Integrated bioinformatic analysis of differentially expressed genes and signaling pathways in plaque psoriasis

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Abstract. Psoriasis is an immune-mediated cutaneous disorder with a high incidence and prevalence. Patients with psoriasis may experience irritation, pain and psychological problems. The cause and underlying molecular etiology of psoriasis remains unknown. In an attempt to achieve a more comprehensive understanding of the molecular pathogenesis of psoriasis, the gene expression profiles of 175 pairs of lesional and corresponding non-lesional skin samples were downloaded from 5 data sets in the Gene Expression Omnibus (GEO) database. Integrated differentially expressed genes (DEGs) were obtained with the use of R software. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were analyzed using the DAVID online analysis tool. The protein-protein interaction (PPI) network was constructed on the STRING platform and hub genes were calculated with the use of Cytoscape software. Finally, GEO2R was used to determine the expression of the hub genes in scalp psoriasis. A total of 373 genes from the 5 data sets were identified as DEGs, including 277 upregulated and 96 downregulated genes. GO analysis revealed that immune responses and epidermal differentiation/development were the most enriched terms in biological processes, extracellular space/matrix was the most enriched term in cellular components, and endopeptidase inhibitor activity was the most enriched term in molecular functions. In the KEGG pathway enrichment, DEGs were mainly enriched in the metabolic and viral infection-associated pathways. A total of 17 hub genes were calculated, including *CSK2*, *CDC45*, *MCM10*, *SPC25*, *NDC80*, *NUF2*, *AURKA*, *CENPE*, *RRM2*, *DLGP5*, *HMMR*, *TTK*, *IFIT1*, *RSAD2*, *IFI6*, *IFI27* and *ISG20*, among which interferon-α-inducible genes were revealed to display a similar expression pattern as that obtained in scalp psoriasis. This comprehensive bioinformatic re-analysis of GEO data provides new insights on the molecular pathogenesis of psoriasis and the identification of potential therapeutic targets for the treatment of psoriasis.

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Abbreviations: GEO, Gene Expression Omnibus; DEG, differentially expressed gene; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction

Key words: psoriasis, bioinformatic analysis, pathogenesis, molecular mechanism

Introduction

Psoriasis, as characterized by a well-demarcated erythematous plaque with silver scales, is a chronic, immune-mediated disorder that mainly affects the skin and joints (1). The worldwide prevalence rates of psoriasis range from 0.9-8.5% in adults and 0-2.1% in children (2). Although this condition rarely poses a threat to life, the irritation, pain and especially the aberrant appearance make these patients susceptible to psychological problems, such as anxiety and depression (3,4). With recent advances in the understanding of psoriasis, an increasing number of therapies have emerged, however a high recurrence rate persists. Therefore, it is important to better understand the underlying molecular pathogenesis of psoriasis in order to identify more effective therapeutic approaches for the control of psoriasis development and progression.

Gene expression microarrays have been widely applied in psoriatic research and represent an important new tool for use in the identification of disease-related molecules associated with psoriasis. Recently, comprehensive analysis of microarray data from multiple centers has become a popular research area. Ainali *et al* investigated gene expression patterns in lesional and non-lesional psoriatic tissue samples from 2 GEO data sets to establish a molecular sub-groups within the clinical phenotype of plaque psoriasis (5). Mei and Mei screened differentially expressed genes based on 4 psoriatic data sets followed by characterization of gene functions and mutual interactions (6). Sevimoglu and Arga analyzed and integrated data from 12 studies to identify the potential candidates for disease biomarkers and therapeutic targets (7).

However, analysis of the unpaired data obtained from lesional and non-lesional samples may lead to a potential bias caused by disease heterogeneity. In order to eliminate or reduce such bias, only paired lesional and the corresponding non-lesional skin samples were selected and analyzed in this study. Information was compiled from 5 original microarray data sets, GSE14905 (8), GSE30999 (9,10), GSE34248 (11), GSE41662 (11) and GSE53552 (12), from the Gene Expression Omnibus (GEO) database. A total of 175 pairs of lesional and non-lesional skin samples from plaque psoriatic patients were selected. With use of bioinformatic methods, integrated differentially expressed genes (DEGs) were identified, followed by the Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Protein-protein interaction (PPI) analysis and hug gene calculation were subsequently performed. Finally, an additional GEO data set, GSE75343 (13), which contained a study of gene expression levels in scalp psoriatic patients, was used as a means to validate whether the hub genes obtained from the aforementioned databases exhibited a similar expression profile as that in scalp psoriatic lesions.

Through integration of the bioinformatic analyses of the gene expression from these 175 pairs of psoriatic skin samples, 377 genes were identified as DEGs, with 277 of these genes being upregulated and 96 genes downregulated. We revealed that these genes covered a wide range of biological functions in epidermal development, keratinization, immune responses, metabolic pathways, cell cycle and extracellular spaces. These results provide a comprehensive understanding of the molecular pathogenesis of the disease, which may guide subsequent studies on psoriasis research.

Materials and methods

Microarray data sets and data calibration. Using the keyword 'psoriasis', data sets using the descriptors 'paired biopsy from both lesional and non-lesional skin' and 'pre-treatment status' were screened. The raw files of 5 enrolled microarray data sets, including GSE14905 (8), GSE30999 (9,10), GSE34248 (11), GSE41662 (11) and GSE53552 (12) (Table I), were downloaded from the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/). In each data set, only pre-treatment psoriatic skin samples and their matched adjacent normal samples were selected, which resulted in 175 pairs of skin samples from psoriatic patients for subsequent analysis. The raw files were processed with R software 3.5.1 (https://www.r-project.org) to convert the gene probe IDs to gene symbol codes. Finally, calibrations of gene expression levels according to the quartile method were performed for subsequent analysis.

DEGs analysis and integration. A differential expression analysis on each GEO series, as based on paired-sample t-tests between psoriatic skin and adjacent normal skin samples, were performed with use of R software. A gene was defined as a differentially expressed gene between the psoriatic and normal sample when the P-value was <0.05 ($P<0.05$) and the gene expression fold change (FC) value was >2 or <0.5 ($|\log2FC|\geq1$), which were illustrated as Volcano plots. An overlap of total, upregulated or downregulated DEGs, plotted as Venn charts, from all 5 data sets were listed for subsequent function analysis.

GO term and KEGG pathway analysis of DEGs. The DAVID knowledgebase (https://david.ncifcrf.gov/), an online gene functional annotation tool, was used to analyze the function and pathway enrichment of candidate genes obtained (14). With this technique, the Fisher exact test P-value was calculated as a result of enrichment degree. The top 10 enrichment GO term or KEGG pathway annotations for both up- or downregulated DEGs obtained in our study were listed.

PPI network and hub gene analyses. The STRING platform, an online tool used for the structural and functional analysis of protein interactions (15), was used to identify interactions among proteins of interest. The corresponding results were analyzed and structured with the use of the Cytoscape software 3.6.1 (https://cytoscape.org). The hub genes, which were considered to be involved in playing pivotal regulatory roles in the PPI network, were subsequently calculated based on the overlapping results obtained by MCC (Maximal Clique Centrality) and DMNC (Density of Maximum Neighborhood Component) topological analysis methods, respectively, with use of the cytoHubba app built in the software (16).

