Psoriasis: Targets and Therapy

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ORIGINAL RESEARCH

High-Throughput RNA Sequencing Reveals the Effect of NB-UVB Phototherapy on Major Inflammatory Molecules of Lesional Psoriasis

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Objective: To identify the narrowband ultraviolet B (NB-UVB)-induced molecular mechanisms that may account for their anti-inflammatory efficacy, gene expression and transcriptome profiling, which were performed using advanced molecular techniques.

Methods: This research was conducted on patients with moderate-to-severe plaque-type psoriasis who received NB-UVB treatment. RNA sequencing (RNA-Seq) was conducted to assay the transcriptomes and identify the differentially expressed transcripts that had been enriched during the major pathway analysis.

Results: Clinical improvement of psoriasis by NB-UVB therapy is linked to the suppression of the "immunological signaling pathways" and "cell cycle regulatory, growth and proliferation pathways" which are critical to the pathogenesis of the disease. In addition, these results were further substantiated by demonstrating that NB-UVB therapy has a significant effect on keratinocyte differentiation and affects the regulation of genes and inflammatory mediators that are related to cell proliferation and apoptosis. Moreover, NB-UVB phototherapy is also involved with the downregulation of toll-like receptors signaling in lesional psoriasis.

Conclusion: NB-UVB is an effective treatment for psoriasis. Our study supports the conclusion that the clinical effectiveness of NB-UVB therapy is based on the suppression of a broad range of inflammatory signaling pathways, gene expression of inflammatory cytokines and increased expressions of anti-inflammatory signaling pathways in psoriatic skin. This is the first study that applied advanced molecular techniques to investigate phototherapy as a new key to unlock genetic knowledge and create novel information. Ultimately, the goal is to increase medical knowledge and improve the patient care of psoriasis.

Keywords: psoriasis, pathogenesis, inflammation, NB-UVB, high-throughput RNA-sequencing, Inflammation, gene, translational study, chronic skin disease, UVB irradiation

Introduction

Psoriasis vulgaris, major chronic immune-mediated skin disease, affects 1–3% of the adult population worldwide.[1] Psoriasis is one of the major complex skin diseases that is significantly linked to genetic predisposition, major histocompatibility alleles, and environmental factors.[2] Over the past few years, the cellular and molecular contributions to psoriasis were further elucidated. Pathways of cytokine and chemokine are directly linked to cellular communication and controlling inflammatory networks in psoriasis. Significantly, the contribution of major gene products to psoriatic disease has also been investigated through targeting of key immune components, These genes span an array of functions involving innate immunity and adaptive immune responses.[3]

Received: 31 August 2021
Accepted: 28 October 2021
Published: 26 November 2021

Treatments of psoriasis are recommended with systemic drugs, biologic agents, topical therapies, and phototherapy. These can control the disease, but there is no long-term cure. NB-UVB phototherapy is suggested as an alternative major treatment for psoriasis. Mechanisms of this intervention on the pathogenesis of the disease have been investigated during the decade involving DNA damage, production of reactive oxygen species, apoptosis, cell cycle arrest, cellular proliferation, vitamin D synthesis, immunosuppression, and cytokine productions.[4–8] Advanced laboratory techniques have already been applied in the dermatological field to identify biomarkers and effective treatment, especially inflammatory skin diseases.[9,10] Recently, next-generation sequencing (NGS) has been introduced with satisfactory results of high efficacy, accuracy, and precision.[11–14] NGS is used for transcriptomic profiling to compensate for the limitations of the sample size, while avoiding the problem caused by the shortcomings of the microarrays.[15] Previous studies have shown that genes in lesional psoriatic skin were reported with upregulated gene functions such as immune response and epidermal proliferation.[16] Significantly, understanding the genetic and immunological basis of psoriasis is expected to lead to the most effective strategy for psoriasis treatment. However, pathogenesis and molecular mechanisms of phototherapy in psoriasis are still controversial and require further studies with advanced genomic techniques.

This study aimed to understand the mechanisms of psoriasis through analyzing differentially expressed genes with RNA-Seq technique by using phototherapy. This research was conducted in patients with moderate-to-severe plaque-type psoriasis who received NB-UVB treatment. RNA sequencing (RNA-Seq) was conducted to assay the transcriptomes and identify differentially expressed transcripts enriched in major pathway analysis. Our results would provide significant molecular results of the pathogenesis of psoriasis and develop new therapeutic modalities for psoriasis treatment in the future. In addition, pathway analysis after phototherapy treatment would be revealed in this analysis.

Materials and Methods

Patients and Methods

This study was designed as a single-center, clinical experiment, a before-and-after study was conducted at the dermatology outpatient Benjakitti Park Hospital in Thailand between June 2018 and June 2020. This study was approved by the Human Ethics Committee of Thammasat University No. MTU-EC-OO-2-027/60, conducted according to the principles of the Declaration of Helsinki. Three patients diagnosed with moderate-to-severe plaque psoriasis were enrolled. Signed informed consent was obtained from all patients before enrollment. Study participants were asked to discontinue any systemic treatment and phototherapy for at least 4 weeks, and topical therapies for at least 2 weeks before entering the study. Pregnant and lactating women were also excluded. Patients completed detailed demographic data, including reporting information on comorbidities and concomitant medications. Patients were treated with NB-UVB phototherapy. Before starting phototherapy, the participants' skin type was evaluated. A starting dose of 200–300 mJ/cm2 was administrated to the patients using a Waldmann UV5002 cabinet (Villingen-Schwenningen, Germany), which was increased 10% to 20% in subsequent session depending on clinical response and skin tolerance until clearance of disease. NB-UVB phototherapy was recommended to be conducted in 3 sessions per week with dose increments continued until lesion clearance.

Disease severity and response to therapy were assessed using the psoriasis area severity index (PASI) score before and during treatment. Clinical samples, including skin biopsy samples, were collected at baseline and after successful treatment (PASI 75 reduction). Patients would be followed up every 2 weeks for the first month, then every 4 weeks until the end of the study. Other side effects were investigated such as erythema, tenderness, burning sensation, pruritic symptom. PASI scores were evaluated before, during, and after treatment. Treatment was continued until a 75% reduction in original PASI score was reached, if the 12-week period had not been completed.

Tissue samples were collected from patients using a 4-mm punch biopsy from psoriasis skin before the start of NB-UVB therapy and 7 days after the last treatment session. To further elucidate molecular alterations in psoriasis, we performed a gene expression study of skin samples. The library preparation process was performed using a TruSeq RNA preparation kit (pair-end, 150 bp), following the manufacturer's instruction (Bioactive, Thailand). Briefly, purified RNA was randomly fragmented for short-read sequencing and fragmented RNA was reverse transcribed into cDNA. Next, ligate adapters onto both ends of the cDNA fragments. After amplifying fragments using PCR, fragments were selected with insert sizes between 200 and

400 bp. The cDNA library was sequenced using NextSeq and results were obtained from the DesingStudio program running on a machine. Total RNA was extracted from the frozen biopsies using TRIzol™ Reagent (Ambion) following the manufacturer's instructions. The NGS was performed by a commercial sequencing facility (Macrogen, Seoul, Korea). Briefly, RNA quality and quantity were checked using an Agilent 2100 Bioanalyzer with an RNA Integrity Number (RIN). Libraries for high throughput sequencing were prepared using the SMARTer Universal Low Input RNA Kit, TruSeq RNA Sample Prep Kit v2. The cDNA was checked for quality and quantity on an Agilent 2100 Bioanalyzer. Libraries sequencing was carried out using a NovaSeq 6000 System (Illumina Inc., San Diego, CA, USA) with 100 bp paired-end reads. The quality control of the sequenced raw reads was analyzed. To reduce biases in the analysis, low-quality reads, adaptor sequence, contaminant DNA, or PCR duplicates were removed. Trimmed reads were mapped to the reference genome (UCSC hg19) with HISAT2, splice-aware aligner. A transcript was assembled by StringTie with aligned reads. Gene differential expression analysis was performed with edgeR Bioconductor statistical library version 3.8 on RStudio.[17,18]

The sequencing results were analyzed for quality control of the sequenced raw reads using FastQC. Overall reads quality, total bases, total reads, GC (%), and basic statistics were calculated. To reduce bias in the analysis, artifacts such as low-quality reads, adaptor sequence, contaminant DNA, or PCR duplicates were removed. Trimmed reads were mapped to reference genome with HISAT2, splice-aware aligner. The transcript was assembled by StringTie with aligned reads. Expression profiles are represented as reading count and normalization value which is based on transcript length and depth of coverage. The FPKM (Fragments Per Kilobase of transcript per Million Mapped reads) value is used as a normalization value.

