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Delineating the healthy human skin UV response and early induction of interferon pathway in cutaneous lupus erythematosus

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Short title: UV induces interferon pathway in lupus skin

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Abbreviations:

CLE,	cutaneous lupus erythematosus
DEG,	differentially expressed gene
DLE,	discoid lupus erythematosus
LE,	lupus erythematosus
MHC,	major histocompatibility complex
qRT-PCR,	quantitative real-time reverse transcriptase PCR
RNP,	ribonucleoprotein
SCLE,	subacute cutaneous lupus erythematosus
SLE,	systemic lupus erythematosus
snRNP,	small nuclear RNP

To the Editor,

UV irradiation is known to trigger and exacerbate cutaneous lupus erythematosus (CLE) in genetically susceptible individuals. UV irradiation is capable of inducing, in a delayed fashion, even systemic organ manifestations of lupus erythematosus (LE) (Hasan et al. 2004; Lehmann and Homey 2009). About half of LE patients have preceding polymorphic light eruption several years before the onset of lupus (Nyberg et al. 1997). UV irradiation induces keratinocyte apoptosis in CLE skin (Kuhn et al. 2006), and endogenous nucleic acids can induce IFN-associated responses in cultured keratinocytes (Scholtissek et al. 2017). However, the exact mechanism of how UV irradiation induces CLE is not known.

To understand the early *in vivo* molecular UV-induced changes in human skin, we performed UVB photoprovocation (Supplementary Figure S1 online) on the intact skin of 20 patients with CLE (discoid and subacute) and on four healthy volunteers with no history of photosensitivity (Supplementary Table S1 online), and analyzed skin biopsies obtained both from photoprovocated and from unexposed skin of the same individual. Skin biopsies (three per subject, i.e. total 42 samples) from three DLE, seven SCLE and four healthy control subjects were analyzed by mRNA sequencing (RNA-Seq; Supplementary Table S1 online). The major variation in gene expression was between the photoprovocated skin samples, irrespective of the diagnostic group, and the unexposed skin samples (Supplementary Figure S2 online). The expression profile of the pre-photoprovocation skin samples and of those kept unexposed (biopsied after photoprovocation) were concordant and were thus combined to serve as controls for the UV-exposed skin samples.

To analyze the *in vivo* UVB effect on healthy skin, we compared the photoprovocated healthy subject samples to the combined control samples from the same individuals, and found 162 differentially expressed genes (DEGs), which we regarded as photoprovocation-response genes of healthy skin (column “Healthy” is “UP” or “DOWN” of Supplementary Table S2 online). Genes related to nucleic acid binding (Figure 1a) and erythematous reactions (Figure 1b) represented the majority of the DEGs. A nucleosome subunit and small nuclear ribonucleoproteins (snRNPs), known as autoantigens in systemic lupus erythematosus (SLE; Figure 1c; H2AFZ, a variant of histone H2A; SNRPB, Sm-B snRNP; SNRPD3, Sm-D snRNP), were up-regulated by photoprovocation. Therefore, even in healthy human skin UVB response, induction of autoantigens occurred.

In comparisons of the *in vivo* UVB effect on healthy, DLE and SCLE skin, we found 411 DEGs in total (Supplementary Table S2 online), of which 13% (54/411) were consistently altered in DLE and SCLE (“Altered” in Supplementary Table S2 online), and 19 out of the 54 were specifically up-regulated genes in DLE and SCLE skin but not in healthy skin; for example, a major histocompatibility complex (MHC) II gene HLA-DPA1 (Figure 2a), an IFN-induced ubiquitin-like modifier ISG15, a proteasome activator subunit PSME2, an ubiquitin-conjugating enzyme UBE2L6, and known IFN-induced protein with tetratricopeptide repeat family genes IFIT1, IFIT2 and IFIT3. The altered genes were also up-regulated in many cell lines by IFN-γ and IFN-α (Figure 2b). Therefore, we conclude that UV exposure of DLE and SCLE skin results in enhanced protein degradation and antigen presentation, suggested to be brought about by IFNs.