GEO2R analysis of gene expression levels. The gene expression levels of hub genes were analyzed in GSE75343 (13), a microarray data set comparing gene expression levels of scalp psoriatic skin and adjacent normal skin samples (Table I). The GEO2R, an online analysis tool built in the GEO website, was used for this analysis. Statistical analyses were performed using paired-sample t-tests and a P-value <0.05 was required for results to be considered statistically significant. Scatter charts were plotted using GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Microarray data standardization and DEG identification in plaque psoriasis. With use of the quartile division method, gene expression levels of each of the 5 GEO series were standardized and the results of pre- and post-standardization are presented in Fig. 1A. After pre-processing of the data, DEGs were analyzed using paired-sample t-tests within each series using a screening criteria of $P<0.05$ and $|\log2FC|\geq1$ (Fig. 1B). The number of DEGs in each series, including up- and downregulated DEGs are presented in Table II. When DEGs in each series were intersected with one another, 373 genes, considered as integrated DEGs were obtained and used for subsequent analysis with 277 of these genes being upregulated and 96 downregulated (Fig. 2). The ratio of the number of upregulated genes to that of downregulated genes was close to 1:1 in each of the GEO data sets, however, in the integrated results this ratio was equal to approximately 3:1, indicating a possible commonality in the upregulated genes during psoriasis development while the downregulated genes differ in individuals.

GO and KEGG pathway enrichment analysis of DEGs. GO enrichment analysis, which is comprised of 3 functional groups (biological processes, cellular components and molecular functions), was performed using the DAVID online tool. Within each of the functional groups, the top 10 enrichment terms for both up- or downregulated DEGs as

Table I. Information for psoriatic GEO data.

GEO series	Platform	Sample	Type	Pair no.	(Refs.)
GSE14905	GPL570	Paired LS and NLS	Plaque psoriasis	28	Yao *et al* (8)
GSE30999	GPL570	Paired LS and NLS	Moderate to severe plaque psoriasis	85	Suárez-Fariñas *et al* (9)
GSE34248	GPL570	Paired LS and NLS	Mild to moderate plaque psoriasis	14	Bigler *et al* (11)
GSE41662	GPL570	Paired LS and NLS	Moderate to severe plaque psoriasis	24	Bigler *et al* (11)
GSE53552	GPL570	Paired LS and NLS	Moderate to severe plaque psoriasis	24	Russell *et al* (12)
GSE75343	GPL570	Paired scalp LS and scalp NLS	Moderate to severe plaque psoriasis with scalp involvement	13	Ruano *et al* (13)

LS, lesional skin; NLS, non-lesional skin.

Table II. DEGs in each GEO series.

GEO series	No. of total DEGs	No. of upregulated DEGs	No. of upregulated DEGs
GSE14905	1195	682	513
GSE30999	1979	1040	939
GSE34248	1670	854	816
GSE41662	2203	1073	1130
GSE53552	2220	1084	1136

DEGs, differentially expressed genes.

identified according to the Fisher's exact test P-value are listed in Tables III and IV. The corresponding visual diagrams are presented in Fig. 3A and B. Within the biological process function group, upregulated DEGs were mainly enriched in GO terms of immune responses, ectoderm development, defense responses, keratinization and epidermal development while downregulated DEGs were mainly enriched in the regulation of system processes, regulation of smooth muscle contraction and muscle organ development. Notably, with the exception of enrichment of the cornified envelope, which is an extremely tough structure formed beneath the cell membrane (17) in the upregulated DEGs group, both up- and downregulated DEGs were enriched in the extracellular space within the cellular component enrichment analysis. Within the molecular function enrichment group, the upregulated DEGs were mainly enriched in chemokine activity, chemokine receptor binding and endopeptidase inhibitor activity, while downregulated DEGs were enriched in cytoskeletal protein binding processes. The KEGG pathway enrichment was performed using the same analysis tool and the results, in which only the pathways for the upregulated genes are displayed by figure, due to the limited number of enrichment pathways in the downregulated group, are presented in Table V and Fig. 4. In upregulated DEGs, signaling pathways were mainly enriched in metabolic pathways, measles, influenza A and chemokine signaling pathways, while aldosterone-regulated sodium reabsorption and PPAR signaling pathways were enriched in downregulated DEGs.

PPI network construction and hub gene selection. The online database STRING was used to obtain PPI information on the 373 DEGs, including the 277 upregulated and 96 downregulated genes and the PPI network, with 2 notable functional modules, was constructed with use of Cytoscape software (Fig. 5A). Hub genes were then calculated using the cytoHubba app from the network we constructed. As a result of these calculations, 17 genes with the highest scores were considered as hub genes and were automatically divided into 2 groups exactly corresponding to the modules in Fig. 5A. One group consisted of *TTK*, *AURKA*, *DLGAP5*, *HMMR*, *CDC45*, *CENPE*, *SPC25*, *MCM10*, *NDC80*, *RRM2*, *CKS2* and *NUF2*, which are genes involved in the cell cycle, mitosis and proliferation. The second group consisted of *IFI6*, *ISG20*, *IFIT1*, *RSAD2* and *IFI27*, all of which belong to IFN-α-inducible genes (Fig. 5B). Notably, these 17 hub genes all belong to the upregulated genes of the DEGs we obtained, which further demonstrated the importance of these upregulated genes in the molecular pathogenesis of psoriasis.

Hub gene expression levels in scalp psoriasis. To investigate whether scalp psoriasis displayed a similar gene expression profile as that of skin psoriasis, 13 pairs of scalp lesional and adjacent non-lesional samples were selected from GSE75343 (Table I). With use of the online analysis tool, GEO2R, expression levels of these 17 hub genes were determined. The results revealed that statistically significant differences were obtained in the expression of *IFI6*, *IFI27*, *RSAD2*, *ISG20*, *MCM10* and *SPC25* (Fig. 6), but not in the other hub genes (data not shown). Further analysis revealed that 4 out of 6 IFN-α-inducible genes exhibited significant differences with regard to gene expression, while in genes involved in the cell cycle, mitosis and proliferation, only 2 of them exhibited differences in gene expression.

