

## INVESTIGATIVE REPORT

# Oxidation Reduction is a Key Process for Successful Treatment of Psoriasis by Narrow-band UVB Phototherapy

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**Narrow-band UVB (NB-UVB) phototherapy is commonly used for treatment of psoriasis, though the mechanisms underlying its efficacy have not been completely elucidated. We used gene expression profiling to characterise gene expression in lesional epidermis from psoriasis patients in the middle and late stages of NB-UVB phototherapy. Increased melanogenesis gene expression was the earliest response to phototherapy. At the end of treatment, genes responding to phototherapy and correlated to treatment outcome were involved in oxidation reduction, growth and mitochondria organisation. Particularly, SPATA18, a key regulator of mitochondrial quality, was significantly down-regulated in psoriasis ( $p < 0.05$ ). Poly(dA:dT) and poly(I:C) stimulation increased SPATA18 level in primary keratinocytes, indicating the importance of mitochondria quality control under innate immune induced oxidative stress. Normalised SPATA18 expression after phototherapy indicates improved mitochondrial quality control and restored cellular redox status. Our data suggest that oxidation reduction is critical for the resolution of psoriatic plaques following NB-UVB phototherapy. Key words: psoriasis; NB-UVB phototherapy; melanogenesis; oxidation reduction.**

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Psoriasis is a common inflammatory disease affecting approximately 2–3% of the world's population (1). It is an immune-mediated skin disorder that also affects nails and joints, and is associated with a wide range of other systemic manifestations such as obesity, diabetes and hypertension (2). Molecular alterations in psoriasis have been characterised using array-based gene expression profiling techniques. It is now clear that psoriatic lesions exhibit significant differences in the expression of thousands of genes compared to normal skin, with alteration in several biological processes such as cell differentiation, metabolism and immune responses (3–5). However, in most studies whole skin biopsies were used

and alterations specific for epidermal keratinocytes or the immune components are therefore not clarified.

Narrow-band UVB (NB-UVB) phototherapy is a well-established first-line treatment for psoriasis and can induce clearance of the disease in around 70% of patients (6). It has been shown that UVB radiation is absorbed by endogenous chromophores such as nuclear DNA, followed by a cascade of events such as production of reactive oxygen species (ROS), induction of apoptosis and modification of cytokine expression (7). Despite the long-term use of NB-UVB phototherapy, its primary mechanisms of action are still unclear.

It has been shown that clinical improvement in chronic plaque-type psoriasis lesions after NB-UVB therapy is accompanied by a decrease in the expression of IL-12, IL-18 and IL-23 (8). Effective NB-UVB phototherapy suppressed multiple parameters of the IL-17 pathway (9). Suppression of the IFN and TH17 pathways by NB-UVB phototherapy was found in resolved psoriatic lesions (10). In addition to immune cells, epidermal keratinocytes have also been considered the major target for NB-UVB therapeutic efficacy since induction of keratinocyte apoptosis in psoriatic lesions was shown to be sufficient for resolution of lesions during NB-UVB phototherapy (11, 12).

In order to gain more insight into the therapeutic action of NB-UVB phototherapy on psoriatic epidermis, we performed a profiling study to investigate global gene expression of epidermal cells before treatment, after one month of treatment, and prior to the last session of treatment (2–3 months). The relationship between gene expression and treatment outcome was studied using Partial Least Squares Discriminant Analysis (PLS-DA) to identify the key therapeutic targets of NB-UVB phototherapy. Results of our study suggest that oxidation reduction is a critical event for the resolution of psoriatic plaques.

## MATERIALS AND METHODS

### *Patient recruitment and tissue sample collection*

Twelve patients diagnosed with plaque type psoriasis were recruited to the study. The study was approved by the Regional Ethics Review Board, Umeå, Sweden (Dnr 08-108 M) and performed in accordance with the Declaration of Helsinki.

