7
MLANA	2.15E-03	2.31E-04	2.516	2.484	Melan-A		Chr9
ALDH3A1*	2.23E-03	3.11E-05	2.136	2.642	Aldehyde dehydrogenase 3 family member A1		Chr17
DCT*	2.23E-03	3.37E-04	2.833	2.379	Dopachrome tautomerase		Chr13
IL20RB*	2.36E-03	1.13E-04	2.544	2.223	Interleukin 20 Receptor Subunit Beta		Chr3
PHGDH*	3.03E-03	8.07E-05		2.417	3.026	Phosphoglycerate dehydrogenase	Chr1
POU3F1*	3.10E-03	1.04E-04	2.174	2.036	POU class 3 homeobox 1		Chr1
NFIX	3.74E-03	2.48E-04	2.035	2.054	Nuclear factor I X		Chr19
CTNNBIP1*	3.95E-03	1.59E-03		2.404	2.168	Catenin beta interacting protein 1	Chr1
SOX21*	4.75E-03	8.14E-05		2.496	2.748	SRY-box transcription factor 21	Chr13
AADACL2*	4.87E-03	4.72E-05	2.012	2.702	Arylacetamide deacetylase like 2		Chr3
FCER1A	8.74E-03	6.83E-04	2.096	2.437	Fc fragment of IgE receptor Ia		Chr1
GPNMB*	9.64E-03	2.18E-04		2.198	2.197	Glycoprotein nmb	Chr7
SERPINA12*	1.37E-02	2.65E-03	2.774	2.477	SERPINA12		Chr14

DSC1*	1.41E-02	2.26E-04	2.271	2.779	Desmocollin 1	Chr18
MAMDC2	1.68E-02	7.13E-04	2.054	2.173	MAM domain containing 2	Chr9
FLG2*	2.23E-02	2.10E-04	2.946	3.650	Filaggrin family member 2	Chr1
KRT31*	2.80E-02	1.47E-02	2.029	3.492	keratin 31	Chr17
PMEL*	2.89E-02	3.35E-04	2.137	2.746	Premelanosome protein	Chr12
CD1A	2.99E-02	8.10E-03		2.107	2.007 CD1a molecule	Chr1
LCE2D*	3.99E-02	4.71E-04		2.790	3.490 LCE2D	Chr1
TYRP1	4.53E-02	9.88E-04	2.531	2.769	Tyrosinase Related Protein 1	Chr9
LCE5A*	4.65E-02	1.93E-03		2.088	2.350 Late Cornified Envelope 5A	Chr1
LOR*	5.81E-02	5.06E-04	2.077	2.733	Loricrin Cornified Envelope Precursor Protein	Chr1
HLA-DQB2*	8.11E-02	2.74E-02		2.542	2.220 HLA Class II Histocompatibility Antigen	Chr6
DQ Beta 2 Chain						
LCE1B*	9.39E-02	5.27E-04		2.410	3.423 Late Cornified Envelope 1B	Chr1
LCE2B*	9.87E-02	2.21E+00		0.001	2.877 Late Cornified Envelope 2B	Chr1
LCE1C	* 9.96E-02		2.93E-04		2.033 3.154 Late Cornified Envelope 1C	Chr1
LCE2A*	1.06E-01	6.24E-04		2.167	3.249 Late Cornified Envelope 2A	Chr1
LCE2C*	1.23E-01	1.38E-03		2.166	3.046 Late Cornified Envelope 2C	Chr1
Down regulated genes						
KRT16	4.90E-06	3.97E-02	4.917	2.151	Keratin 16	Chr17
CXCL10	5.85E-06	4.42E-05	7.199	3.841	C-X-C Motif Chemokine Ligand 10	Chr4
FPR1	1.28E-05	5.83E-07	3.739	2.992	Formyl Peptide Receptor 1	Chr19
KRT6C*	3.14E-05	3.07E-02	5.158	2.324	Keratin 6C	Chr12
CXCL9	3.64E-05	6.27E-04	6.408	3.299	C-X-C Motif Chemokine Ligand 9	Chr4
S100A9*	4.02E-05	6.55E-02	5.395	2.876	S100 Calcium Binding Protein A9	Chr1
FCN1	4.22E-05	2.28E-04		3.891	2.604 Ficolin 1	Chr9
EPSTI1	7.55E-05	7.06E-04	4.462	2.381	Epithelial Stromal Interaction 1	Chr13
GZMB	7.55E-05	2.91E-04	3.640	2.481	Granzyme B	Chr14
CCL8	2.28E-04	1.43E-03	5.669	3.972	C-C Motif Chemokine Ligand 8	Chr17
WARS	2.28E-04	1.77E-05	4.916	3.317	Tryptophanyl-TRNA Synthetase 1	Chr14
MX1	2.28E-04	2.07E-04	4.226	2.127	MX Dynamin Like GTPase 1	Chr21
CXCL11	2.28E-04	9.99E-06	5.839	4.824	C-X-C Motif Chemokine Ligand 11	Chr4
GBP1	2.28E-04	2.96E-05	5.033	3.027	Guanylate Binding Protein 1	Chr1
LILRB3	2.64E-04	3.03E-04	3.604	2.433	Leukocyte Immunoglobulin Like	Chr19
Receptor B3						
GBP5	2.67E-04	5.61E-05	4.203	3.133	Guanylate Binding Protein 5	Chr1
GBP4	3.35E-04	1.32E-04	3.997	2.397	Guanylate Binding Protein 4	Chr1
CXCL8	3.88E-04	4.13E-03	3.064	2.684	C-X-C Motif Chemokine Ligand 8	Chr4
IFI44L	4.55E-04	3.18E-03	3.881	2.085	Interferon Induced Protein 44 Like	Chr1
ISG20	4.55E-04	5.79E-05	3.968	2.150	Interferon Stimulated Exonuclease	Chr15
Gene 20						
ISG15	5.29E-04	1.31E-03	4.401	2.203	ISG15 Ubiquitin Like Modifier	Chr1
UBD*	6.77E-04	4.87E-04	4.368	2.879	Ubiquitin D	Chr6
IFI30	8.01E-04	3.07E-04	3.894	2.260	IFI30 Lysosomal Thiol Reductase	Chr19
FCER1G	8.34E-04	1.64E-04		3.631	2.360 Fc Fragment Of IgE Receptor Ig	Chr1
KRT6A*	8.74E-04	1.38E-02		3.884	2.337 Keratin, Type II Cytoskeletal 6A	Chr12
IFIT3	1.02E-03	6.49E-05	2.870	2.039	Interferon Induced Protein With	Chr19