Discussion

Psoriasis, one of the most common skin ailments, afflicts millions of people worldwide. In addition to its negative

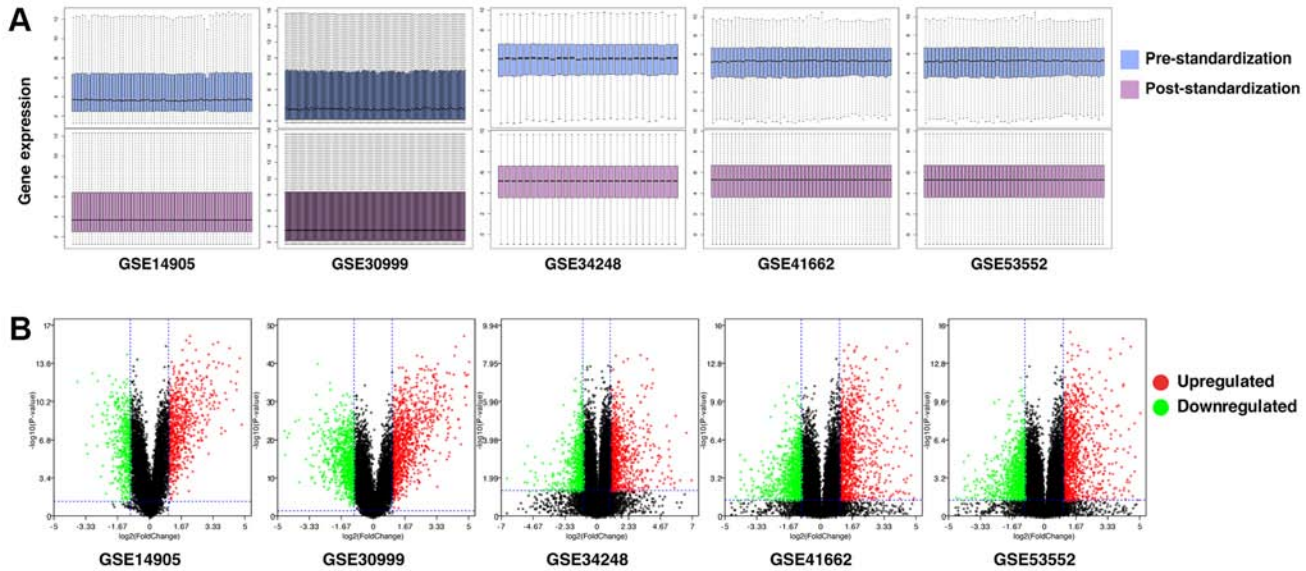

Figure 1. Data standardization and DEG identification. (A) Pre-standardization gene expression levels of each data set are presented as blue boxplots and post-standardization values are presented as purple boxplots. (B) The upregulated DEGs (red dots) and downregulated DEGs (green dots) of each data set were identified with the use of criteria of P<0.05 and |log2FC|≥1. DEGs, differently expressed genes.

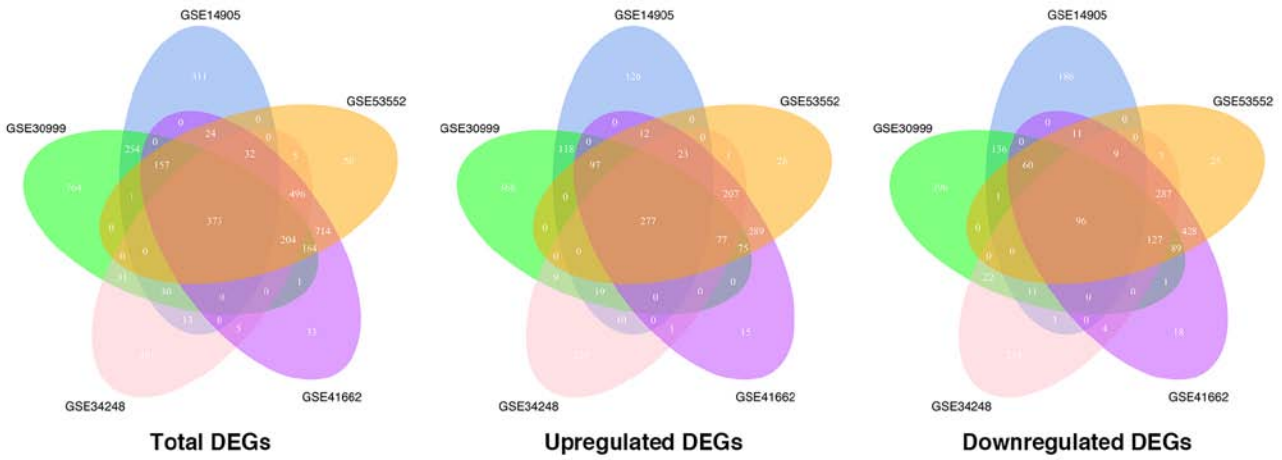

Figure 2. DEG integration of each data set. DEGs of each data set are overlapped and presented as a Venn plot, including total, upregulated and downregulated genes. DEGs, differently expressed genes.

aspects on physical and mental health, the cost of psoriasis places a huge burden on both individuals and society (17). Although dozens of medications are available for relief of the symptoms of this disease, no cure for psoriasis currently exists. Therefore, it is clear that the identification of pivotal molecules that play critical roles in the pathogenesis of this disease for potential development of therapeutic targets represents an important area of investigation.

Gene expression microarrays provide a comprehensive view of genome-wide expression profiles of clinical samples and have been widely used to analyze genes which are differentially expressed in psoriasis. However, few studies exist which have integrated such high-throughput gene expression microarray data of paired lesional and non-lesional skin samples. In the present study, gene expression profiles of 175 pairs of psoriatic skin samples and the corresponding normal tissues from 5 GEO data sets were integrated and analyzed with use of bioinformatic methods. Our results demonstrated several important pathways and the pivotal genes associated with the molecular pathogenesis of psoriasis.

Psoriasis is an immune-mediated inflammatory cutaneous disease characterized by an overt proliferation and differentiation of keratinocytes (1). Our GO biological process enrichment results, especially with regard to upregulated genes, included immune responses, keratinization, inflammatory responses and keratinocyte differentiation, all of which are commonly accepted components of the pathogenesis and pathological changes of psoriasis. In addition, the enrichment in defense responses and responses to wound healing processes indicates two important psoriatic precipitating factors: infection (18) and trauma (Koebner phenomena) (19), respectively, both of which are associated with the activation of innate immunity involved in the initial pathogenesis of psoriasis (20). Additional reported risk factors include smoking (21), alcohol (22) and obesity (23). In the cellular component enrichment analysis, in addition to mitosis-associated components such as chromosome

Table III. GO analysis of upregulated genes associated with psoriasis.