Informed oral and written consent was obtained from all subjects. Detailed descriptions of NB-UVB treatment and other experimental procedures are available in Appendix S1<sup>1</sup>. A total of six 4-mm diameter punch biopsies were taken from lesions on each psoriasis patient: 2 prior to UV treatment (Pre-UV), 2 at the middle stage of UV treatment (after one month of treatment, Mid-UV) and 2 before the last treatment session (Post-UV). Twelve healthy age-, sex- and skin type-matched volunteers were also recruited and 2 punch biopsies were taken from the buttocks. Collected biopsies were processed for gene profiling study. Clinical data on these patients is shown in Table S1<sup>1</sup>.

In order to examine gene expression in non-involved normal skin from psoriasis patients, material from our previous study (13) including another 15 patients and 15 healthy age- and sex-matched controls were also used (not processed for gene profiling study).

#### *Microarray profiling and differential expression analysis*

Laser Capture Microdissection (LCM) was performed to collect the epidermis for RNA extraction. Illumina HumanHT-12 v4 Expression BeadChips (Illumina Inc., San Diego, CA, USA) were used for gene expression profiling, following manufacturer's protocol. Normalisation of microarray data for differential expression was performed using linear models and differential expression for microarray data (LIMMA) package (14), the statistical language R and extension taken from Bioconductor. Differential analysis using LIMMA (15) or Significance Analysis of Microarrays (SAM) was performed with the software tool Mev 4.8.1 (MultiExperiment Viewer). LIMMA was performed to identify differentially expressed genes in psoriasis compared to matched healthy controls. SAM analysis was used to identify genes significantly affected by phototherapy in paired samples. Partial least squares discriminant analysis (PLS-DA) was performed using SIMCA software (Simca v. 13, MKS Umetrics AB, Sweden) to study the correlation between treatment outcome and changes in gene expression in response to phototherapy, and also to identify the best discriminating genes for separating the patients with unsatisfactory treatment outcome from patients with good or excellent treatment outcome. The lists of genes were subjected to functional annotation analysis using Database for Annotation, Visualization, and Integrated Discovery (DAVID) (16, 17). Gene ontology analysis was focused on biological processes and cellular compartments and pathway analysis was focused on KEGG pathway (Kyoto Encyclopedia of Genes and Genomes). Gene expression data were confirmed by quantitative real-time PCR (qRT-PCR) and/or immunohistochemistry.

#### *Cell culture and stimuli*

Human epidermal keratinocytes adult (HEKa, Life technologies, Carlsbad, USA) were treated with double-stranded DNA (dsDNA) analogue poly(dA:dT) (poly(deoxyadenylic-deoxythymidylic acid sodium salt) or poly(I:C) (Polyinosinic acid: Polycytidylic acid, double-stranded RNA (dsRNA) analogue). Real time RT-PCR and western blotting were performed to study mRNA and protein levels of selected genes. For detailed descriptions, see Appendix S1<sup>1</sup>.

## RESULTS

### *NB-UVB phototherapy is effective for most patients*

Treatment comprising approximately 24 sessions was given during 2–3 months. Clinical improvement was

assessed by evaluating erythema, desquamation and induration following phototherapy. Response assessment on the 11 patients that fulfilled treatment showed 7 patients with excellent clinical response, 2 with good response and 2 with unsatisfactory response. Even if lesions in patients with excellent clinical response had almost been resolved before the last session of treatment, affected skin could easily be distinguished from normal skin at biopsy. One patient (patient 5) left at the middle stage of phototherapy, thus only Pre-UV and Mid-UV samples could be collected.

