Dose and time effects of solar-simulated ultraviolet radiation on the in vivo human skin transcriptome*

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Summary
Background Terrestrial ultraviolet (UV) radiation causes erythema, oxidative stress, DNA mutations and skin cancer. Skin can adapt to these adverse effects by DNA repair, apoptosis, keratinization and tanning.

Objectives To investigate the transcriptional response to fluorescent solar-simulated irradiation (FSSR) in sun-sensitive human skin in vivo.

Methods Seven healthy male volunteers were exposed to 0, 3 and 6 standard erythemal doses (SED). Skin biopsies were taken at 6 h and 24 h after exposure. Gene and microRNA expression were quantified with next generation sequencing. A set of candidate genes was validated by quantitative polymerase chain reaction (qPCR); and wavelength dependence was examined in other volunteers through microarrays.

Results The number of differentially expressed genes increased with FSSR dose and decreased between 6 and 24 h. Six hours after 6 SED, 4071 genes were differentially expressed, but only 16 genes were affected at 24 h after 3 SED. Genes for apoptosis and keratinization were prominent at 6 h, whereas inflammation and immunoregulation genes were predominant at 24 h. Validation by qPCR confirmed the altered expression of nine genes detected under all conditions; genes related to DNA repair and apoptosis; immunity and inflammation; pigmentation; and vitamin D synthesis. In general, candidate genes also responded to UVA1 (340–400 nm) and/or UVB (300 nm), but with variations in wavelength dependence and peak expression time. Only four microRNAs were differentially expressed by FSSR.

Conclusions The UV radiation doses of this acute study are readily achieved daily during holidays in the sun, suggesting that the skin transcriptional profile of typical holiday makers is markedly deregulated.

What’s already known about this topic?
- The skin’s transcriptional profile underpins its adverse (i.e. inflammation) and adaptive molecular, cellular and clinical responses (i.e. tanning, hyperkeratosis) to solar ultraviolet radiation.
- Few studies have assessed microRNA and gene expression in vivo in humans, and there is a lack of information on dose, time and waveband effects.
Terrestrial solar ultraviolet radiation (UVR) is the most important environmental factor affecting skin physiology. Its spectrum comprises ≤5% of ultraviolet (UV)B (~295–315 nm) and ≥95% of UVA (315–400 nm). The relative photobiological effects of UVB and UVA depend on their absorption by cutaneous chromophores.1–4

Solar UVR readily induces erythema, and action spectroscopy shows that UVB is orders of magnitude more potent than UVA per unit dose (J m⁻²).4–6 UVB also readily induces DNA damage that can lead to skin cancer, if such damage is not removed via apoptosis or DNA repair mechanisms.3–6 However, UVB also has beneficial effects and initiates the cutaneous synthesis of vitamin D. UVA penetrates deeper into the skin than UVB, and has a role in skin photoageing.7 UVA can also generate reactive oxygen species that trigger DNA damage.3,4,5 Skin may adapt to UVR exposure by increasing keratinocyte cell division (stratum corneum thickening) and by tanning (melanogenesis).6,9 Adaptive pigmentation is biphasic. UVA induces immediate pigment darkening that is mediated by photo-oxidation of pre-existing melanin and redistribution of melanosomes. This is followed by persistent pigment darkening that lasts 2–3 days. Then, there is delayed tanning, mainly by UVB-induced de novo melanin synthesis and an increase in the number of active melanocytes. However, the tanning response is generally insufficient to prevent UVR mutagenic effects10,11 and erythema in lighter skin types.12

The molecular consequences of UVR exposure, both adverse and beneficial can be reflected in the skin’s transcriptional profile. UVB-induced cutaneous gene expression has been investigated,5,4,6 but the response of microRNAs (miRNAs), small noncoding regulatory RNAs, is less well characterized.13 However, few studies have been performed in vivo in humans,3 and have usually only evaluated a single UVR dose of a specific waveband14–16 at one time point after exposure.15–17

The main aim of the current study was to investigate the effects of different doses of fluorescent solar-simulated radiation (FSSR) over time (6 h and 24 h) on the skin’s transcriptional profile, including miRNAs, of seven healthy sun-sensitive volunteers. A secondary aim was to assess the effects of UVB (300 nm) and UVA1 (340–400 nm) from samples taken from a previous study.18
NCBI hg19 (Entrez Gene) database. The small RNA sequencing data were analysed as previously described.\textsuperscript{12}

**Validation of the expression levels of candidate genes**

Validation of 44 genes was performed using quantitative polymerase chain reaction (qPCR) with the TaqMan Real-Time PCR system (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). The selection criteria are specified in Table S3 (see Supporting Information). ΔCt was calculated by subtracting mean of two housekeeping genes (UBE2D2 + TBP) Ct to the candidate gene Ct.