Term	Count	Rich factor (%)	P-value	Functional group
GO:0006955 immune response	39	16.04938272	4.38742E-13	BP
GO:0007398 ectoderm development	17	6.995884774	2.50742E-08	BP
GO:0006952 defense response	29	11.93415638	4.97398E-08	BP
GO:0031424 keratinization	9	3.703703704	1.40384E-07	BP
GO:0008544 epidermis development	15	6.172839506	3.81508E-07	BP
GO:0009611 response to wounding	25	10.28806584	5.4487E-07	BP
GO:0006954 inflammatory response	18	7.407407407	4.12036E-06	BP
GO:0030855 epithelial cell differentiation	12	4.938271605	4.1551E-06	BP
GO:0030216 keratinocyte differentiation	9	3.703703704	4.20155E-06	BP
GO:0009913 epidermal cell differentiation	9	3.703703704	8.13933E-06	BP
GO:0006935 chemotaxis	12	4.938271605	1.83209E-05	BP
GO:0001533 cornified envelope	6	2.469135802	1.19845E-05	CC
GO:0005576 extracellular region	49	20.16460905	0.000143627	CC
GO:0005615 extracellular space	23	9.465020576	0.000294727	CC
GO:0031262 Ndc80 complex	3	1.234567901	0.001187266	CC
GO:0044421 extracellular region part	25	10.28806584	0.004874319	CC
GO:0005792 microsome	10	4.115226337	0.00688315	CC
GO:0042598 vesicular fraction	10	4.115226337	0.008255849	CC
GO:0001772 immunological synapse	3	1.234567901	0.008416898	CC
GO:0000777 condensed chromosome kinetochore	5	2.057613169	0.009278963	CC
GO:0000793 condensed chromosome	7	2.880658436	0.010321628	CC
GO:0008009 chemokine activity	7	2.880658436	3.62741E-05	MF
GO:0004867 serine-type endopeptidase inhibitor activity	9	3.703703704	3.72441E-05	MF
GO:0042379 chemokine receptor binding	7	2.880658436	5.23427E-05	MF
GO:0004866 endopeptidase inhibitor activity	10	4.115226337	0.000171511	MF
GO:0030414 peptidase inhibitor activity	10	4.115226337	0.00025684	MF
GO:0004252 serine-type endopeptidase activity	9	3.703703704	0.001273205	MF
GO:0008236 serine-type peptidase activity	9	3.703703704	0.003155874	MF
GO:0017171 serine hydrolase activity	9	3.703703704	0.003376462	MF
GO:0005125 cytokine activity	9	3.703703704	0.005452577	MF
GO:0004175 endopeptidase activity	13	5.349794239	0.005566809	MF

GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function.

kinetochore and the Ndc80 complex, enrichment in the extracellular matrix of both up- or downregulated genes revealed the significance of this component. The extracellular matrix (ECM) is a collection of non-cellular molecular networks that regulate diverse cellular functions, such as growth, migration and homeostasis (24,25). The ECM is composed of interstitial matrix and basement membrane, both of which are reported to be involved in the development of psoriasis. Findings from a guttate psoriasis prognosis study, indicate that psoriasis disease progression is believed to be governed by the triggering of humoral immune responses, which could produce extracellular antibodies to neutralize the streptococcal lytic enzyme and prevent disruption of the laminin layer in the basement membrane caused by the enzyme (26). Recently, neutrophil extracellular traps (NETs), which are web-like structures consisting of DNA associated with histones, antimicrobial peptides and enzymes (27,28), were reported to act as a source of autoantigens which contribute to the occurrence of several autoimmune diseases (29,30), including psoriasis. For example, Lin *et al* reported that mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis (31). In molecular function enrichment analysis, it was observed that in upregulated genes, several GO terms indicated endopeptidase inhibitor activity, which contains a family of serine protease inhibitors (serpins). Serpins, such as *SERPINA3*, *SERPINB4*, *SERPINA1*, *SERPINB12*, *SERPINB3* and *SERPINB13* in our enrichment gene list represent a broad family of protease inhibitors that utilize conformational changes to inhibit target enzymes (32); and it has been suggested that these serpins play a role in psoriatic pathogenesis. Similar to the results obtained in our analysis, Johnston *et al* detected upregulation of two endogenous protease inhibitors, serpins A1 and A3, both of which are present in psoriasis vulgaris and generalized pustular psoriasis. These serpins may play a counter-regulated role to control the activity of IL-36, whose activation requires N-terminal peptide cleavage by neutrophil serine protease (33).

Table IV. GO analysis of downregulated genes associated with psoriasis.

Term	Count	Rich factor (%)	P-value	Functional group
GO:0044057 regulation of system process	6	6.976744	0.008882	BP
GO:0006940 regulation of smooth muscle contraction	3	3.488372	0.010755	BP
GO:0007517 muscle organ development	5	5.813953	0.011225	BP
GO:0030003 cellular cation homeostasis	5	5.813953	0.020798	BP
GO:0051241 negative regulation of multicellular organismal process	4	4.651163	0.030364	BP
GO:0055080 cation homeostasis	5	5.813953	0.03044	BP
GO:0042698 ovulation cycle	3	3.488372	0.031344	BP
GO:0006937 regulation of muscle contraction	3	3.488372	0.035764	BP
GO:0019432 triglyceride biosynthetic process	2	2.325581	0.036656	BP
GO:0008016 regulation of heart contraction	3	3.488372	0.040411	BP
GO:0005576 extracellular region	25	29.06977	0.00021	CC
GO:0005615 extracellular space	12	13.95349	0.001648	CC
GO:0044421 extracellular region part	14	16.27907	0.002814	CC
GO:0031012 extracellular matrix	6	6.976744	0.047088	CC
GO:0008092 cytoskeletal protein binding	8	9.302326	0.009079	MF
GO:0003779 actin binding	6	6.976744	0.018296	MF
GO:0004857 enzyme inhibitor activity	5	5.813953	0.037901	MF
GO:0003995 acyl-CoA dehydrogenase activity	2	2.325581	0.068241	MF
GO:0042803 protein homodimerization activity	5	5.813953	0.07167	MF
GO:0008201 heparin binding	3	3.488372	0.084388	MF
GO:0030246 carbohydrate binding	5	5.813953	0.084603	MF

GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function.