### *Clear alterations in gene expression was seen in lesional psoriatic epidermis*

Epidermal gene expression profiles of psoriatic lesions (Pre-UV) were compared with matched healthy controls, and 3,389 genes (1,601 up-regulated and 1,788 down-regulated) were found to be significantly dysregulated in psoriasis (LIMMA adjusted  $p$ -value  $< 0.001$ , fold change  $> 1.5$ ). Of these, S100A9 (S100 calcium binding protein A9) showed the greatest up-regulation with a fold change of 141.4 compared to healthy controls, and the most down-regulated gene was *PAMR1* (peptidase domain containing associated with muscle regeneration 1) with 10.8 fold decreased expression in psoriatic lesions. DAVID functional annotation study was performed for the top 100 up-regulated or down-regulated genes, respectively. Seventy-nine of the 100 most up-regulated genes, and 85 of the 100 most down-regulated genes had a DAVID ID-number and were included in the analysis. Genes up-regulated in psoriasis were mainly involved in keratinization/differentiation (19 genes, 24% of 79 genes with DAVID ID) and defence response/immune response (13 genes, 16%), whereas genes down-regulated in psoriasis were mainly involved in cell adhesion (11 genes, 13% of 85 genes with DAVID ID). DAVID functional annotation of the 1,601 up-regulated and 1,788 down-regulated genes was also performed, and the top 5 enriched gene ontology terms in biological process, cellular component and KEGG pathways, respectively, are shown in Table SIII<sup>1</sup>. Interestingly, gene ontology terms for cellular component revealed that genes encoding mitochondrial proteins were up-regulated and genes encoding extracellular matrix proteins down-regulated in psoriasis, indicating altered mitochondrial function and cell-matrix interactions in psoriasis.

### *Melanogenesis genes were early responder genes to phototherapy*

Next we investigated gene expression changes in response to NB-UVB phototherapy. To identify genes whose response to phototherapy were putatively involved in the early phases of plaque resolution, we first studied changes in gene expression after

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one month of treatment. SAM differential expression analysis for matched samples was performed to identify significantly affected genes (Mid-UV vs. Pre-UV). Surprisingly, only 26 genes were significantly modified after one month of phototherapy (20 genes up-regulated and 6 genes down-regulated, false discovery rate (FDR) < 0.05; fold change > 1.5) (Table I). Genes encoding key melanogenesis enzymes TYR (tyrosinase) and TYRP1 (tyrosinase-related protein 1) were selected for microarray data confirmation using qRT-PCR. Expression of melanocyte transcription factors MITF (microphthalmia-associated transcription factor), SOX10 (sex determining region Y-box 10) (18, 19) and melanocyte stimulating hormone receptor MC1R (melanocortin 1 receptor) (20) following phototherapy were also studied. Fig. 1A showed that in accordance with microarray data, TYR and TYRP1 were significantly up-regulated (Mid-UV vs. Pre-UV,  $p < 0.001$ ) and higher gene expression could also be seen at the end of phototherapy (Post-UV vs. Mid-UV). Expression of MITF and SOX10 was also found to be significantly up-regulated following phototherapy, whereas expression of MC1R was not significantly affected at the early stage of lesion resolution (Mid-UV vs. Pre-UV).

Down-regulation of pigmentation signalling pathways in lesional psoriatic skin has recently been shown (21). From Fig. 1A, we could also see that except for

Table I. List of early responder genes to narrow band-UVB phototherapy

Gene symbol	Fold change	Function
<b>Up-regulated</b>		
<i>TYR</i>	2.24	Pigmentation, oxidation reduction
<i>MLANA</i>	2.22	Melanosome biogenesis
<i>LPPR4</i>	2.19	Cell morphogenesis
<i>TYRP1</i>	2.16	Pigmentation, oxidation reduction
<i>SNCA</i>	1.84	Immune response, oxidation reduction
<i>HSPA12A</i>	1.83	Protein turnover
<i>KITLG</i>	1.81	Cell motion, melanocyte differentiation
<i>RFTN2</i>	1.74	Raftlin family member 2
<i>NOV</i>	1.72	Regulation of cell growth
<i>SRPX</i>	1.66	Cell adhesion
<i>QPRT</i>	1.66	Oxidoreduction coenzyme metabolic process
<i>GMPR</i>	1.65	Oxidation reduction, purine metabolism
<i>CSPG4</i>	1.62	Angiogenesis
<i>BACE2</i>	1.60	Proteolysis
<i>FEZ1</i>	1.59	Cell morphogenesis, cell motion
<i>TPCN2</i>	1.56	Ion transport, pigmentation
<i>APIS2</i>	1.54	Intracellular protein transport, endocytosis
<i>FHOD3</i>	1.54	Cytoskeleton organisation
<i>APIS2</i>	1.53	Protein sorting
<i>BACE2</i>	1.51	Proteolysis
<b>Down-regulated</b>		
<i>DENND2C</i>	0.56	GDP-GTP exchange factor
<i>GDA</i>	0.58	Purine metabolism
<i>ATP1A1</i>	0.59	ATP biosynthetic process
<i>P2RY2</i>	0.65	Cellular ion homeostasis
<i>GNG12</i>	0.66	Chemokine signalling
<i>GRHL1</i>	0.67	Transcription

GDP-GTP: guanosine diphosphate/guanosine triphosphate.