**Wavelength dependence of candidate genes: ultraviolet A\textsubscript{1} and B**

Wavelength dependence was investigated from previous data generated by our group.\textsuperscript{18} Briefly, the skin of healthy skin type I/II volunteers was irradiated with 1 MED (equivalent to 1.6–2.5 SED) of UVA1 (340–400 nm) (n = 9) or UVB (300 nm) (n = 5) (Table S3; see Supporting Information). Gene expression was assessed with Agilent Whole Human Genome Oligo Microarrays (Agilent Technologies, Waldbronn, Germany) (44 K) at 6 h and 24 h from skin biopsies. Intensity signals were background-corrected and normalized using quartile normalization (see Bolstad et al., 2003).\textsuperscript{23} The microarray data are deposited at NCBI GEO: accession number GSE45493.

**Differential expression**

Data analyses were done in R3.1.0 and R3.2.3 environments.\textsuperscript{24} mRNA and miRNA differential expression were analysed by fitting negative binomial models using the R package DESeq2 v.1.14.1.\textsuperscript{15} The following contrasts were tested: 6 h–3 SED vs. 6 h–0 SED; 6 h–6 SED vs. 6 h–0 SED; 24 h–3 SED vs. 24 h–0 SED; 24 h–6 SED vs. 24 h–0 SED and 24 h–0 SED vs. 6 h–0 SED. The models were adjusted for participant identification (ID) and batch variables. Effect sizes are expressed as log\textsubscript{2} fold changes (Log2FC). Multiple testing was controlled with the false discovery rate (FDR) method (genes) and with the Bonferroni correction (miRNAs). After multiple testing corrections, no statistically significant differences were observed among expression levels in unexposed control skin biopsies (0 SED) collected at 6 h and at 24 h.

The association between gene expression levels assessed by qPCR (ΔCt) and FSSR-exposure groups was tested with linear mixed models adjusting for participant ID as a random effect. Effect size is reported as the minus coefficient of the model (−ΔΔCt), which can be regarded as a Log2FC. ANOVA with repeated measurements followed by Tukey post hoc tests was used to test for expression differences among the groups in the microarray experiment.\textsuperscript{18} Effect size is reported as Log2FC.

**Functional enrichment analysis**

Genes with a P-value < 1E-03 were selected for functional enrichment analysis. Gene-set enrichment analysis was performed with the Functional Annotation Clustering option of the Database for Annotation, Visualization and Integrated Discovery version 6.7 (https://david-d.ncifcrf.gov/).\textsuperscript{26,27} Enrichment for transcription-factor regulation was assessed with Enrichr (https://amp.pharm.mssm.edu/Enrichr/).\textsuperscript{28,29}

**microRNA – gene regulatory networks**

The regulatory networks of miRNAs and genes were analysed using MAGIA2 (http://gencomp.bio.unipd.it/magia2/).\textsuperscript{10} This tool combines expression profile analysis with silico regulatory interaction predictions. Experimentally validated miRNA-targeted gene pairs were retrieved from miRWalk 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/). Matrices of non-normalized counts for mRNAseq and smallRNAseq can be found at Mendeleev Data (https://doi.org/10.17632/mhxmn9b2pt.1).

**Results**

**Gene expression after exposure to fluorescent solar-simulated radiation**

**Discovery phase: mRNAseq**

The number of differentially expressed genes at 5% FDR increased with higher FSSR dose and earlier time after
exposure (Table 2 and Fig. 1). More genes were differentially expressed after 6 SED compared with 3 SED (6 h: 4071 vs. 132 genes; 24 h: 1583 vs. 16 genes) (Tables S4–S7; see Supporting Information). More than 87% of the genes expressed after 3 SED were also differentially expressed at 6 SED (Fig. 2). Only a fraction of the genes deregulated at 6 h were still affected at 24 h (3 SED: 7.5%; 6 SED: 29-7%). Ten genes (seven downregulated and three upregulated) showed altered expression patterns under all conditions (Fig. 2).