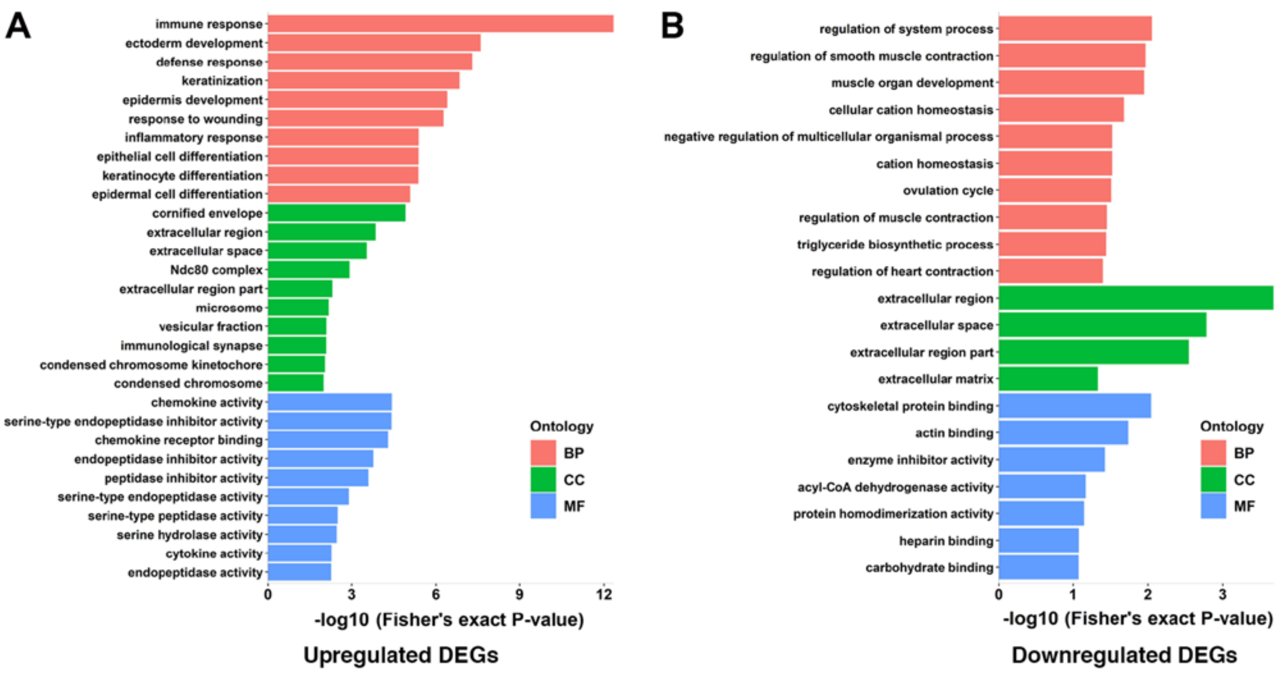

Figure 3. GO enrichment of DEGs. (A) GO enrichment of upregulated DEGs in 3 functional groups: biological processes (red), cellular components (green) or molecular functions (blue). These groups are ranked and presented as bar plots according to their Fisher's exact P-value. (B) GO enrichment of downregulated DEGs. DEGs, differently expressed genes; GO, gene ontology.

Such a negative regulatory effect, although unlikely to balance the protease expression revealed in the study by Lin *et al* (31), may provide for new insights into the development of psoriasis therapy.

Table V. KEGG analysis of DEGs associated with psoriasis.

Regulation	ID	Term	Count	Rich factor (%)	P-value	Genes
Upregulated	hsa05164	Influenza A	11	4.280155642	0.00022787	IRF7, OAS3, CXCL8, IL1B, RSAD2, OAS1, OAS2, MX1, STAT1, TMPRSS4, CXCL10
Upregulated	hsa05162	Measles	9	3.501945525	0.00073285	CCNE1, PRKCQ, IRF7, OAS3, IL1B, OAS1, OAS2, MX1, STAT1
Upregulated	hsa04062	Chemokine signaling pathway	10	3.891050584	0.00162187	CXCL1, CCL22, CCL20, CXCL13, CXCL9, CXCL8, CXCR2, STAT1, CCL18, CXCL10
Upregulated	hsa05160	Hepatitis C	7	2.723735409	0.01355117	IFIT1, IRF7, OAS3, CXCL8, OAS1, OAS2, STAT1
Upregulated	hsa05168	Herpes simplex infection	8	3.112840467	0.01820027	CDK1, IFIT1, IRF7, OAS3, IL1B, OAS1, OAS2, STAT1
Upregulated	hsa00240	Pyrimidine metabolism	6	2.33463035	0.01847329	TYMP, NT5C3A, RRM2, UPP1, PNP, CMPK2
Upregulated	hsa04620	Toll-like receptor signaling pathway	6	2.33463035	0.01989591	IRF7, CXCL9, CXCL8, IL1B, STAT1, CXCL10
Upregulated	hsa05146	Amoebiasis	6	2.33463035	0.01989591	ARG1, CXCL8, IL1B, SERPINB4, SERPINB13, SERPINB3
Upregulated	hsa01100	Metabolic pathways	27	10.50583658	0.03117996	XDH, GDA, KYNU, HSD17B2, NT5C3A, GALNT6, CYP2C18, UPP1, AASS, PNP, CMPK2, ARG1, TYMP, HPSE, ALOX12B, FUT2, SPTLC2, DHRS9, HYAL4, ST6GALNAC1, SQLE, RRM2, AKR1B10, LIPG, GK, SMPD3, PLA2G4D
Upregulated	hsa04110	Cell cycle	6	2.33463035	0.0359891	CCNB1, CCNE1, CDK1, CDC45, TTK, CDC20
Upregulated	hsa04060	Cytokine-cytokine receptor interaction	8	3.112840467	0.05301106	CCL20, CXCL13, CXCL9, CXCL8, IL1B, CXCR2, IL7R, CXCL10
Upregulated	hsa04623	Cytosolic DNA-sensing pathway	4	1.556420233	0.06819191	IRF7, IL1B, AIM2, CXCL10
Upregulated	hsa04668	TNF signaling pathway	5	1.945525292	0.07110534	CXCL1, NOD2, CCL20, IL1B, CXCL10
Upregulated	hsa04115	p53 signaling pathway	4	1.556420233	0.07600871	CCNB1, CCNE1, CDK1, RRM2
Upregulated	hsa04622	RIG-I-like receptor signaling pathway	4	1.556420233	0.08420339	ISG15, IRF7, CXCL8, CXCL10
Downregulated	hsa04960	Aldosterone-regulated sodium reabsorption	3	3.488372093	0.01441893	HSD11B1, NR3C2, ATP1A2
Downregulated	hsa03320	PPAR signaling pathway	3	3.488372093	0.03821865	ACOX2, LPL, ACADL

KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

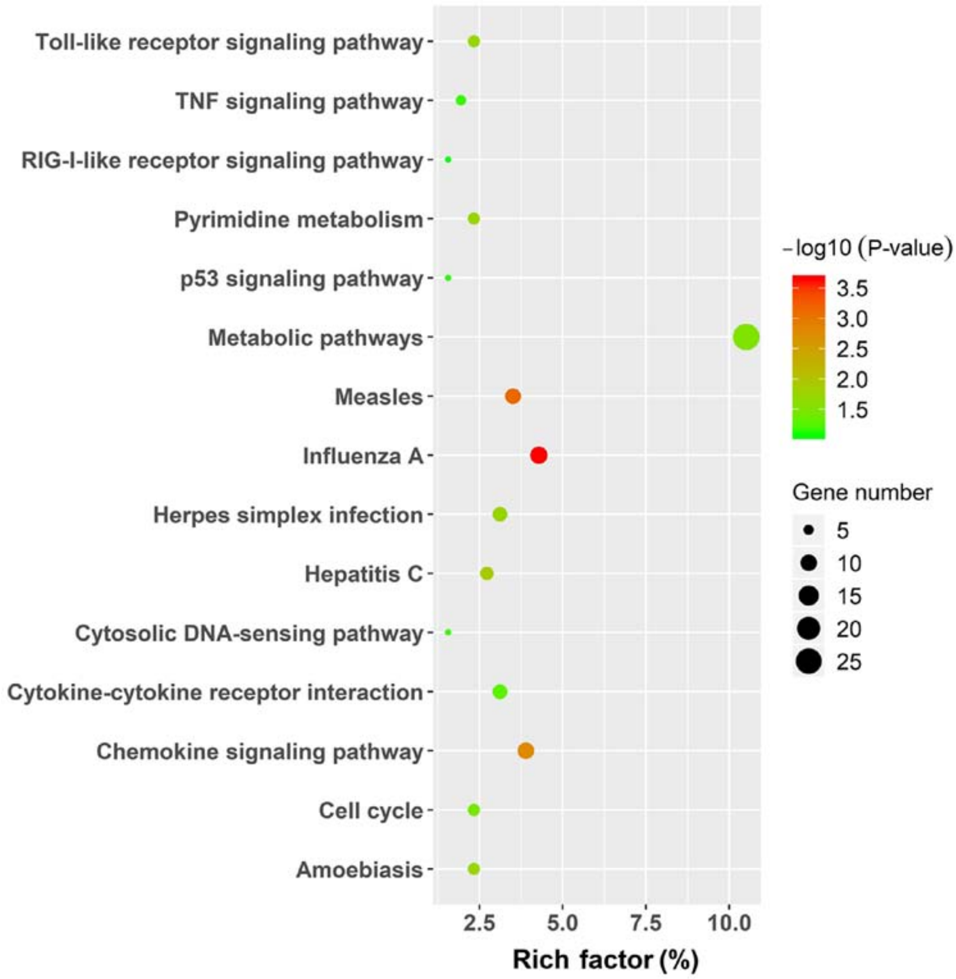

Figure 4. KEGG pathway enrichment of upregulated DEGs. The upregulated DEGs were mainly enriched in metabolic and viral infection KEGG pathways. Rich factor (%) is the ratio of the number of differentially expressed genes annotated in a pathway (as indicated in the y-axis) to the number of all genes annotated in this pathway. DEGs, differently expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

The results from our KEGG pathway analysis revealed that a high enrichment in metabolic and viral infection pathways was present in upregulated genes. There were 26 genes enriched in metabolic pathways, including *XDH*, *GDA*, *KYNU*, *HSD17B2*, *NT5C3A*, *GALNT6*, *CYP2C18*, *UPP1*, *AASS*, *PNP*, *CMPK2*, *ARG1*, *TYMP*, *HPSE*, *ALOX12B*, *FUT2*, *SPTLC2*, *DHRS9*, *HYAL4*, *ST6GALNAC1*, *SQLE*, *RRM2*, *AKR1B10*, *LIPG*, *GK*, *SMPD3* and *PLA2G4D*. Among these genes, *ALOX12B*, one of the lipoxygenases, is reported to play an important role in the regulation of epithelial proliferation, differentiation, wound healing and inflammatory skin diseases (34). *PLA2G4D*, a member of phospholipase A2, was revealed to have a strong gene expression in the upper spinous layer of psoriatic epidermis, while in normal skin the expression of *PLA2G4D* was not detected (35). The expression or functions of the other genes in our list have received little attention with regard to their roles in cutaneous disorders. Therefore, these genes may provide important new research targets for the understanding and treatment of psoriasis. Most of the genes enriched in viral infection KEGG pathways are IFN-α-inducible genes which belong to one group of the hub genes in the PPI network.

The PPI network was constructed by Cytoscape software and hub genes were then determined. With this analysis, 17 genes were identified and divided into 2 groups according to protein-protein interactions. One group of these were IFN-α-inducible genes, which were also enriched in KEGG viral infection pathways, and included *IFI6*, *IFI27*, *IFIT1*, *RSAD2* and *ISG20*. A role for IFN-α in psoriasis development has been gradually revealed. For example, Garcia-Romo *et al* demonstrated that in the initial phase of disease development, cutaneous accumulated plasmacytoid pre-dendritic cells become activated and produce IFN-α, which then drives the stimulation of autoimmune T cells in pre-psoriatic skin (30). Such a mechanism provides an explanation for the role of innate immunity in connecting environmental triggers, such as viral or bacterial infection and wound healing with disease-associated autoimmune T cells. This also clarifies the reason for an absence of IFN-α in our analysis, since the samples we selected were all from chronic plaque psoriasis patients. The expression of IFN-α-inducible genes in our study was also observed in scalp psoriatic samples, demonstrating an important role for IFN-α in the pathogenesis of psoriasis within different skin areas. The other group of genes including, *CSK2*, *CDC45*, *MCM10*, *SPC25*, *NDC80*, *NUF2*, *AURKA*, *CENPE*, *RRM2*, *DLGP5*, *HMMR* and *TTK*, were associated with the regulation of the cell cycle, mitosis and proliferation (36,37). Notably, there are two kinases in this group of hub genes:

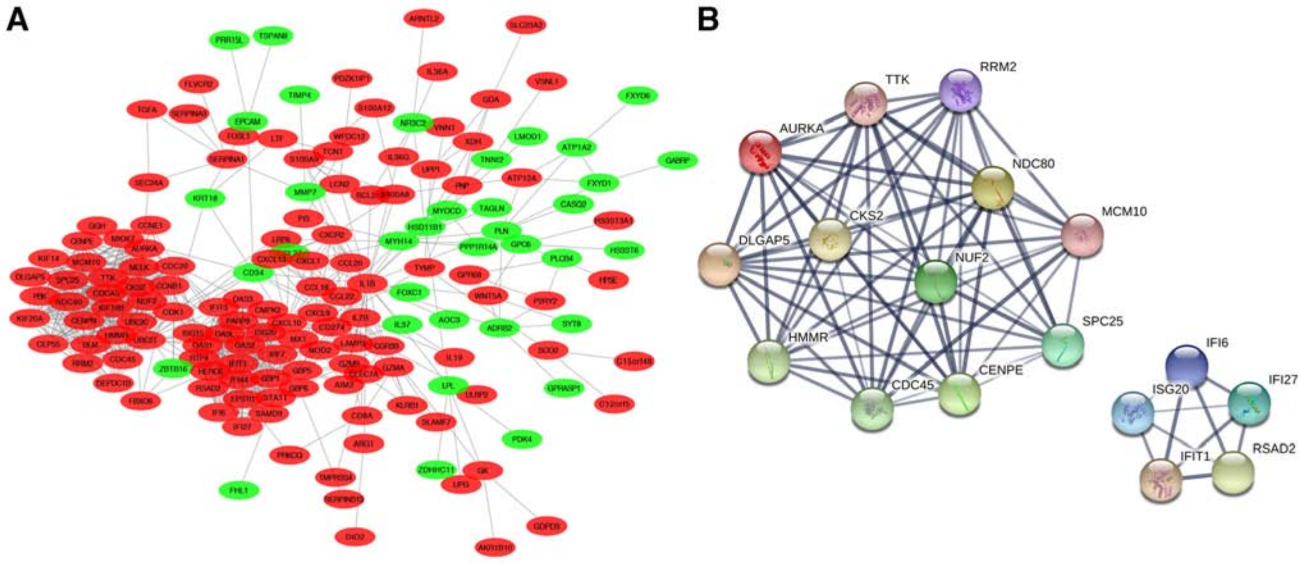

Figure 5. PPI network and hub genes. (A) The PPI network was constructed and formatted with upregulated genes revealed in red ellipses and downregulated genes in green ellipses. (B) Hub genes, represented as circles, were separated into 2 groups and the interaction evidence degree between proteins is presented as the gray scale of the lines. PPI, protein-protein interaction.