Tetratricopeptide Repeats 3						
SERPINB1	1.10E-03	1.49E-04	3.408	2.104	Serpin Family B Member 1	Chr6
IFIT2	1.25E-03	9.84E-05	3.138	2.101	Interferon Induced Protein With	Chr10
Tetratricopeptide Repeats 2						
CCR7	1.44E-03	1.78E-04	2.403	2.009	C-C Motif Chemokine Receptor 7	Chr17
SELE	2.31E-03	2.90E-02	2.683	2.055	Selectin E	Chr1
IL6	2.36E-03	8.74E-02	3.504	2.623	Interleukin 6	Chr7
RSAD2	2.61E-03	7.12E-05	3.246	3.103	Radical S-Adenosyl Methionine	Chr2
Domain Containing 2						
PLEK	2.69E-03	1.37E-03	3.676	2.155	Pleckstrin	Chr2
IDO1	2.90E-03	3.32E-04	4.047	3.335	Indoleamine 2,3-Dioxygenase 1	Chr8
CD163	5.68E-03	9.86E-04	3.102	2.105	CD163 Molecule	Chr12
IL1B	8.36E-03	1.06E-03	2.912	2.671	Interleukin 1 Beta	Chr2
ADAMTS4	1.02E-02	6.27E-02	3.592	2.361	ADAM Metallopeptidase With	Chr1
Thrombospondin Type 1 Motif 4						
PLA2G2A	1.06E-02	2.35E-03	2.845	2.436	Phospholipase A2 Group IIA	Chr1
DEFB4A	1.14E-02	1.14E-02	4.951	3.447	Defensin Beta 4A	Chr8
CCL19	1.17E-02	3.49E-03		3.336	2.886 C-C Motif Chemokine Ligand 19	Chr9
MMP12	2.16E-02	3.31E-03	2.638	2.464	Matrix Metallopeptidase 12	Chr11
KRT17*	2.38E-02	1.37E-02	3.444	2.210	Keratin 17	Chr17
MMP1	1.16E-01	1.59E-02	2.179	2.107	Matrix Metallopeptidase 1	Chr11

Table 2 Histospecific genes identified by genecards .

System	Genes
Hematologic/immune	fcgr1a,cd1a,fpr1,fcn1,epsti1,gzmb,lilrb3,gbp5, cxcl8,ifi44l,gbp4 Ubd,fcgr1g,ifit3, serpinb1,ifit2,ccr7,rsad2,plek,cccl19,gbp1
Neurologic	ephb6,nfix,mlana,tyrp1,wars,mx1
Skin	axin2,tuftelin 1,wfdc5,keratin 15,apcdd1,exph5,nmu ,cdhr1,rapgef1 aldh3a1,dct,phgdh,pou3f1,ctnnbip1,sox21 ,aadacl2,gpnmb,serpina12 flg2 ,krt31,pmel,lce2d,lce5a,Lor,hla-dqb2,lce1b,lce1c,lce2a,lce2c il20rb,lce2b,krt6c,s100a9, krt6a,krt17,dsc1
Muscle	cccl8,il1b,mmp1
Respiratory	cxcl10,cxcl9,cxcl11,,isg20,isg15,ido1
Secretory	IFI30,sele,il6,adamts4,defb4a,mmp12,krt16
Digestive	col7a1
Reproductive	mamdc2,pla2g2a
Internal	cd163

Table 3 The enriched terms for DDGs.