Functional and Pathway Analysis

Metascape was employed to perform the gene enrichment and functional annotation analyses.[19] In addition, functional annotation of these genes was performed using the Database for Annotation, Visualization, and Integrated Discovery.[20] The lists of significant genes expressed in psoriasis lesions before and after NB-UVB therapy were investigated with Ingenuity Pathway Analysis software (IPA) to identify signaling pathways represented by these genes.[21] IPA was used in the analysis of biological information, common differential genes, canonical pathways, and upstream regulators. Functional gene analysis was performed using the upstream regulator function in IPA. In addition, the score was acquired by the -log of right-tailed Fisher's exact test.[22] Overlap P-value and activation Z-score were calculated. $P < 0.05$ was considered to be significant. While, Z-score > 2 or <-2 was considered as significant with a calculation based on the expression association between regulators and genes, and weighted correction.[22]

Results

To investigate the pathophysiological mechanisms underlying remission of psoriasis with phototherapy, transcriptome profiling was performed with lesion samples before and after the intervention. At the end of treatment, all patients showed clearance of plaque and reached stable remission, which were the hallmark of the end of acute psoriatic inflammation. The characteristics of patients with psoriasis are shown in Table 1.

Differentially expressed coding genes (DEGs) were then identified by comparing the post-treatment with the pre-treatment transcriptomes of each patient. A heatmap and the volcano plot of DEGs show global transcriptome changes in all patients after NB-UVB treatment (Figure 1A and B). A total of 684 DEGs were identified when comparing before and after the intervention, which were 271 upregulated (39.62%) and 413 downregulated (60.38%). The top up- or down-regulated DEGs after NB-UVB therapy are shown in Tables S1 and S2.

Functional Enrichment and Annotation

Functional enrichment and annotation for DEGs was analyzed using Metascape with the major sources (Figure 2).[19] To gain an in-depth understanding, major

Table 1 Baseline Characteristics of Patients with Psoriasis

Patient	Age	Sex	PASI Before	PASI After	BMI	Skin Lesion	Scalp Lesion	Nail Lesion	Alcohol	Smoking
1	57	Male	12.5	2	24.4	Yes	Yes	Yes	No	No
2	23	Male	12.2	1	20.3	Yes	Yes	Yes	No	No
3	39	Male	35.5	5	20.5	Yes	No	Yes	No	No

genes and functions investigated with DAVID Bioinformatics Resources are shown in Table 2.[20]

NB-UVB Therapy Affects Recognized Therapeutic Targets in Psoriasis and Pathway Analysis

Phototherapy can affect recognized therapeutic targets in psoriasis. IPA was conducted to identify related signaling pathways involved in the mechanism of NB-UVB therapy in psoriasis. Canonical pathway analysis with the IPA software was performed and nine significant pathways were identified (Figure 3A and B). The top significantly changed canonical pathways were "role of IL-17A in psoriasis pathway", "PPAR signaling" "IL-6 signaling", and "p38 MAPK signaling pathway".

All major signaling pathways that were affected by NB-UVB therapy in psoriasis shown in Table 3 and Figure S1. It can be categorized into 4 groups, including NB-UVB regulated pathways that are "immunological signaling pathways", "Cell cycle regulatory, growth, and proliferation pathways", "Therapeutic targets for psoriasis", and others.

Regulatory Network Analysis

Upstream regulator analysis, based on expected causal effects between upstream regulators and targets, was analyzed to predict upstream molecules associated with changes in gene expression. Upstream regulators have positive z-scores, indicating that their downstream effects were activated whereas upstream regulators with negative z-scores were inhibited downstream effects. The top upstream regulators predicted to be inhibited by z-score are presented in Table 4. TNF was the most predicted inhibited upstream regulators by Z-score after NB-UVB. The top upstream regulators that were predicted to be activated are presented in Table 5. Interestingly, top upstream regulators associated with a molecule of cytokine were analyzed as shown in Table 6. In addition, the top upstream regulators associated with transmembrane receptors were ranked by absolute z-score and are presented in Table 7. Moreover, the top 50 upstream regulators were predicted to be inhibited and activated attached in Tables S3 and S4.

In the Regulatory effects network, STING1, TLR9, IL-17C, IL-1A, IL-36A, CD2, IL-17R, NFKBIZ, and TNIP1 were identified as the upstream regulators, which target 27 downstream DEGs, resulting in downregulated effects on immunological cells (phagocytes and granulocytes), proliferation of epithelial cells, growth of connective tissue, and migration of tumor cell lines. Interestingly, STING1 was proposed as a major upstream regulator that can regulate immunological and proliferation of epidermal cells, implying that our analysis may provide insight into new targets for psoriasis (Figure 4).

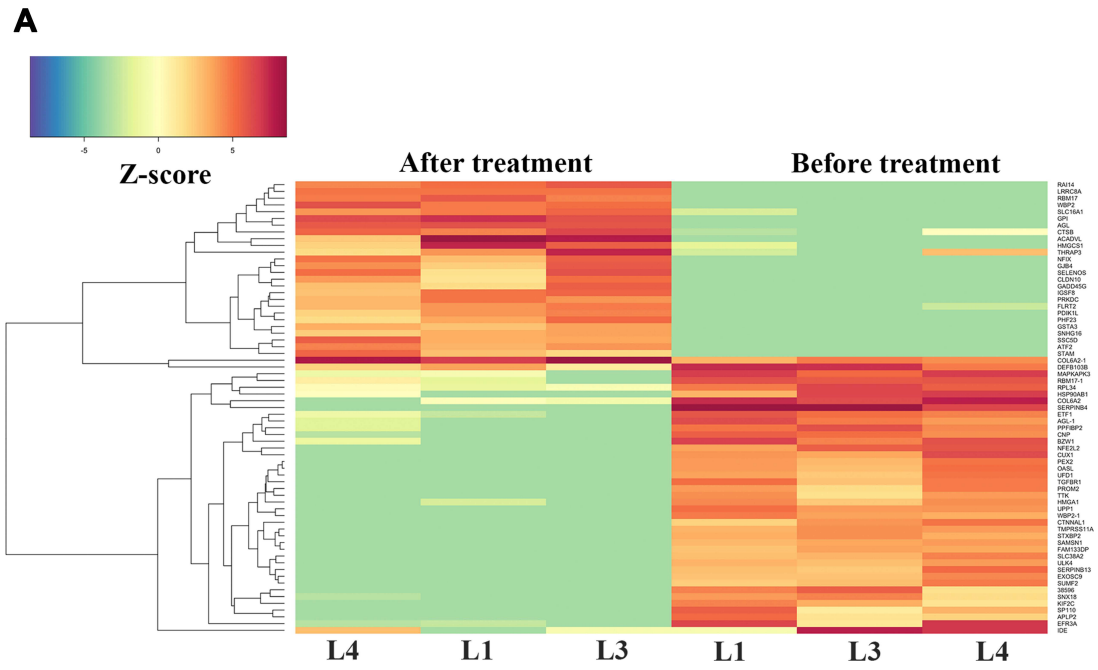

Figure 1 Continue.