TYRP1, melanogenesis related genes were significantly down-regulated in psoriatic lesions (Pre-UV) compared to matched healthy controls. In order to map gene expression in non-involved normal skin of psoriatic patients, we next studied TYR gene expression in another set of psoriasis samples containing paired lesional and non-involved normal skin biopsies from 15 psoriasis patients, and also healthy skin biopsies from 15 matched healthy controls (13). cDNA synthesized from RNA extracted from whole skin biopsies was used in this study. From Fig. 1B, similar TYR expression levels were seen in non-involved psoriasis skin and healthy controls ( $p = 0.17$ ). Immunohistochemical analysis of TYR expression was performed, showing more TYR positive cells in the basal layer of non-involved normal skin in psoriasis patients compared to matched healthy controls. Similarly, more TYRP1 and MITF positive cells were also found in non-involved normal skin of psoriasis patients (Fig. 1C).

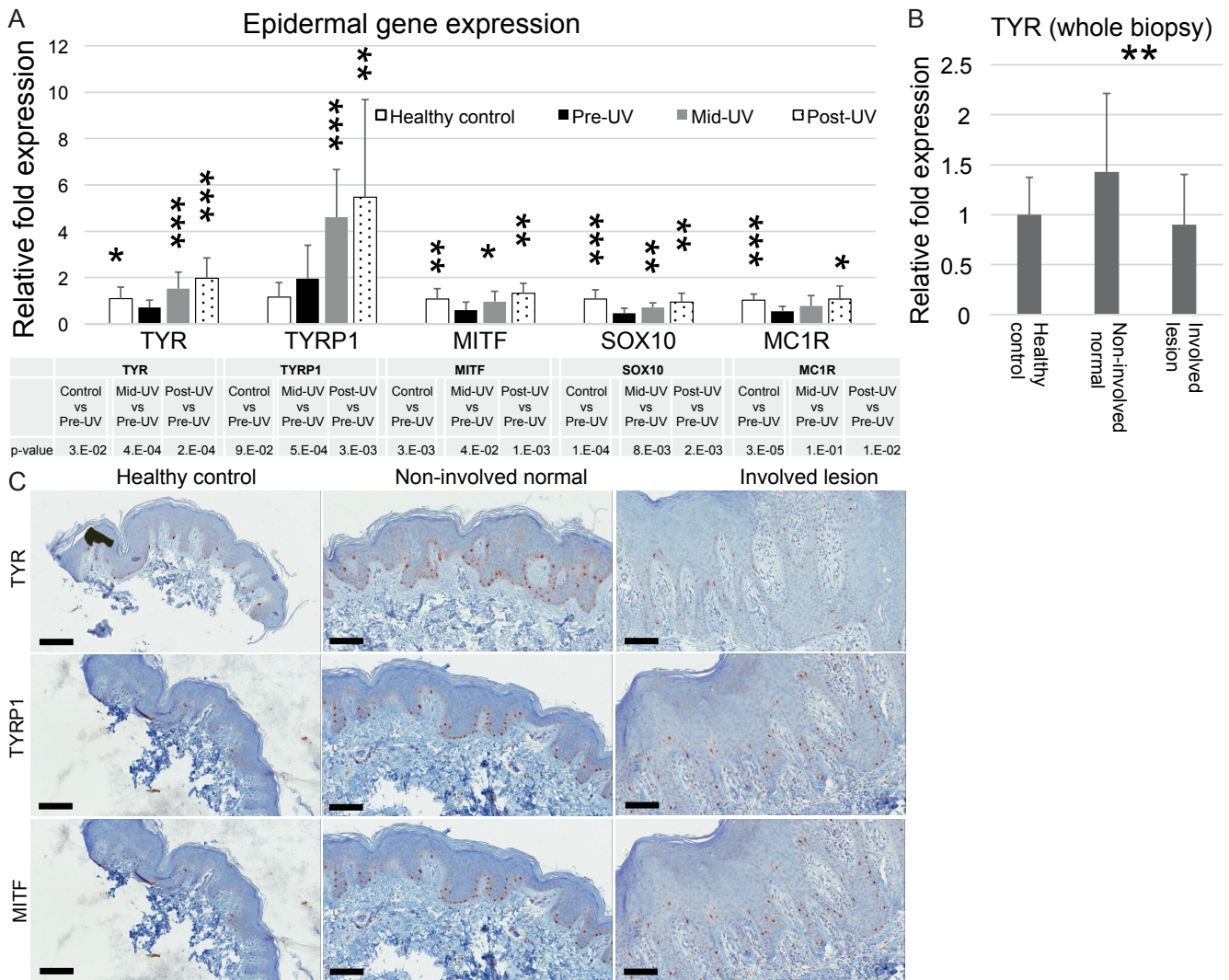
To determine whether up-regulation of melanogenesis genes was simply an UV-induced photo protective response or whether it contributed to lesional resolution, we then studied the relationship between gene expression and clinical improvement. An overall higher fold up-regulation of expression of melanogenesis genes following phototherapy was found in patients with better treatment outcome (Fig. S1<sup>1</sup>).