The induction of the IFIT1/2/3 mRNAs by qRT-PCR (quantitative real-time reverse transcriptase PCR) was confirmed in a larger set of skin samples (Supplementary Table S1

online), and the difference was significant for IFIT1/2 in SCLE (Figure 2c; $P < 0.05$). The IFIT1/2/3 protein expression in the skin lesions was concordant with the mRNA expression (Figure 2d and Supplementary Table S3 online). Typically, IFIT1 was mostly expressed in basal keratinocytes while IFIT2 and IFIT3 were more evenly distributed in the epidermis. IFIT2 is a mediator of apoptosis via a mitochondrial pathway (Stawowczyk et al. 2011), and photoprovocation induced consistently apoptotic keratinocytes in all but one individual, typically more strongly in 60% of the SCLE samples (Supplementary Figure S3 and Table S3 online). Since IFIT2 can bind to AU-rich element (Yang et al. 2012), which can be found around the 3'-end of mammalian mRNAs (Chen and Shyu 1995), and is involved in post-transcriptional regulation (Berchtold et al. 2008), IFIT2 might increase the amount of fragmented endogenous RNAs, which induce IFN responses in keratinocytes (Scholtissek et al. 2017), sustaining the skin lesion.

We also measured serum IFN-α values before and after photoprovocation (Supplementary Figure S1a online) with Simoa and serum IFN-γ values with Luminex. No significant increase in serum IFN levels was found during photoprovocation of small skin areas. However, when combining the serology results with the IFIT gene expression data, the majority (8/10) of the SCLE patients showed strong up-regulation of the IFIT genes and serum IFN-α levels up to 0.62 pg/ml, while only one third of the DLE patients showed up-regulation of the IFIT genes and measurable IFN-α or IFN-γ in their serum, even prior to photoprovocation (Supplementary Table S3 online). Typically, SCLE is the most UV-sensitive subtype of LE, and SCLE patients frequently fulfil four or more of the SLE criteria. No remarkable serum levels of any other cytokines measured were found except for one DLE case who had elevated IL-17A (103 pg/ml) and the highest IFN-γ levels.

In summary, our findings delineate the healthy human skin UV response genes, including induction of autoantigens, and show that UV irradiation upregulates in particular the IFIT family genes of the hundreds of IFN-stimulated genes and the MCH II gene HLA-DPA1 in CLE skin but not in healthy skin. Our findings revisit the importance of UV avoidance and the constitutive IFN production in autoimmune disorders (Funabiki et al. 2014), and emphasize early targeting of the IFN pathway also in cutaneous lupus, with for example monoclonal anti-IFN antibodies already in clinical trials for SLE (Kalunian 2016; Merrill et al. 2011).

DATA AVAILABILITY

The data analyzed during this study are included in this published article and its supplementary information files. The raw RNA sequences generated during this study are not publicly available due to GDPR but are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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The study was approved by the Ethics Committee of Medicine of the Hospital District of Helsinki and Uusimaa (HUS), Finland, (191/13/03/01/2010) and all patients and healthy volunteers provided their written informed consent. This work was supported by Finska Läkaresällskapet, Finnish Rheumatism Association, and Swedish Research Council. DD acknowledges support from the ANR (CEI7001002) and Immunoqure AG for provision of IFN-α mAbs for Simoa assays. The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project b2014069. Mrs. Alli Tallqvist is acknowledged for technical assistance in tissue biopsy preparation and immunohistochemistry, and Mrs. Ingegerd Fransson for RNA extraction. JK is a recipient of the Royal Society Wolfson Research Merit Award. Figures 1c and 2a were made by the user data mapping feature of KEGG database (Kanehisa et al. 2017); the copyright permission number is 180344.

AUTHOR CONTRIBUTIONS

Conceptualization: JP, SKo, JK and AR

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Formal Analysis: SKa, JP, SKo, TS, KM, KKi, VB, DD KKr, JP and AR

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Supervision: JK and AR

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FIGURE LEGENDS

Figure 1. Inspection of normal UV photoprovocation-response genes.

(a) Classification of the up-regulated (top) and down-regulated (bottom) genes by photoprovocation based on PANTHER protein classes.

(b) Ten first Human Phenotype Ontology terms, in which the up-regulated genes by photoprovocation were enriched significantly. Numbers on each bar represent the number of member genes of the term (at the denominator), and the number of the response genes among the member (at the numerator). The specific response genes are checked in the matrix.