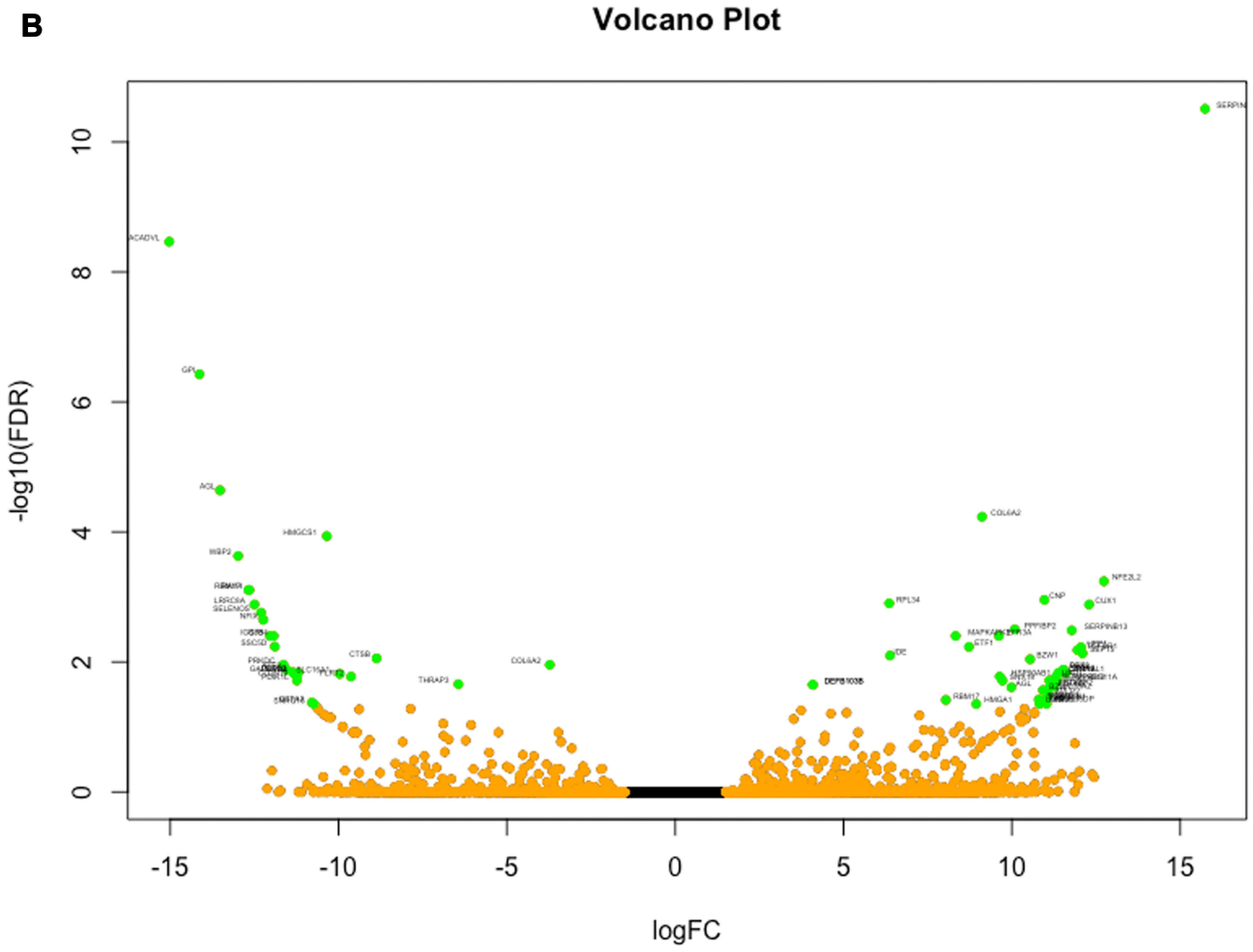

Figure 1 (**A**) Heatmap visualization of z-scores for DEGs identified in samples before and after intervention. (**B**) Volcano plot of RNA-Seq data. The Volcano plot can show differential RNA expression between the two groups (expression of after phototherapy versus before phototherapy). The horizontal line represents a P-value of 0.05 (log10 scaled). The red dots represent significant differentially-expressed genes, and the black dots represent gene expression that was not significantly different.

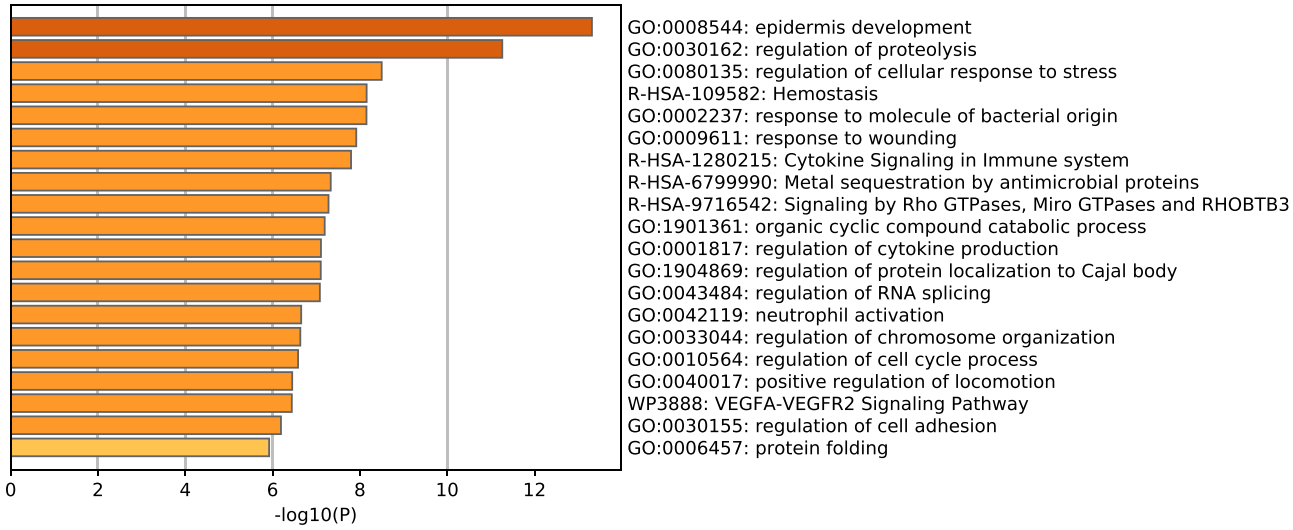

Figure 2 Functional enrichment and annotation for DEGs analyzed using Metascape with major sources, including GO Biological Processes, Reactome Gene Sets and WikiPathways.