#### *Multiple biological processes were significantly modified after phototherapy*

At the end of phototherapy, 407 genes were significantly up-regulated and 428 genes significantly down-regulated (SAM differential expression analysis for matched samples Post-UV vs. Pre-UV, FDR < 0.05, fold change > 1.5). Only 17% of the significantly down-regulated genes in psoriasis lesions before treatment were significantly up-regulated by phototherapy (309/1,788), and 25% of the significantly up-regulated genes were significantly down-regulated following phototherapy (396/1,601). DAVID functional annotation analysis showed that the majority of genes up-regulated by therapy were involved in pigmentation, cell adhesion, cell motion, cell morphogenesis and oxidation reduction, whereas genes that were down-regulated by therapy were involved in ectoderm development, carbohydrate catabolic process, translation, homeostatic processes and the immune response. Lists of genes are shown in Table SIV<sup>1</sup>.

#### *Successful treatment of psoriasis is linked to oxidation reduction*

After phototherapy, clinical improvement could be seen in 9 patients whereas 2 patients were unsuccessfully treated. To identify the correlation between changes in gene expression following phototherapy and clinical



**Fig. 1.** Dysregulation of melanogenesis genes in psoriasis. (A) Epidermal gene expression in healthy untreated skin (Healthy control) and psoriatic lesion prior to (Pre-UV), during (Mid-UV) and before the last session of NB-UVB phototherapy (Post-UV) was measured by quantitative real-time PCR (qRT-PCR). *RPL13A* was used as an internal control gene. The pooled set of data from 12 controls was set to 1. The mean and SD of relative mRNA fold expression are shown. Independent sample *t*-test was performed for comparing psoriasis with healthy controls and a paired-sample *t*-test was performed for comparing gene expression following treatment ( $***p < 0.001$ ,  $**p < 0.01$ ,  $*p < 0.05$ ). One patient stopped treatment halfway, and only 11 samples are thus included in the Post-UV group. (B) Tyrosinase (TYR) mRNA expression in healthy control, non-involved normal skin from psoriatic patients, and involved lesional skin. qRT-PCR results showed decreased expression of TYR in involved lesional skin compared to paired non-involved psoriasis skin ( $p < 0.01$ ). Similar TYR gene expression could be seen in non-involved psoriasis skin compared to matched healthy controls ( $p = 0.17$ ). (C) Immunohistochemical staining of TYR, tyrosinase-related protein 1 (TYRP1) and microphthalmia-associated transcription factor (MITF) in skin sections from healthy controls, non-involved normal skin from psoriasis patients and involved psoriatic lesions. Increased numbers of TYR, TYRP1 and MITF positive cells were found in non-involved psoriasis skin. Scale bar: 100  $\mu$ m.