(c) Healthy skin UV photoprovocation-response genes indicated in the known SLE pathway [KEGG: hsa05322]. Rectangle boxes indicate molecules in this pathway. White box genes do not exist in human. Yellow box genes were up-regulated by photoprovocation in control skin samples.

Figure 2. Altered UV-photoprovocation-response genes in DLE and SCLE.

(a) The up-regulated genes (yellow box) in the known SLE pathway [KEGG: hsa05322].

(b) Ten most enriched gene sets in the LINCS L1000 ligand perturbation dataset, and the altered genes corresponding to the enriched gene sets; each gene set consists of differentially regulated genes induced by a ligand in a cell-line.

(c) Up-regulation of IFIT1/2/3 mRNA expression in photoprovocated SCLE (in all but two) and DLE skin lesions, by qRT-PCR validation.

(d) IFIT protein expression in skin samples by immunohistochemistry. IFIT proteins are more strongly expressed in SCLE than healthy control after UV-provocation. Note the strong expression (dark brown color) especially in basal keratinocytes and in areas with apoptotic keratinocytes (i.e. cells with pyknotic nuclei and eosinophilic cytoplasm). Scale bar = 100 µm.

Figure 1

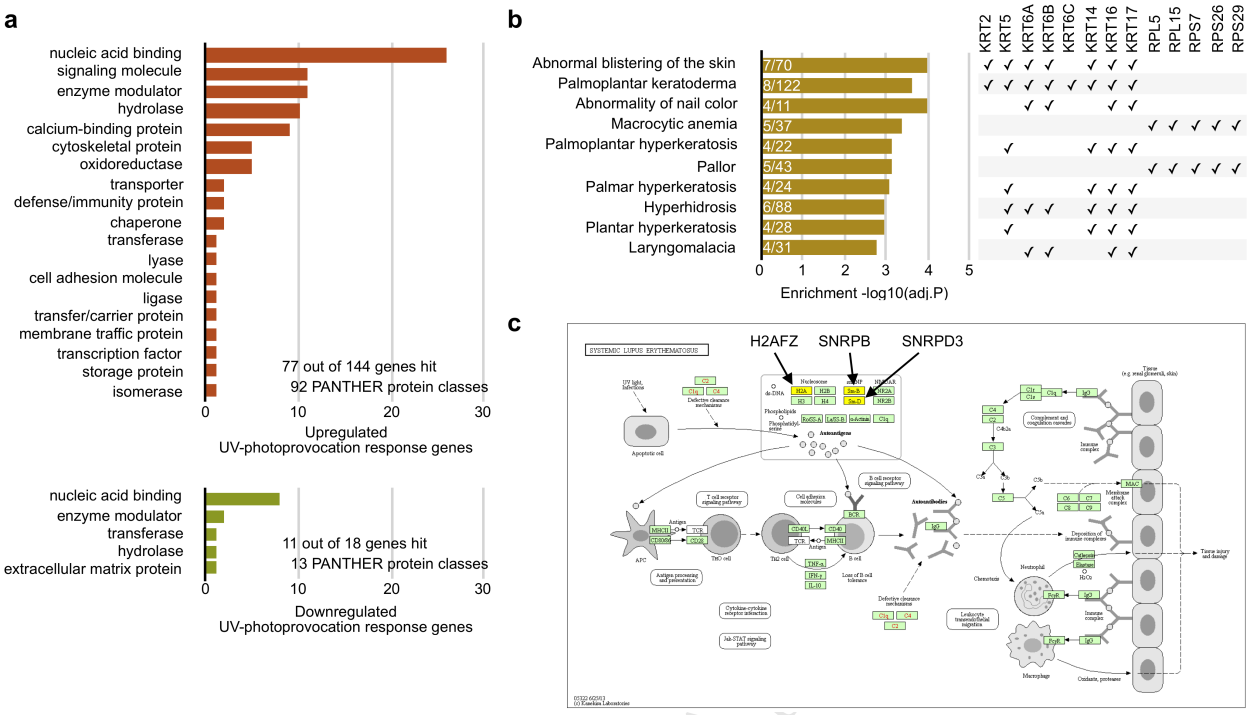

Figure 2