Table 2 Significant Down-Regulated Genes Involved with Major Pathogenesis of Psoriasis are Affected by NB-UVB Phototherapy in Psoriasis Lesion Using DAVID Bioinformatics Resources

	Gene	Symbol	Log Ratio	p-value
Epidermis development	ATP2C1	ATPase secretory pathway Ca2+ transporting 1(ATP2C1)	−7.457	0.0133
	S100A7	S100 calcium binding protein A7(S100A7)	−3.664	−3.664
	CRABP2	Cellular retinoic acid binding protein 2(CRABP2)	−7.383	0.000648
	KLK7	Kallikrein related peptidase 7(KLK7)	−3.767	0.0453
	KRT16	Keratin 16(KRT16)	−5.091	0.000284
	KRT17	Keratin 17(KRT17)	−4.672	0.0364
	PLOD1	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1(PLOD1)	−4.411	0.00324
	SPRR1A	Small proline rich protein 1A(SPRR1A)	−2.587	0.0212
	SPRR1B	Small proline rich protein 1B(SPRR1B)	−1.74	0.039
	SPRR2A	Small proline rich protein 2A(SPRR2A)	−4.449	0.000943
	SPRR2B	Small proline rich protein 2B(SPRR2B)	−2.58	0.0104
	SPRR2D	Small proline rich protein 2D(SPRR2D)	−2.551	0.00634
	SPRR2F	Small proline rich protein 2F(SPRR2F)	−3.517	0.000398
Cell division	MAD2L2	MAD2 mitotic arrest deficient-like 2 (yeast)(MAD2L2)	−7.187	0.0318
	SETDB2	SET domain bifurcated 2(SETDB2)	−9.154	0.0467
	BIRC5	Baculoviral IAP repeat containing 5(BIRC5)	−9.644	0.00027
	CSNK1A1	Casein kinase 1 alpha 1(CSNK1A1)	−9.284	0.046
	CDC25A	Cell division cycle 25A(CDC25A)	−5.608	0.00641
	CENPF	Centromere protein F(CENPF)	−2.811	0.00328
	CCNA2	Cyclin A2(CCNA2)	−3.178	0.00721
	CCNB1	Cyclin B1(CCNB1)	−2.671	0.0318
	KIF20B	Kinesin family member 20B(KIF20B)	−2.833	0.0274
	KIF2C	Kinesin family member 2C(KIF2C)	−11.028	0.00018
	PTTG1	Pituitary tumor-transforming 1(PTTG1)	−10.162	0.0324
	PPP1CC	Protein phosphatase 1 catalytic subunit gamma(PPP1CC)	−5.216	0.0313
	PPP2R2D	Protein phosphatase 2 regulatory subunit Bdelta(PPP2R2D)	−8.906	0.0426
	RNF8	Ring finger protein 8(RNF8)	−6.833	0.0194
	STAG2	Stromal antigen 2(STAG2)	−10.386	0.0404
	TACC1	Transforming acidic coiled-coil containing protein 1(TACC1)	−8.934	0.0252

Cell-cell adherent junction	LASP1	LIM and SH3 protein 1(LASP1)	−9.115	0.0121
	LMO7	LIM domain 7(LMO7)	−2.211	0.0156
	RANBP1	RAN binding protein 1(RANBP1)	−2.683	0.0301
	ANLN	Anillin actin binding protein(ANLN)	−10.681	0.000294
	ANXA1	Annexin A1(ANXA1)	−2.04	0.0175
	BZW1	Basic leucine zipper and W2 domains 1(BZW1)	−10.922	0.0000952
	BZW2	Basic leucine zipper and W2 domains 2(BZW2)	−5.762	0.0206
	CDH3	Cadherin 3(CDH3)	−1.694	0.0316
	CALD1	Caldesmon 1(CALD1)	−3.857	0.0308
	CTNND1	Catenin delta 1(CTNND1)	−10.396	0.0437
	CHMP5	Charged multivesicular body protein 5(CHMP5)	−1.815	0.0446
	DSC2	Desmocollin 2(DSC2)	−5.116	0.0278
	FLOT1	Flotillin 1(FLOT1)	−5.013	0.0476
	HSP90AB1	Heat shock protein 90 alpha family class B member 1(HSP90AB1)	−9.639	0.0000464
	KTN1	Kinectin 1(KTN1)	−3.715	0.00133
	PPME1	Protein phosphatase methylesterase 1(PPME1)	−8.728	0.0251
	RPL34	Ribosomal protein L34(RPL34)	−6.358	0.000000907
	STK24	Serine/threonine kinase 24(STK24)	−5.568	0.0243
	TMPO	Thymopoietin(TMPO)	−8.71	0.017
	TJP1	Tight junction protein 1(TJP1)	−9.751	0.0424
	TWF1	Twinfilin actin binding protein 1(TWF1)	−5.981	0.028

(*Continued*)

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Table 2 (Continued).

	Gene	Symbol	Log Ratio	p-value
Inflammatory response and Immunity	**Cytokine and chemokine activity**			
	SP100	SP100 nuclear antigen(SP100)	−3.352	0.0499
	IL36RN	Interleukin 36 receptor antagonist(IL36RN)	−2.585	0.0486
	IL36A	Interleukin 36, alpha(IL36A)	−10.156	0.00224
	IL36G	Interleukin 36, gamma(IL36G)	−3.612	0.00203
	NAMPT	Nicotinamide phosphoribosyltransferase(NAMPT)	−1.623	0.0429
	CXCL8	C-X-C motif chemokine ligand 8(CXCL8)	−9.997	0.00121
	Positive regulation of NF-κB transcription factor activity			
	CLU	Clusterin(CLU)	−7.093	0.0325
	IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta(IKBKB)	−8.495	0.0236
	NPM1	Nucleophosmin(NPM1)	−1.676	0.0427
	RPS6KA4	Ribosomal protein S6 kinase A4(RPS6KA4)	−7.07	0.0494
	Innate immunity			
	S100A12	S100 calcium binding protein A12(S100A12)	−8.161	0.0441
	S100A8	S100 calcium binding protein A8(S100A8)	−4.491	0.0106
	S100A9	S100 calcium binding protein A9(S100A9)	−5.832	0.00372
	LCN2	Lipocalin 2(LCN2)	−5.05	0.0173
	Type I interferon signaling pathway			
	OAS2	2′-5′-oligoadenylate synthetase 2(OAS2)	−2.737	0.00906
	OASL	2′-5′-oligoadenylate synthetase like(OASL)	−11.523	0.0000271
	SP100	SP100 nuclear antigen(SP100)	−3.352	0.0499
	ADAR	Adenosine deaminase, RNA specific(ADAR)	−11.034	0.0117
	Others			
	CLEC7A	C-type lectin domain family 7 member A(CLEC7A)	−9.153	0.00128
	MEFV	Mediterranean fever(MEFV)	−7	0.012
	POLR3F	RNA polymerase III subunit F(POLR3F)	−4.442	0.0386
	C1QBP	Complement C1q binding protein(C1QBP)	−1.994	0.0269
	HMGB3	High mobility group box 3(HMGB3)	−4.795	0.0153
	IFI16	Interferon gamma inducible protein 16(IFI16)	−1.806	0.04
	JAML	Junction adhesion molecule like(JAML)	−5.983	0.00513
	HLA-DPA1	Major histocompatibility complex, class II, DP alpha 1(HLA-DPA1)	−2.442	0.0253
	PTPN22	Protein tyrosine phosphatase, non-receptor type 22(PTPN22)	−3.779	0.0309
	TNIP3	TNFAIP3 interacting protein 3(TNIP3)	−5.305	0.00153
	ACKR2	Atypical chemokine receptor 2(ACKR2)	−3.938	0.034
	GBP5	Guanylate binding protein 5(GBP5)	−3.794	0.0417