In general, a slight increase in the number of upregulated vs. downregulated genes was observed. At 6 SED, the effect size of upregulated genes was slightly more pronounced than the effect of downregulated. When the analysis was restricted to five volunteers with available samples in all conditions, results were similar (Table S8 and Fig. S2; see Supporting Information).

Gene-set enrichment analysis

At 6 h after 6 SED, genes for keratinization, apoptosis, transcription/translation, and cytoskeleton organization were detected (Table S9; see Supporting Information). Analyses at 24 h showed the same pathways, except for keratinization and apoptosis, as well as pathways for inflammation, immunoreactivity (interleukins, tumour necrosis factor, nuclear factor kappa, interferon-gamma), and hyaluronan biosynthesis (Table S10). Fewer pathways were detected at 3 SED (Tables S11 and S12).

Transcription-factor enrichment analysis

The highest-ranking transcription factors in all conditions were: MYC (oncogene); MAX (forms a complex with MYC); and SIN3A (antagonizes MYC) (Tables S13–S16; see Supporting Information). Other transcription factors were detected that related to DNA repair, apoptosis and cell proliferation (P53, E2F) and to skin barrier and metalloproteinases (KLF4, FLJ and ZNF384). USF, involved in keratinocyte growth, was only detected at 6 h with both doses; whereas transcription factors related to immune and inflammatory responses (CEBPB, RUNX1, STAT3 and RELA) ranked highest at 24 h.

Candidate genes: validation by quantitative polymerase chain reaction and wavelength-dependence analyses

We carried out qPCR validation for 44 candidate genes: nine genes were detected under all conditions (see Fig. 3a), and genes in pathways of specific interest identified in our previous studies including DNA repair and apoptosis (n = 3) (see Fig. 3b), immunity and inflammation (n = 5) (see Fig. 3c), pigmentation (n = 20) (see Fig. 3d) and vitamin D synthesis (n = 7) (see Fig. 3e) (see Tables S2 and S17, Supporting Information). Moreover, wavelength dependence of nominally replicated genes was investigated using previous microarray expression data generated by our group (Fig. 3; Table S3). Genes detected in all conditions showed the same direction of the effect in the validation study. All showed at least nominal significance, and three were still significant after Bonferroni correction. Their functions are described in Table S18 (see Supporting Information). In general, expression patterns were similar with UVA1 and UVB irradiation. We also found an increased expression of the DNA repair gene POLH at 6 h. The genes related to immunity and inflammation (CD83, IL1A, IL20, IL6 and TNF) were at least nominally significant with peak induction at 24 h. Among vitamin D genes, only a

Table 2. Summary of the number of statistically significant genes at 5% false discovery rate (FDR) and their effect size after different fluorescent solar-simulated radiation dose and time after exposure

| Time, dose (sample size*), direction of the effect | Genes at 5% FDR, n | Mean Log2FCb | Median Log2FCb | Min Log2FCb | Max Log2FCb |
|------------------------------------------------|--------------------|--------------|----------------|-------------|-------------|
| 6 h                                             |                    |              |                |             |             |
| 3 SED vs. 0 SED (7 vs. 5)                        |                    |              |                |             |             |
| All                                            | 132                | 0.576        | 0.552          | 0.269       | 1.322       |
| Downregulated                                   | 58                 | −0.622       | −0.577         | −0.333      | −1.322      |
| Upregulated                                     | 74                 | 0.539        | 0.528          | 0.269       | 1.031       |
| 6 SED vs. 0 SED (6 vs. 5)                        |                    |              |                |             |             |
| All                                            | 4071               | 0.651        | 0.606          | 0.199       | 2.002       |
| Downregulated                                   | 1766               | −0.609       | −0.571         | −0.220      | −1.825      |
| Upregulated                                     | 2305               | 0.684        | 0.640          | 0.199       | 2.002       |
| 24 h                                            |                    |              |                |             |             |
| 3 SED vs. 0 SED (7 vs. 7)                        |                    |              |                |             |             |
| All                                            | 16                 | 0.892        | 0.816          | 0.582       | 1.592       |
| Downregulated                                   | 10                 | −0.949       | −0.822         | −0.662      | −1.592      |
| Upregulated                                     | 6                  | 0.798        | 0.765          | 0.582       | 0.982       |
| 6 SED vs. 0 SED (6 vs. 7)                        |                    |              |                |             |             |
| All                                            | 1583               | 0.650        | 0.592          | 0.209       | 1.967       |
| Downregulated                                   | 586                | −0.571       | −0.546         | −0.209      | −1.411      |
| Upregulated                                     | 997                | 0.697        | 0.651          | 0.231       | 1.967       |