ONTOLOGY	ID	Description	p-value	geneID	Count
BP	GO:0008544	epidermis development	1.19541E-17	COL7A1/DCT/DSC1/KRT6A/KRT15/KRT16	22
		/ keratinocyte differentiation		KRT17/KRT31/LOR/POU3F1/SOX21/EXPH5	
				LCE2B/APCDD1/LCE5A/KRT6C/LCE1B/LCE1C	
				LCE2A/LCE2C/LCE2D/FLG2	BP GO:0009615 response to virus
5.02351E-12	GBP1/IFIT2/IFIT3/IL1B/IL6/CXCL10/ISG20/RSAD2				

				CXCL9/MMP12/MX1/CCL8/CCL19/ISG15/IFI44L	15
BP	GO:0030593	neutrophil chemotaxis	1.69908E-11	CCR7/FCER1G/IL1B/CXCL8	10
				CXCL10/CXCL9/S100A9/CCL8	
				CCL19/CXCL11	
KEGG	hsa04061	Viral protein interaction with cytokine and cytokine receptor	1.51065E-09	CCR7/IL6/CXCL8/CXCL10/CXCL9	9
				CCL8/CCL19 CXCL11/IL20RB	
MF	GO:0042379	chemokine receptor binding	1.85516E-08	DEFB4A/CXCL8/CXCL10/CXCL9	7
				CCL8/CCL19/CXCL11	
BP	GO:0006959	humora limmune response	2.69486E-08	CCR7/DEFB4A/FCN1/IL1B/IL6	12
				CXCL8/CXCL10/KRT6A/CXCL9	
BP	GO:0070098	chemokine-mediated signaling pathway	8.16999E-08	CCR7/CXCL8/CXCL10/CXCL9	7
				CCL8/CCL19/CXCL11	
BP	GO:0060337	type I interferon signaling pathway	1.3893E-07	IFIT2/IFIT3/ISG20/MMP12	7
				MX1/ISG15/RSAD2	
KEGG	hsa04657	IL-17 signaling pathway	4.47045E-07	DEFB4A/IL1B/IL6/CXCL8/CXCL10/MMP1/S100A9	7
MF	GO:0001664	G protein-coupled receptor binding	5.19828E-07	DEFB4A/FCN1/FPR1/CXCL8/CXCL10	10
				CXCL9/CCL8/CCL19/CXCL11/NMU	
MF	GO:0005200	structural constituent of cytoskeleton	6.72691E-06	KRT6A/KRT15/KRT16/KRT17/KRT31/LOR	6

Table 4 Genes of interest.

Up-regulated			Down-regulated		
Gene	Fold change		Gene	Fold change	
	Control-lesioned	Heal-lesioned		Control-lesioned	Heal-lesioned
Immune					
fcer1a	2.096	2.437	fpr1	3.739	2.992
cd1a	2.107	2.007	fcn1	3.891	2.604
		Epst	4.462	2.381	
			Gzmb	3.640	2.481
			gbp5	4.203	3.133
			cxcl8	3.064	2.684
			ifi44l	3.881	2.085
			gbp4	3.997	2.397
			gbp1	5.033	3.027
			Ubd	4.368	2.879
			fcer1g	3.631	2.360
			ifit3	2.870	2.039
			serpinb1	3.408	2.104
			ifit2	3.138	2.101
			ccr7	2.403	2.009
			rsad2	3.246	3.103
			Plek	3.676	2.155
			ccl19	3.336	2.886

Neurologic

ephb6	2.531	2.508	wars	4.916	3.317
Nfix	2.035	2.054	mx1	4.226	2.127
Mlana	2.516	2.484			
tyrp1	2.531	2.769			

Reproductive

mamdc2	2.054	2.173	pla2g2a	2.845	2.436
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Figures

Figure 1

a Heat map display of differential genes between control normal skin and lesioned skin group.b Heat map display of differential genes in the healed and lesioned skin groups.c Venn diagram Expression profiles of total RNA in control normal and lesioned groups, healed skin and lesioned groups were taken to intersect.UP represents upregulated genes,and down represents downregulated genes.

Figure 2

Bubble diagram. 12 groups of representative enrichment function terms. The different colors represent the significance of differential enrichment results, larger values represent smaller fdr values, and the size of the circle represents the number of enriched genes, with larger circles representing a larger number.

Figure 3

a Interaction network of 82 nodes and 429 edges with interaction scores > 0.4 obtained after uploading the DDGs to the STRING database and visualizing the network with Cytoscape software. Nodes stand for genes and edges stand for associations across genes. Red means uplifted genes and blue means downlifted genes. (b, c,d) Three critical components were recognized using cytohubba and MCODE plugins in the Cytoscape software.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Rawdata.csv](#)