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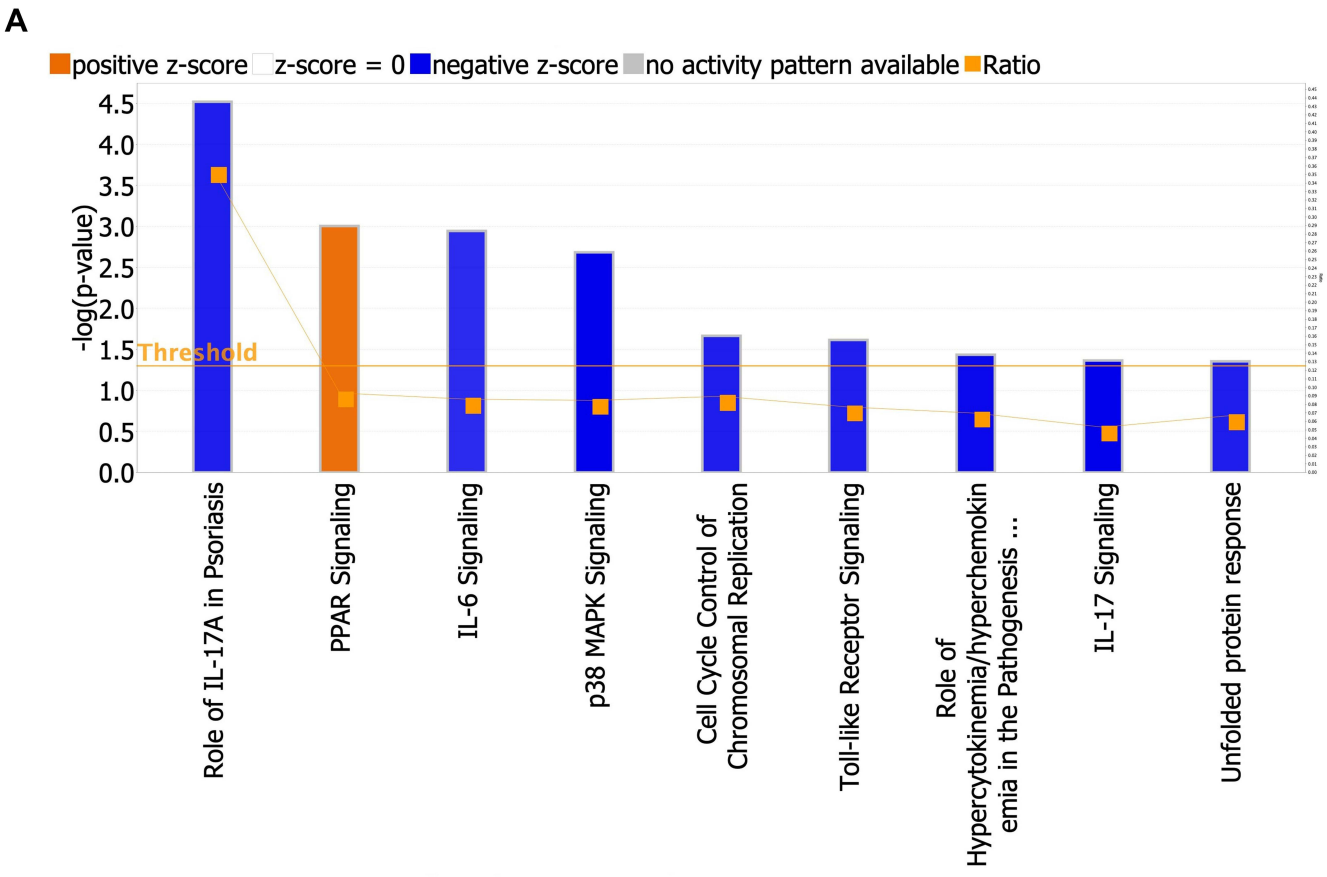

Figure 3 Continue.

Discussion

UVB radiation has been considered to be an effective treatment for psoriasis for decades. This is a potent immunosuppressive intervention that can improve clinical outcomes with prominent inhibition of cell-mediated immune response.[3] Previous researches have been conducted to investigate the efficacy of NB-UVB in psoriasis. However, immunopathogenesis and genetic aspects have not been completely elucidated.[23–25] In this study, considering that psoriasis is an autoinflammatory disease with systemic inflammation, this result suggests that NB-UVB in psoriasis treatment is significantly associated with a change of functional enrichment and annotation with significant enrichment in clusters of immune responses, including "cytokine signaling in immune response", "regulation of cytokine production", and "neutrophil activation". In addition, NB-UVB strongly suppressed genes of the epidermis development, immunity, and inflammatory response. The expressions of other genes involved in cell division and cell–cell adherent junction were also inhibited after the intervention. To gain a greater understanding at a systems level of phototherapy, we focused on canonical pathways analysis. The result showed that NB-UVB treatment played a significant role in psoriasis improvement, associated with "immunological signaling pathways" and "cell cycle regulatory, growth, and proliferation pathways".

Within our data, after NB-UVB treatment in lesional psoriasis, immunological signaling pathways were inhibited with the down expression of inflammatory and cytokine-related genes. "Role of IL-17A in psoriasis and IL-17 pathway" were major inhibited canonical pathways after NB-UVB treatment within the results with significant downregulation of expression of genes. These results are in agreement with previous studies suggesting that the mechanism of UVB phototherapy is directly involved in the IL-17 signaling pathway.[8,23,25–27] A published study in 2017 revealed additional mechanisms of UVB phototherapy with blockage of IL-17A/TNF-α-induced IL-6, IL-8, and CXCL-1 production and decreased an expression of IL-17RA and IL-17RC on fibroblasts through the TGF-β1/Smad3 pathway.[28] The result is evidence of a correlation