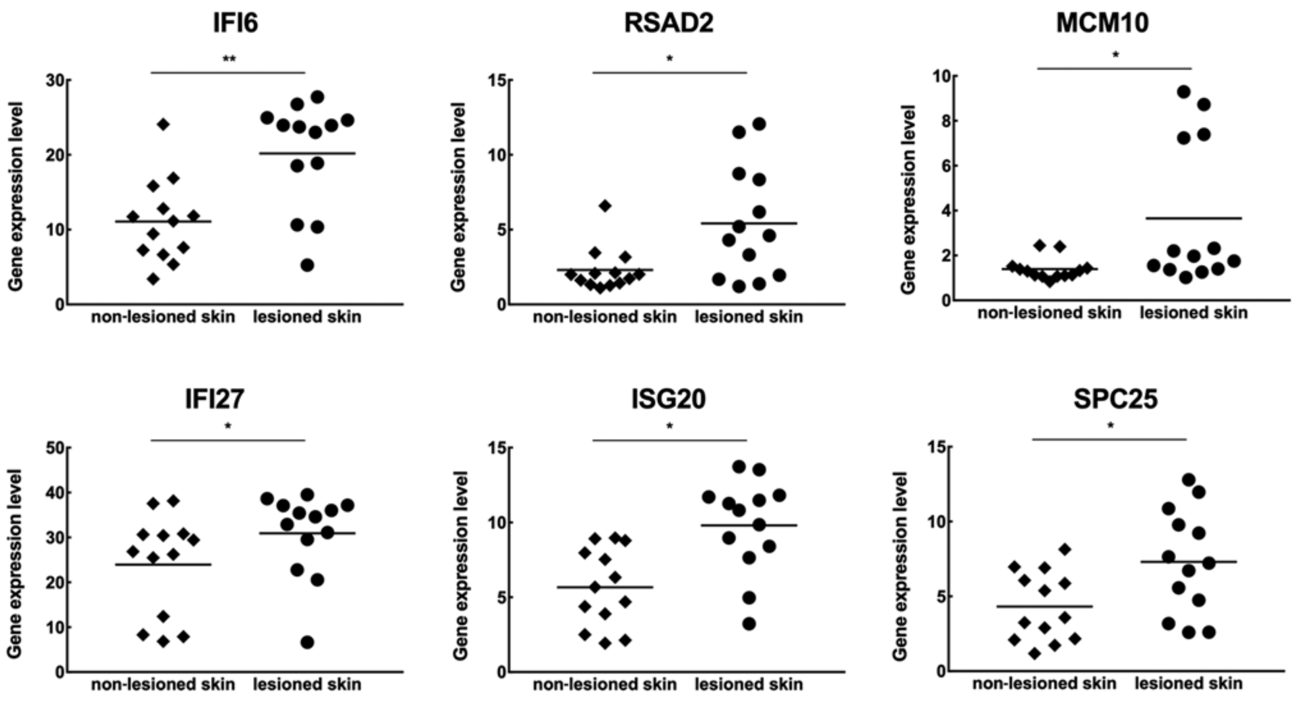

Figure 6. Gene expression levels in scalp psoriasis. Hub genes with significantly different expression levels between lesional and non-lesional scalp samples are plotted. The asterisk (*) indicates statistically significant differences between non-lesional scalp and lesional scalp samples (*P<0.05, **P<0.01).

Aurora kinase A, essential for chromosome segregation (38) and TTK, whose expression is at high levels in tissues which contain large numbers of proliferating cells (39). While the relationship between these kinases and psoriasis development is yet unclear, there exists a potentially important role that they may play in the pathogenesis of psoriasis. In contrast to the results in the IFN-α-inducible gene group, the expression of most hub genes associated with the cell cycle and proliferation in scalp psoriatic samples (except for *MCM10* and *SPC25*), revealed no significant differences from that of paired control samples. Within the scalp area a large proportion of follicles are in anagen, which may contribute to a set of highly expressed genes associated with the cell cycle and proliferation. This can be contrasted with that of skin samples from other areas where most hair follicles are in catagen or telophase. Histologically, in the initial stages of this disease scalp psoriasis mainly affects the interfollicular epidermis with perifollicular inflammation (13), while later stages include destruction of hair follicles with perifollicular fibrosis and hair loss (40). Based on these findings, it was hypothesized that the expression of these cell cycle-related genes, which are assumed to be at high expression levels in psoriatic samples, is relatively reduced in scalp psoriatic samples where hair follicle destruction occurs as compared to normal scalp tissues, as reflected in our results.

Although a similar bioinformatical study on psoriasis has been performed (7), in our present study, we limited our data sets to paired lesional and non-lesional psoriatic skin samples and performed DEG analysis with use of paired-sample t-tests in each data set. An overlap method was subsequently employed to combine these DEG results as a means to obtain an overall set of DEGs corresponding with that of each data set. With use of these strict screening methods, we consider that our results have a relatively high degree of specificity for detecting pivotal disease-associated molecules, however the resultant low sensitivity would be considered as a limitation of this study. In our future research, if ethical approval is obtained, RT-qPCR validation of these identified target genes in clinical samples will be conducted. In conclusion, through a comprehensive bioinformatic re-analysis of these original GEO data, an overall view regarding the molecular pathogenesis of psoriasis and the potential for identification of therapeutic targets for this disease was provided.

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Availability of data and materials

All data generated or analyzed in the present study are included in this published article.

Authors' contributions

YJZ collected the online microarray data and the corresponding clinical information and drafted the manuscript. YJZ and YZS performed the bioinformatic and statistical analysis. XHG and RQQ contributed to the study design and performed the proofreading and revision of the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