Log2FC: Log2 fold change; SED, standard erythemal doses. *Samples not included: ICE_003_A, ICE_004_A, ICE_004_E, ICE_004_F; *Absolute |Log2FC for genes differently expressed at 5% FDR; *Absolute |Log2FC.
decreased expression of CYP2R1 was validated by qPCR at 6 h and 24 h. Their expression was similarly affected by UVA1 and UVB.

Fourteen of 20 pigmentation genes were nominally significant, of which three survived Bonferroni correction. They all were downregulated after FSSR, except for EDNRB, which was upregulated at 24 h. In general, they responded similarly to UVA1, UVB or both, except for TYR and LYST, which were downregulated by FSSR at 6 h but upregulated by UVA1 at 24 h.

microRNA expression after exposure to fluorescent solar-simulated radiation in skin

Discovery phase: microRNAseq

The FSSR-induced effect on miRNA expression was of smaller magnitude than that of gene expression (Fig. 4). Only four miRNAs were differentially expressed after multiple testing correction (Fig. 5; Table S19, see Supporting Information). Hsa-miR-146b-5p and hsa-miR-223-3p were upregulated at 6 h after 6 SED. At 24 h, the levels of hsa-miR-223-3p were still high, whereas the levels of hsa-miR-146b-5p had almost returned to baseline. The expression patterns of hsa-miR-204-5p and hsa-miR-142-5p were more complex.

microRNA – gene regulatory networks

We investigated the miRNA-gene regulatory networks. All miRNAs, except for hsa-miR-142-5p, showed significant correlations
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with some predicted target genes or transcription factors (Fig. S3; see Supporting Information). The following miRNA-gene negative significant correlations were found: hsa-miR-146b-5p and TMEM237, TMEM132E, LANCL1, SLC6A4; hsa-miR-204-5p and IL1B; hsa-miR-223-3p and HLF. A list of experimentally validated targeted genes for these four miRNAs is shown in Table S20 (see Supporting Information). Some of them are: IL6 (hsa-miR-223-3p),36 KIT (hsa-miR-146b-5p)37 and PRKCB (hsa-miR-142-5p).38

**Discussion**

The transcriptional profile of human skin in vivo was investigated after exposure to FSSR. Dose was the main driver of transcriptional changes, and the number of differentially expressed genes decreased with time. At 6 h post 6 SED, 4071 genes were differentially expressed, which represents around 20% of the human transcriptome, and highlights the significant impact of UVR on the skin. Previous studies have also reported marked transcriptional changes in human skin after a single exposure of narrow or broadband UVB.14–16,18 In contrast, only 21 genes were differently expressed in blood in the same volunteers after whole body exposure to 3 SED of FSSR.19 Although some pathways detected at 6 h and 24 h were the same, others were more prominent at a specific time point suggesting a sequential response to FSSR. Apoptosis and keratinization were the first responses at 6 h, followed by inflammation, immunoactivation and hyaluronan biosynthesis, at 24 h. In mice, chronic UVR exposure increases the accumulation of hyaluronan in the extracellular matrix of keratinocytes.39 In agreement with this pattern, at 6 h we...
observed an enrichment of USF transcription factors, which promote keratinocyte growth; whereas at 24 h, transcription factors related to immune and inflammatory responses were found (CEBPB, RUNX1, STAT3 and RELA). Some of the transcription factors detected in the enrichment analysis had been previously associated with UVR: STAT3, KLF4 and E2F.

The qPCR analyses confirmed the expression levels of 30 of the 44 candidate genes, including those related to DNA repair and apoptosis, immunity and inflammation, pigmentation and vitamin D. The FSSR emission spectrum comprises 5% UVB, which accounts for 79% of its erythemally effective energy.