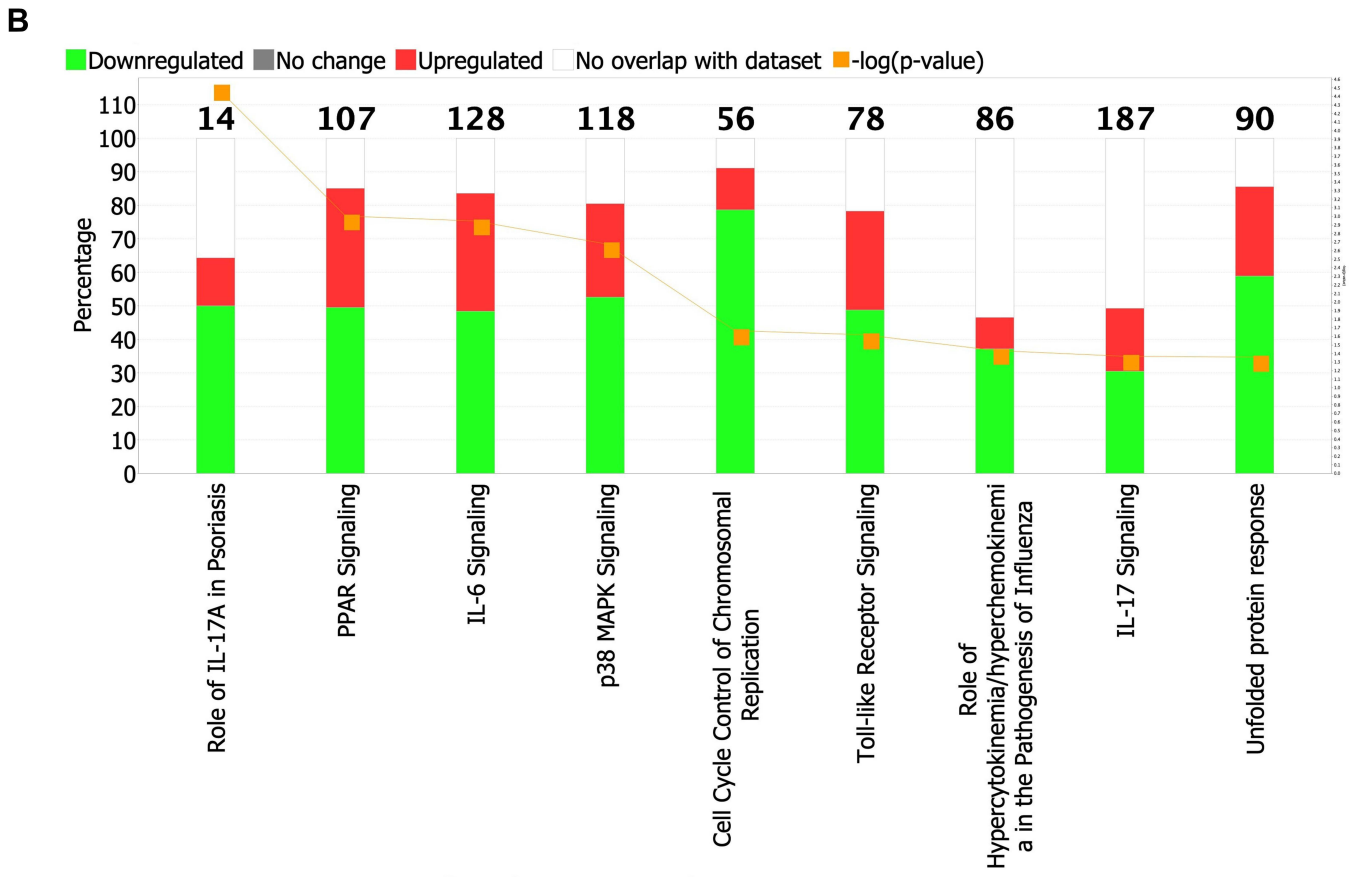

Figure 3 (**A**) Pathway analysis. Enrichment of top clusters for significant DEGs was analyzed with −log (FDR) = 1.3 and an absolute value of z-score > 2. The height of the bars represents the probability that the DEGs in the dataset are related to each pathway. The Orange bars show predicted pathway activation, and the blue bars show predicted pathway inhibition. The Orange points connected by a line represent the ratio which presents the proportion of DEGs in the datasets that map to each canonical pathway. (**B**) Pathway analysis with the percentage of upregulated DEGs (red segment of bar), the percentage of downregulated DEGs (green segment of bar) and overlaps in each pathway. Percentage of non-overlapped DEGs to the pathways is shown by the white segment of the bar. The number of molecules in each known pathway are shown on the top of the bar.

between regulated genes in major significant pathways and response to treatment. Interestingly, most well-known psoriasis-related genes were inhibited with phototherapy in lesional psoriasis, such as CXCL8, DEFB4A/DEFB4B, S100A7, S100A8, S100A9, DEFB103B.[25,29,30] Furthermore, a significant reduction of inflammatory-related genes involved in IL-17 signaling was demonstrated, particularly IL-36A, IL-36G, HRAS, HSP90AB1, LCN2, NOS2. Results also align with previous researches suggesting that IL-36G is hallmark psoriasis gene in psoriasis lesions.[31,32] Notedly, the inhibited genes of IL-36 cytokines were predominantly expressed in other pathways in this analysis, including the downregulated of IL-17 signaling, p38 MAPK signaling, acute phase response signaling, toll-like receptor signaling, the role of hypercytokinemia/hyperchemokinemia in the pathogenesis of influenza, and LXR/RXR activation. According to this result, we proposed that one of the mechanisms of NB-UVB in psoriasis treatment was to inhibit IL-36 cytokines that were classified in an IL-1 family involved in the innate and adaptive immunity.[33,34] In addition, this major finding is consistent with previous research showing that mutations of IL-36RN were a demonstrated risk for generalized pustular psoriasis and alteration of IL-36 cytokines was reported with increased severity of the disease in the early onset.[35] As a result, our research highlights that IL-36 cytokines are interesting genetic markers that should be investigated in further study.

We extended these results to investigate the most prominent impact of NB-UVB therapy on the significant decrease in gene expression of inflammatory cytokines in transcriptomic analysis. It may be hypothesized that the effect of phototherapy on the expression of cytokines in lesional psoriasis may be explained by the reduction in the release of the proinflammatory mediators from inflamed skin.[36–38] The upstream regulator candidates were presented with predicted inhibition of inflammatory cytokines which were reported in pathogenesis of psoriasis,

Table 3 Effect of NB-UVB Phototherapy on Major Pathways in Psoriasis

Name	p-value	Activation z-Score	Molecules Affected by NB-UVB Within These Pathways	
			Downregulated	Upregulated
Immunological signaling pathways				
Role of IL-17A in psoriasis	3.26E-05	−2.236	CXCL8, DEFB4A/DEFB4B, S100A7, S100A8, S100A9	
IL-6 signaling	1.12E-03	−2.111	CXCL8, HRAS, IKBKB, IL36A, IL36G, IL36RN, MAP4K4, SOCS3, STAT3	COL1A1, CSNK2A2
p38 MAPK signaling	2.232E-03	−2.53	IL36A, IL36G, IL36RN, MAPKAPK3, PLA2G4D, RPS6KA4, TGFBR1, H3C14	ATF2, EEF2K
Acute phase response signaling	6.45E-03	−1.058	HRAS, IL36RN, IL36G, SOCS3, SERPINA3, STAT3, CRABP2, SOD2, IKBKB, IL36A	OSMR, FN1
Toll-like receptor signaling	2.30E-02	−2.236	IKBKB, IL36A, IL36G, IL36RN, MAP4K4, UBD	
Role of hypercytokinemia /hyperchemokinemia in the pathogenesis of influenza	3.90E-02	−2.449	CXCL8, IL36A, IL36G, IL36RN, OAS2	S1PR1
IL-17 signaling	4.67E-02	−2.53	CXCL8, HRAS, HSP90AB1, IL36A, IL36G, LCN2, NOS2 DEFB103A/DEFB103B DEFB4A/DEFB4B	ATF2
Cell cycle regulatory, growth, and proliferation pathways				
ATM signaling	7.24E-03	0.816	CCNB1, PPP1CC, CDC25A, RNF8	ATF2, GADD45G, MDM4, BRAT1
Ferroptosis signaling pathway	3.06E-02	0.707	CTSB, ALOX15, SLC11A2	H2AFY, HRAS, STAT3, CHAC1, NFE2L2
Kinetochore metaphase signaling pathway	7.08E-04	−1.897	BIRC5, CCNB1, KIF2C, H2AFY, MAD2L2, PPP1CC, PTTG1, STAG2, TTK, MACROH2A1	CDC27
Senescence pathway	6.82E-03	−1.807	HRAS, CCNB1, CDC25A, RPS6KA4, HBP1, SOD2, PDK2, MAPKAPK3, IKBKB, CXCL8, PDHA1, ITSN2, TGFBR1, ZFP36L1	GADD45G, CDC27, ZFP36L1
Cell cycle control of chromosomal replication	2.29E-02	−2.236	CDC45, CDK16, DNA2, POLD1, TOP2A	
FAT10 cancer signaling pathway	1.04E-02	−1.342	IKBKB, MAD2L2, STAT3, TGFBR1, UBD	
Therapeutic target in psoriasis				
PPAR signaling	9.59E-04	2.53	HRAS, HSP90AB1, IKBKB, IL36A, IL36G, IL36RN, MAP4K4, NCOR1, PPARD, CITED2	
LXR/RXR activation	2.48E-02	1.89	CLU, IL36A, IL36G, IL36RN, NCOR1, NOS2, S100A8	APOE
Sirtuin signaling pathway	4.55E-02	0.577	NAMPT, HIST2H3C, STAT3, NOS2, NDUFA5, GABPA, SOD2, VDAC1, CXCL8, PDHA1, PFKM, NFE2L2	PRKDC, GADD45G
Other				
Neuroprotective role of THOP1 in alzheimer's disease	1.98E-02	−1.342	KLK7, SERPINA3, TMPRSS11D, PRSS22, IDE, TMPRSS11A	PRKAR1A, ACE
Unfolded protein response	2.29E-02	−2.236	CANX, CEBPA, NFE2L2, PDIA6, XBP1, DNAJC10	