References

1. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD, Wu JJ, Mehta NN, Finlay AY and Gottlieb AB: Psoriasis. Nat Rev Dis Primers 2: 16082, 2016.
2. Parisi R, Symmons DP, Griffiths CE and Ashcroft DM; Identification and Management of Psoriasis and Associated ComorbidiTy (IMPACT) project team: Global epidemiology of psoriasis: A systematic review of incidence and prevalence. J Invest Dermatol 133: 377-385, 2013.
3. Martinez-Garcia E, Arias-Santiago S, Valenzuela-Salas I, Garrido-Colmenero C, Garcia-Mellado V and Buendia-Eisman A: Quality of life in persons living with psoriasis patients. J Am Acad Dermatol 71: 302-307, 2014.
4. Egeberg A, Thyssen JP, Wu JJ and Skov L: Risk of first-time and recurrent depression in patients with psoriasis: A population-based cohort study. Br J Dermatol 180: 116-121, 2019.
5. Ainali C, Valeyev N, Perera G, Williams A, Gudjonsson JE, Ouzounis CA, Nestle FO and Tsoka S: Transcriptome classification reveals molecular subtypes in psoriasis. BMC genomics 13: 472, 2012.
6. Mei R and Mei X: Screening of skin lesion-associated genes in patients with psoriasis by meta-integration analysis. Dermatology 233: 277-288, 2017.
7. Sevimoglu T and Arga KY: Computational systems biology of psoriasis: Are we ready for the age of omics and systems biomarkers? OMICS 19: 669-687, 2015.
8. Yao Y, Richman L, Morehouse C, de los Reyes M, Higgs BW, Boutrin A, White B, Coyle A, Krueger J, Kiener PA and Jallal B: Type I interferon: Potential therapeutic target for psoriasis? PLoS One 3: e2737, 2008.
9. Suárez-Fariñas M, Li K, Fuentes-Duculan J, Hayden K, Brodmerkel C and Krueger JG: Expanding the psoriasis disease profile: Interrogation of the skin and serum of patients with moderate-to-severe psoriasis. J Invest Dermatol 132: 2552-2564, 2012.
10. Correa da Rosa J, Kim J, Tian S, Tomalin LE, Krueger JG and Suárez-Fariñas M: Shrinking the psoriasis assessment gap: Early gene-expression profiling accurately predicts response to long-term treatment. J Invest Dermato 137: 305-312, 2017.
11. Bigler J, Rand HA, Kerkof K, Timour M and Russell CB: Cross-study homogeneity of psoriasis gene expression in skin across a large expression range. PLoS One 8: e52242, 2013.
12. Russell CB, Rand H, Bigler J, Kerkof K, Timour M, Bautista E, Krueger JG, Salinger DH, Welcher AA and Martin DA: Gene expression profiles normalized in psoriatic skin by treatment with brodalumab, a human anti-IL-17 receptor monoclonal antibody. J Immunol 192: 3828-3836, 2014.
13. Ruano J, Suárez-Fariñas M, Shemer A, Oliva M, Guttman-Yassky E and Krueger JG: Molecular and cellular profiling of scalp psoriasis reveals differences and similarities compared to skin psoriasis. PLoS One 11: e0148450, 2016.
14. Sherman BT, Huang da W, Tan Q, Guo Y, Bour S, Liu D, Stephens R, Baseler MW, Lane HC and Lempicki RA: DAVID Knowledgebase: A gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis. BMC Bioinformatics 8: 426, 2007.
15. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, *et al*: The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 45: D362-D368, 2017.
16. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT and Lin CY: cytoHubba: Identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 8 (Suppl 4): S11, 2014.
17. Ishida-Yamamoto A and Iizuka H: Structural organization of cornified cell envelopes and alterations in inherited skin disorders. Exp Dermatol 7: 1-10, 1998.
18. Telfer NR, Chalmers RJ, Whale K and Colman G: The role of streptococcal infection in the initiation of guttate psoriasis. Arch Dermatol 128: 39-42, 1992.
19. Morhenn VB: The relationship of wound healing with psoriasis and multiple sclerosis. Adv Wound Care (New Rochelle) 7: 185-188, 2018.
20. Sweeney CM, Tobin AM and Kirby B: Innate immunity in the pathogenesis of psoriasis. Arch Dermatol Res 303: 691-705, 2011.
21. Armstrong AW, Harskamp CT, Dhillon JS and Armstrong EJ: Psoriasis and smoking: A systematic review and meta-analysis. Br J Dermatol 170: 304-314, 2014.

22. Qureshi AA, Dominguez PL, Choi HK, Han J and Curhan G: Alcohol intake and risk of incident psoriasis in US women: A prospective study. Arch Dermatol 146: 1364-1369, 2010.
23. Jensen P and Skov L: Psoriasis and obesity. Dermatology 232: 633-639, 2016.
24. Frantz C, Stewart KM and Weaver VM: The extracellular matrix at a glance. J Cell Sci 123: 4195-4200, 2010.
25. Theocharis AD, Skandalis SS, Gialeli C and Karamanos NK: Extracellular matrix structure. Adv Drug Deliv Rev 97: 4-27, 2016.
26. McFadden J, Fry L, Powles AV and Kimber I: Concepts in psoriasis: Psoriasis and the extracellular matrix. Br J Dermatol 167: 980-986, 2012.
27. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y and Zychlinsky A: Neutrophil extracellular traps kill bacteria. Science 303: 1532-1535, 2004.
28. Brinkmann V and Zychlinsky A: Neutrophil extracellular traps: Is immunity the second function of chromatin? J Cell Biol 198: 773-783, 2012.
29. Kessenbrock K, Krumbholz M, Schönermarck U, Back W, Gross WL, Werb Z, Gröne HJ, Brinkmann V and Jenne DE: Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med 15: 623-625, 2009.
30. Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, Punaro M, Baisch J, Guiducci C, Coffman RL, *et al*: Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med 3: 73ra20, 2011.
31. Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S, Villanueva EC, Shah P, Kaplan MJ and Bruce AT: Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. J Immunol 187: 490-500, 2011.
32. Law RH, Zhang Q, McGowan S, Buckle AM, Silverman GA, Wong W, Rosado CJ, Langendorf CG, Pike RN, Bird PI and Whisstock JC: An overview of the serpin superfamily. Genome Biol 7: 216, 2006.
33. Johnston A, Xing X, Wolterink L, Barnes DH, Yin Z, Reingold L, Kahlenberg JM, Harms PW and Gudjonsson JE: IL-1 and IL-36 are dominant cytokines in generalized pustular psoriasis. J Allergy Clin Immunol 140: 109-120, 2017.
34. Krieg P and Fürstenberger G: The role of lipoxygenases in epidermis. Biochim Biophys Acta 1841: 390-400, 2014.
35. Chiba H, Michibata H, Wakimoto K, Seishima M, Kawasaki S, Okubo K, Mitsui H, Torii H and Imai Y: Cloning of a gene for a novel epithelium-specific cytosolic phospholipase A2, cPLA2delta, induced in psoriatic skin. J Biol Chem 279: 12890-12897, 2004.
36. Kudalkar EM, Scarborough EA, Umbreit NT, Zelter A, Gestaut DR, Riffle M, Johnson RS, MacCoss MJ, Asbury CL and Davis TN: Regulation of outer kinetochore Ndc80 complex-based microtubule attachments by the central kinetochore Mis12/MIND complex. Proc Natl Acad Sci USA 112: E5583-E5589, 2015.
37. Santaguida S and Musacchio A: The life and miracles of kinetochores. EMBO J 28: 2511-2531, 2009.
38. DeLuca KF, Meppelink A, Broad AJ, Mick JE, Peersen OB, Pektas S, Lens SMA and DeLuca JG: Aurora A kinase phosphorylates Hec1 to regulate metaphase kinetochore-microtubule dynamics. J Cell Bio 217: 163-177, 2018.
39. Mills GB, Schmandt R, McGill M, Amendola A, Hill M, Jacobs K, May C, Rodricks AM, Campbell S and Hogg D: Expression of TTK, a novel human protein kinase, is associated with cell proliferation. J Biol Chem 267: 16000-16006, 1992.
40. George SM, Taylor MR and Farrant PB: Psoriatic alopecia. Clin Exp Dermatol 40: 717-721, 2015.