improvement, we performed PLS-DA. The profile of 3,389 genes which are significantly dysregulated in psoriasis was studied in PLS-DA. Fold changes in gene expression following phototherapy (Post-UV/Pre-UV) were calculated and used for PLS-DA to identify the best discriminators which can separate patients with unsatisfactory response (patients 6 and 7) from patients with good/excellent response to phototherapy. PLS-DA allowed us to identify the most important therapeutic actions of UV by identifying correlation patterns that discriminate groups and estimate the relative importance of each included variable for the

discrimination. The top 100 best discriminators were selected and shown in Table SV<sup>1</sup>. DAVID functional annotation study showed that the top 3 enriched gene ontology terms in biological process are oxidation reduction, growth and mitochondrial organisation.

*SPATA18 expression is correlated with clinical improvement*

PLS-DA revealed that up-regulation of SPATA18 (spermatogenesis associated 18) was highly correlated with clinical improvement. Since clinical improvement

is correlated to oxidation reduction and mitochondrial organisation, and SPATA18 is a key regulator of mitochondrial quality under oxidation stress (22), SPATA18 expression in healthy controls and psoriatic lesions under phototherapy was further validated using qRT-PCR. As shown in Fig. 2A, SPATA18 was significantly down-regulated in psoriasis ( $p < 0.05$ ) compared to matched healthy controls, and also significantly up-regulated following phototherapy (Post-UV vs. Pre-UV,  $p < 0.01$ ). In Fig. 2B it is shown that in patients with good/excellent response, SPATA18 expression levels increased following phototherapy, whereas in 2 patients with unsatisfactory response (patients 6 and 7), expression of SPATA18 was down-regulated.