https://doi.org/10.2147/PTTS.S335913 DovePress

Table 4 Top 20 Upstream Regulators Were Predicted to Be Inhibited by z-Score

Upstream Regulator	Molecule Type	Activation z-Score	p-value of Overlap
TNF	Cytokine	−4.94	0.00000301
IL1A	Cytokine	−4.357	0.0000528
IL1B	Cytokine	−4.144	0.00000018
Poly rI:rC-RNA	Biologic drug	−4.144	0.000042
IFNG	Cytokine	−3.942	0.000000325
EHF	Transcription regulator	−3.873	0.000000127
Lipopolysaccharide	Chemical drug	−3.85	7.64E-08
IL22	Cytokine	−3.535	2.29E-08
IL21	Cytokine	−3.485	0.00224
IRF3	Transcription regulator	−3.405	0.00345
CEBPB	Transcription regulator	−3.241	4.26E-08
OSM	Cytokine	−3.203	6.43E-15
AREG	Growth factor	−3.162	0.000253
STAT1	Transcription regulator	−3.149	0.00000179
IFNL1	Cytokine	−3.101	0.00021
Thapsigargin	Chemical toxicant	−3.079	0.0104
IRF7	Transcription regulator	−3.078	0.000676
LIF	Cytokine	−3.062	0.0278
CSF2	Cytokine	−3.057	0.0000525
Isotretinoin	Biologic drug	−3.053	0.000000251

Abbreviation: poly rI:rC RNA, polyinosinic-cytidylic acid RNA.

including TNF, IL-1A, IL-1B, IFNG, IL-22, IL-21, OSM, IFNL1 after the intervention. These results are consistent with others reported in the literature, suggesting that NB-UVB therapy has effects on the expression of TNF and IFN-γ inducing other cytokines.[39–41] Improvement in psoriatic skin following phototherapy may involve the decreased expression of inflammatory cytokine IFN-γ.[40] Recent researches have shown that IFN-mediated

Table 5 Top 20 Upstream Regulators Were Predicted to Be Activated by Z-Score

Upstream Regulator	Molecule Type	Activation z-Score	p-value of Overlap
KMT2D	Transcription regulator	3.081	0.0000284
IL1RN	Cytokine	2.935	0.0152
Irgm1	Other	2.932	0.00235
mir-21	microRNA	2.876	0.00000408
Zinc	Chemical drug	2.815	0.003
GATA1	Transcription regulator	2.813	0.0525
Sn50 peptide	Chemical toxicant	2.611	0.00264
SPARC	Other	2.556	0.0451
Bexarotene	Chemical drug	2.537	0.00491
KLF3	Transcription regulator	2.496	0.175
Napabucasin	Chemical drug	2.449	0.0111
miR-483-3p (miRNAs w/seed CACUCCU)	Mature microRNA	2.437	0.00191
IL10RA	Transmembrane receptor	2.435	0.000967
TNIP1	Other	2.433	0.00117
SOCS1	Other	2.418	0.0234
Baicalein	Chemical drug	2.407	0.00191
PIK3CG	Kinase	2.391	0.0365
NS-398	Chemical reagent	2.369	0.032
BCL6	Transcription regulator	2.359	0.0246
MRTFA	Transcription regulator	2.335	0.1

Table 6 Top Upstream Regulators Associated with a Molecule of Cytokine by Z-Score

Upstream Regulator	Activation z-Score	p-value of Overlap
TNF	−4.94	0.00000301
IL1A	−4.357	0.0000528
IL1B	−4.144	0.00000018
IFNG	−3.942	0.000000325
IL22	−3.535	2.29E-08
IL21	−3.485	0.00224
OSM	−3.203	6.43E-15
IFNL1	−3.101	0.00021
LIF	−3.062	0.0278
CSF2	−3.057	0.0000525
IL1RN	2.935	0.0152

signaling cascade was associated with activation of the receptor-associated JAK-STAT signaling pathway, and with major signaling pathways, the NF-κB signaling pathway, and p38 MAPK signaling pathway.[42–46] UVB radiation could regulate anti-inflammatory effects with alteration on expression of IL-1/ IL-6 inflammatory axis by different mechanisms.[47] Another finding in our study was that OSM, a cytokine secreted by skin-infiltrating T lymphocytes, was in downregulated genes of an upstream regulator network after phototherapy. Significantly, IL-10RA was shown as the upstream regulator candidate with predicted activation. In sum, our study suggests that NB-UVB irradiation alleviates inflammation by decreasing the expression of inflammatory cytokines and enhances the synthesis of an anti-inflammatory cytokine receptor. Our evidence suggested that "IL-6 signaling" was a major inhibited pathway after treatment with phototherapy. Previous studies investigated the effect of UVB on an alteration of production and expression of IL-6 and mechanisms of transcription of the IL-6 gene through signal transduction of JAK/STAT, MAPK, and NF-κB signaling pathway.[47,48] The results from this study suggest that the efficacy of NB-UVB treatment is directly linked to mechanisms of action through downregulated immunological signaling pathways, including downregulated "p38 MAPK signaling" and "pathway of acute phase response signaling". Although, UVB irradiation can activate epidermal p38 MAPK signaling and induces a local pro-inflammatory response in the early stage.[49,50] NB-UVB treatment can induce chronic effect of UVB which may cause downregulation of p38 MAPK pathway. Downregulated p38 MAPK signaling after NB-UVB in this study may propose p38 MAPK as a potential target for treatment with decreased stress response genes, inflammatory cytokines, and apoptosis with the indirect mechanisms.

Table 7 Top Upstream Regulators Associated with a Transmembrane Receptor by Z-Score

Upstream Regulator	Activation z-Score	p-value of Overlap
TLR3	−2.895	0.0238
IFNAR1	−2.771	0.00703
TLR9	−2.744	0.0302
TNFRSF1A	−2.439	0.113
TLR4	−2.334	0.12
CD40	−2.159	0.213
CD2	−2	0.0367
IL10RA	2.435	0.000967

The abnormal proliferation of keratinocytes in psoriatic lesions is important in the pathogenesis of psoriasis. Keratinocyte can absorb NB-UVB irradiation and is considered to be the major target for regulating plaque mitigation in chronic psoriasis.[51] We propose that keratinocyte differentiation and proliferation can be regulated with genes in signaling pathways and inflammatory mediators related to cell proliferation and apoptosis. ATF2 and GADD45G gene were presented with upregulation in major signaling pathways after phototherapy. GADD45 gene induced by DNA damage and other stress signals associated with growth arrest, cell cycle control, and apoptosis were reported with upregulated after exposure to ultraviolet (UV) radiation.[52] Our results also support previous research, reporting that GADD45 has an important role in maintaining genomic integrity and DNA repair in keratinocytes exposed to UV.[53] ATF2, which can also activate targeting genes of GADD45, is a regulator of apoptosis and expression of proteins of controlling cell cycle and programmed cell death.[54,55] It was claimed that this gene can be activated following UV irradiation.[56] However, there was no specific data reported that this gene functioned in association with NB-UVB in psoriasis treatment. Moreover, we reported inhibited expression of IL-22 and IL-21, which are immuno-inflammatory mediators for psoriasis, may have a positive effect on clinical outcomes linked to the clearing of lesional psoriasis after NB-UVB.[6] IL-22 is considered as the primary promoter of keratinocyte and epidermal hyperproliferation, and dermal inflammation that is

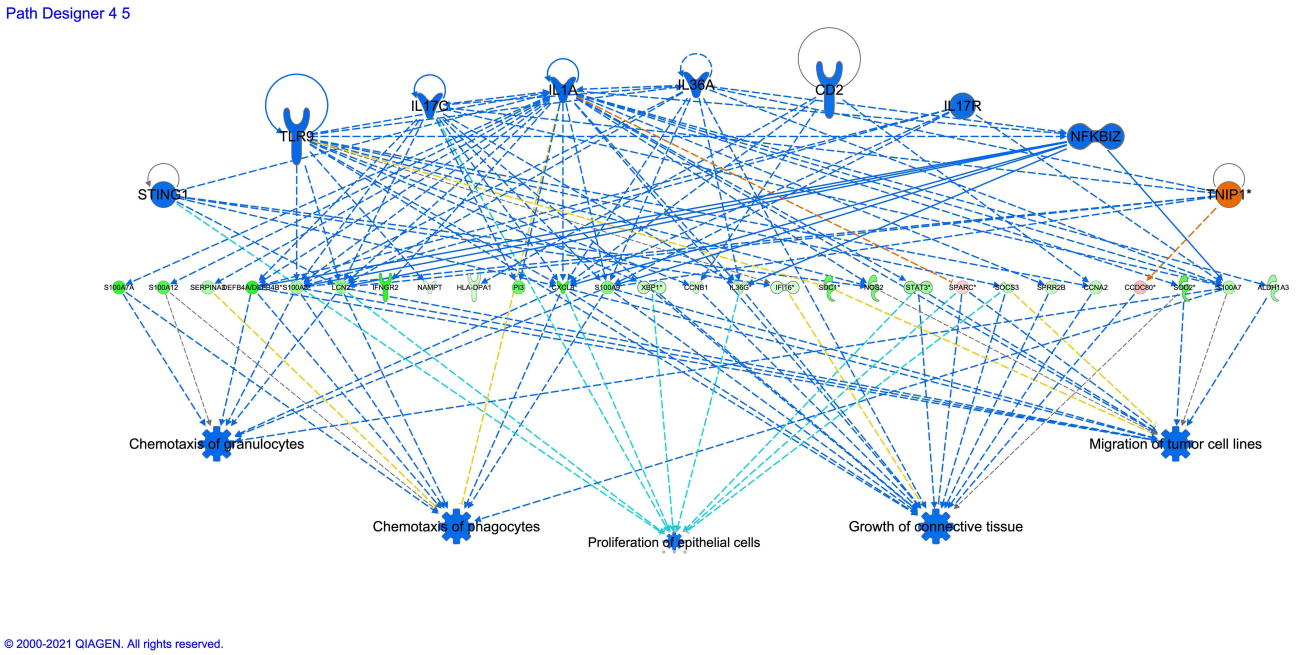

Figure 4 Regulatory effects analysis. The Regulatory effects network was generated by connections between predicted upstream regulators and DEGs and predicted downstream functions or diseases.

correlated with psoriasis severity.[57–61] In addition, blockage of IL-21 could reduce the epidermal thickness and the expression of Th1 and Th17 genes.[62]

We showed that effective treatment with NB-UVB phototherapy in patients with psoriasis led to the downregulation of toll-like signaling pathways in lesional psoriasis. These are also responding with inhibited molecules of TLR in upstream regulator analysis by NB-UVB, particularly TLR3, TLR9, and TLR4. The effect of UV phototherapy can provide significant benefits for psoriatic treatment by blocking downstream pathways of TLR3 signaling and the function of TLR9.[63,64] The downregulation of TLR9 following NB-UVB could affect the immunosuppressive state induced by the UV-induced ligands and downregulating pDCs. Additionally, inhibition of this signaling pathway could suppress immunological function and psoriatic lesion formation, including autoreactive T cells and other inflammatory cytokines.[64,65] Importantly, our results could emphasize that effect of NB-UVB phototherapy can provide significant benefit in the treatment of psoriasis with inhibited downstream signaling pathways of TLR9, TLR3, TLR4. This major result may lead the way to a novel concept of psoriasis treatment. In addition, upstream regulators associated with transmembrane, including CD40 and CD2, were inhibited in responding to the anti-inflammation effect after phototherapy. CD40, expressed in keratinocytes involving signaling transduction activation, a proliferation of cytokine production, and activated CD4+ T cells.[66,67] CD2, protein encoded within the psoriasis susceptibility region in the MHC, directly linked to disease susceptibility and Th1/Th17 cells.[68,69] It is conceivable that clinically effective NB-UVB therapy has an association with the downregulation of CD40 and CD2.

Limitations in the present study lie in the small sample size and the requirement of genetic confirmation in further studies. We decided to use the methodology of NGS and to complete the genome-wide coverage for transcriptome. We also conducted an advanced pathways analysis. Accordingly, we implemented the IPA to compensate for the limitations of our sample size. Importantly, our methodology and sample size calculation carefully followed the previous research of Krueger et al, as published in the Journal of Investigative Dermatology (JID) in 2012. We thus proceeded to investigate the transcriptional profiling and pathogenesis of psoriasis using RNA-seq.[15] However, to the best of our knowledge, this is the first study that investigates the impact of phototherapy and provides a comprehensive gene expression profiling and molecular interaction network for lesional psoriasis with RNA-seq technique and advanced pathway analysis. Combining the molecular profiling of a clinical study with in-depth bioinformatics analysis could provide additional insights into the pathogenesis of psoriasis and mechanisms of

phototherapy. Cutting-edge, systemic, and holistic disease understanding connecting the gaps between diagnostics and treatment, particularly phototherapy will emerge, decoding the immunopathogenesis behind psoriasis in the next future.

Conclusion

Our research provided the first comprehensive results of transcriptome with RNA sequencing and a bioinformatics analysis in NB-UVB phototherapy before and after psoriasis treatment. NB-UVB irradiation can inhibit the expression of the inflammatory signaling pathways, control the gene expression of inflammatory cytokines and increase the expression of anti-inflammatory signaling pathways. The use of NB-UVB phototherapy for its effect on the down-regulation of the toll-like signaling pathways was proposed for the treatment of lesional psoriasis. In addition, the effect of phototherapy on keratinocyte differentiation was emphasized in the resulting regulation of genes and inflammatory mediators that are related to cell proliferation and apoptosis. Our study illustrates how RNA-seq-based transcriptomics can shed light on the mechanism of phototherapy treatment at the molecular level. Ultimately, this research may increase our store of novel genetic knowledge and improve the care of patients with psoriasis.

Acknowledgments

This paper and the research behind it would not have been possible without the exceptional support from Asst Prof. Saranyoo Ponnikorn. The authors gratefully acknowledge the financial support provided by Thammasat University Research Fund under the TU Research Scholar, Contract No. 1/2560 and Chulabhorn International College of Medicine, Contract no T1/2563.

Author Contributions

All authors who have contributed to the data analysis, drafting or revising of this article have agreed on the selection of the journal to which this article will be submitted. They have furthermore given their final approval of the version to be published and have agreed to be accountable for all aspects of the work.

Disclosure

The authors state no conflicts of interest.

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