#### *SPATA18 is involved in innate immune defence*

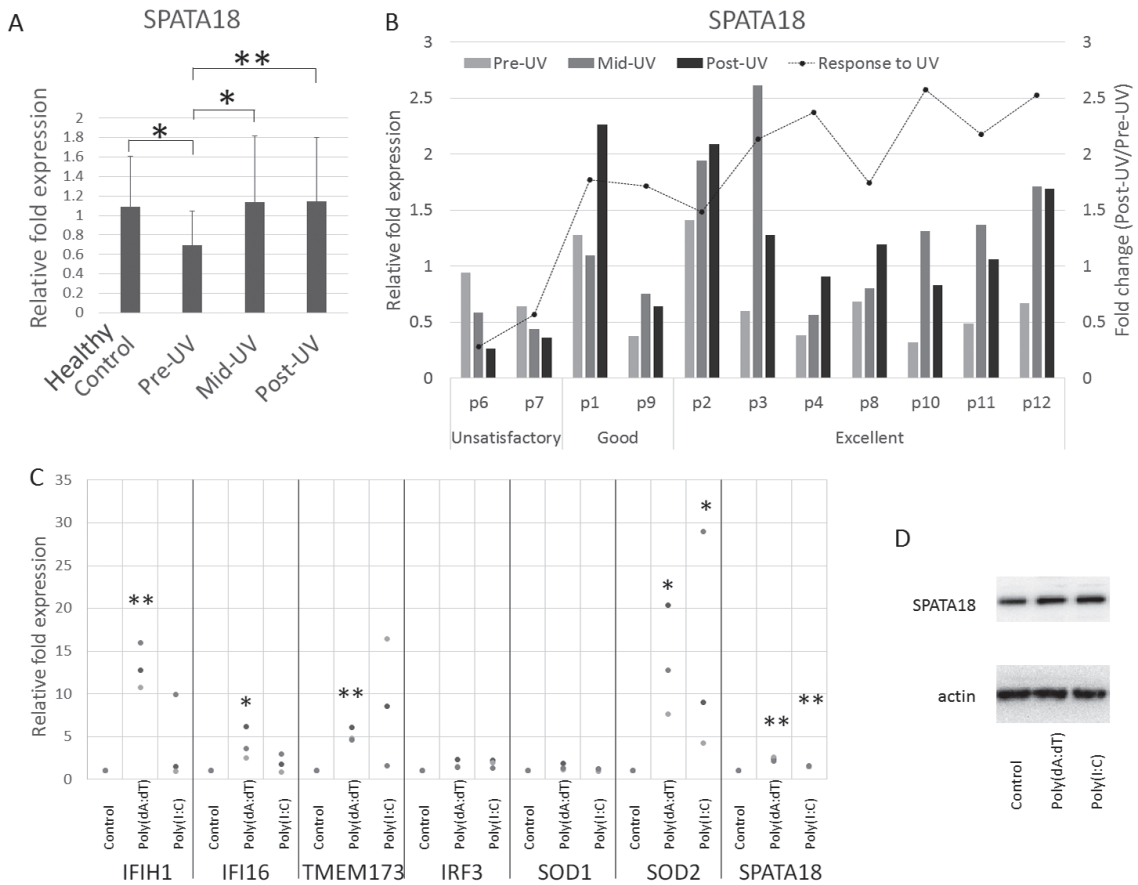
Altered innate immune response has been suggested as an initiating factor triggering the development of psoriasis. It is well known that levels of ROS, important second messengers involved in the innate immune response (23), are increased in psoriasis (24). Therefore, we wanted to determine whether SPATA18 is affected by innate immune-induced ROS induction. Primary HEK293 cells were thus treated with poly(dA:dT) or poly(I:C), and expression of SPATA18 in response to these innate immune triggers was studied. In addition, we examined mRNA expression patterns for some well described pattern recognition receptors, such as IFI16 (interferon, gamma-inducible protein 16) and IFIH1 (interferon induced with helicase C domain 1) (25, 26), as well as the key adaptor of innate immune signalling (TMEM173, transmembrane protein 173) and the key transcription factor required for the induction of interferon- $\beta$  (IRF3, interferon regulatory factor 3) (27). Expression of SOD1 (superoxide dismutase 1, soluble) and SOD2 (superoxide dismutase 2, mitochondrial), 2 important antioxidant enzymes was also examined and used as indicators of ROS production (23). From Fig. 2C we can see that when stimulated with synthetic dsDNA, keratinocytes responded with increased mRNA expression levels of all of the examined DNA sensors and the adaptor. Highly increased SOD2 expression indicated an increased mitochondrial ROS production following dsDNA stimulation. Meanwhile, we observed over 2-fold induction of SPATA18 mRNA (mean fold change = 2.36) after 24 h of treatment with dsDNA ( $p < 0.01$ ). In cultured keratinocytes treated with dsRNA, a fold change of 1.52 induction in SPATA18 gene expression was seen ( $p < 0.01$ ). Increased abundance of SPATA18 protein in poly(dA:dT) or poly(I:C) stimulated cells was also found (Fig. 2D). These results suggest that keratinocytes which are functionally reactive to dsDNA/dsRNA ligand also show increased SPATA18 levels.

## DISCUSSION

Gene expression patterns obtained with LCM and microarray analysis in this study revealed that psoriatic epidermis exhibits significant differences in gene expression compared to healthy controls. In the initial stages of phototherapy, expression of only 26 genes was significantly affected, the most highly affected genes being involved in the melanogenesis pathway. UV irradiation is a well-known triggering factor for melanogenesis, a process generating melanin. Our data showed that several melanogenesis-related genes were significantly down-regulated in psoriasis compared to control samples and that their up-regulation following phototherapy was seen in patients with better clinical improvement. The association between melanogenesis and treatment outcome could be an epiphenomenon of phototherapy, since it might be easier for UV to reach the melanocytes in the basal area of the epidermis that is on the way to clearance. We, however, speculate that with the multifunction of melanin, melanogenesis could be actively involved in resolving psoriasis. It has been shown that the presence of melanin in cells facilitates the apoptotic effect of UV (28). With melanin's antioxidant and anti-inflammatory properties, increased melanogenesis after phototherapy could help abate oxidative stress and inflammation characteristics of psoriatic lesions. Being the main early responder genes at the early stage of phototherapy and also associated with treatment outcome after phototherapy, melanogenesis could actively contribute to the resolution of the disease.

Plasma levels of both 5-S-cysteinyl-dopa (a metabolite reflecting melanocyte activity) and thiobarbituric reactive substances (a typical lipid peroxidation marker) have been found to be increased in psoriasis (29). Down-regulation of pigmentation genes was recently shown in psoriatic lesions, probably due to altered IL-17 and TNF expression (21). Our study revealed that compared to healthy controls, melanogenesis was increased in non-involved psoriatic skin, whereas decreased in involved psoriatic lesions, further suggesting a role for melanogenesis in psoriasis pathogenesis.

Gene ontology studies showed that several genes related to oxidation reduction were significantly up- or down-regulated in psoriasis, and after treatment expression of these genes was significantly modified. In addition, PLS-DA showed that genes involved in oxidation reduction, growth and mitochondrial organisation were significantly affected following phototherapy, and their modification highly correlated to treatment outcome. Efficacy of antioxidant strategies for treating psoriasis (24) also supports our finding that oxidation reduction is a key process in treating psoriasis. Our results indicate that a balanced redox process, as well as normalised mi-



**Fig. 2.** Involvement of SPATA18 in psoriasis pathogenesis. (A) SPATA18 mRNA levels were significantly downregulated in psoriatic epidermis (Pre-UV) compared to healthy controls ( $p < 0.05$ ). Increased SPATA18 expression could be seen following phototherapy ( $*p < 0.05$ ,  $**p < 0.01$ ). (B) SPATA18 gene expression was closely associated with clinical improvement. In patients with unsatisfactory clinical outcome (p6 & p7), expression of SPATA18 was down-regulated following phototherapy. (C) SPATA18 mRNA was expressed in primary human epidermal keratinocytes adult (HEKa) cells and up-regulated upon poly(dA:dT) or poly(I:C) stimulation ( $**p < 0.01$ ). Expression of IFI16, IFIH1, TMEM173, IRF3, SOD1 and SOD2 in response to poly(dA:dT) or poly(I:C) stimulation was also studied. Gene expression value from 3 independent experiments were shown. (d) Western blotting results showed increased SPATA18 protein levels in HEKa cells after stimulation with Poly(dA:dT) or Poly(I:C) after 24 h.  $\beta$ -actin was used as loading control.

tochondrial function are key steps in resolving psoriasis lesions by the use of NB-UVB phototherapy.

In addition to their well-appreciated role in cellular metabolism and programmed cell death, mitochondria appear to function as centrally positioned players in the innate immune system. Mounting evidence suggest that following cellular damage and stress, mitochondria facilitate innate immunity by generating ROS (23). ROS induction was seen in cells stimulated with poly(I:C), and in cells with abnormal mitochondria, higher levels of ROS and type I IFN were seen when treated with poly(I:C) stimulation (30). Mitochondrial quality control is of significant importance for maintaining the steady and healthy state of our bodies (22). Here, we found that a key mitochondrial quality control gene, SPATA18, was up-regulated upon innate immune trigger. SPATA18 is a p53-inducible protein which can repair or eliminate ROS-damaged unhealthy mitochondria, leading to the improvement of mitochondrial functions (22). Therefore, it is likely that increased

SPATA18 expression is required in control of mitochondrial quality under innate immune triggered ROS damage. SPATA18 upregulation following phototherapy could thus result in normalised mitochondria quality control and indicate a re-balanced redox status.

Taken together, assessment of the psoriatic transcriptome in lesional epidermis revealed that psoriasis lesions exhibit significant differences in gene expression compared to healthy controls and that phototherapy can successfully target hundreds of genes involved in diverse biological processes. Our study set-up does not allow differentiating between therapeutic mechanistic effects and epiphenomenal effects of treatment, and the observed changes in gene expression could be due to short-term UV effect unrelated to the anti-psoriatic treatment effect. Nevertheless, it is clear that induction of melanogenesis is an early event for the resolution of psoriatic lesions, and we have identified oxidation reduction in lesional skin as a key process for clinical improvement induced by NB-UVB phototherapy.

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