We thus explored the UVA1 and UVB wavelength dependence of these 30 genes, when exposures were 1 MED. UVB at 300 nm was chosen because it represents the peak of the human erythema and DNA damage action spectra, and UVA1 because it represents the majority (~75%) of solar UVA. Waveband comparisons have to be interpreted with caution because of the design differences in the studies: different volunteers and transcriptomic platforms. The common link with all spectra was erythemal exposure; in other words, the molecular damage was sufficient to trigger a clinical response. This resulted in similar directions in transcriptome response, even though the UVB/UVA1 exposures were lower in MED terms (3–6 MED for FSSR and 1–6–2 MED for individual 1 MED UVB/UVA1), but with a stronger magnitude with UVB. This suggests that chromophores with preferential UVB absorption are more important than chromophores with UVA1 maxima for a wide range of solar UVR effects.

The decreased expression of CYP2R1 after exposure to FSSR, UVA1 and UVB is of particular interest. This gene encodes 25-hydroxylase that converts vitamin D synthesized in skin to 25-hydroxyvitamin D \([25(OH)D]\). Skin also converts 25(OH)D into 1,25(OH)D (active vitamin D) by 1-α-hydroxylase (CYP27B1), which was found to be upregulated in our study. Toxicity does not occur when vitamin D is generated by UVR. It is thought this is the consequence of photodegradation of pre-vitamin D and vitamin D and their conversion into products lacking calcemic activity. Our findings suggest that 25(OH)D and 1,25(OH)D levels are also under photoenzymatic control in skin; and that this could, in part, explain the homeostatic regulation of serum 25(OH)D.

At 6 h after FSSR, many of the core pigmentation genes were downregulated, except for EDNRB, a receptor involved in keratinocyte and melanocyte interactions, which was upregulated at 24 h. Upregulation of EDNRB after UVB exposure has been reported in cultured melanocytes. In general, these effects were observed with FSSR, UVA1 and/or UVB. The exceptions were LYST, that participates in melanosome transport, and TTR, the rate-limiting enzyme in melanin synthesis, which were downregulated by FSSR exposure mainly at 6 h, but upregulated by UVA1 at 24 h. These are compatible with
a redistribution of melanosomes by UVA1 within 24 h after exposure, and would suggest that delayed tanning, that involves an increase in the number of functional melanocytes, is not initiated until 6 h post FSSR.9 Choi et al. analysed the expression of pigmentation genes 3 days after repeated (every weekday for 2 weeks) exposure to SSR, UVA and UVB.17 PMEL, TYR, TRP1 or KIT were among the genes with the highest induction after SSR or UVB, but not UVA. To our knowledge, there are no other studies reporting repression of pigmentation genes at 6 h after UVR. The significance of this needs further investigation, but it could be a protective mechanism against the production of excessive melanin that may result in toxicity.9 Minimal overlap, and with no consistent direction of the effect, was observed between the other genes reported in Choi et al.17

Nine out of the 10 genes differentially expressed in all FSSR conditions were validated. AEN (upregulated) encodes a protein required for p53-dependent apoptosis.48 EPHB1 (downregulated) encodes an ephrin receptor tyrosine kinase that mediates cell–cell communication by interacting with ephrin ligands.49 These participate in development, maintenance and repair processes in cutaneous biology.49,50 GRIPI (downregulated) is required for the formation and integrity of the dermoepidermal junction.51 PRKCB (downregulated) activates TYR, the key and rate-limiting enzyme in pigmentation.52 All these genes were also Similarly affected by UVA1 and/or UVB wavebands.

In contrast to genes, the expression of miRNAs was not greatly affected by FSSR. It is possible that mRNA regulation is less influenced by FSSR or that effects occur at different time points. In vitro studies have shown both acute and long-term changes in mRNA levels after UVR.13 Another explanation could be that the precision of the RNAseq was lower for miRNAs.

Four miRNAs were differentially expressed by FSSR. Hsa-miR-146b-5p and hsa-miR-223-3p were upregulated, as seen in a cellular model exposed to nonsolar UVC (254 nm)53 and in UVB irradiated murine skin,54 respectively. The miRNA-gene network analysis identified SLCA4, a seroton transporter, as a potential target of hsa-miR-146b-5p. Activation of the serotonin pathway has been suggested to mediate UBV-induced immunosuppression.55 In our data, hsa-miR-204-5p levels were inversely correlated with IL1B levels. The interaction between hsa-miR-204-5p and IL1B has previously been validated,56 and hsa-miR-204-5p is known to participate in skin wound healing.57 hsa-miR-142-5p has been found to be upregulated in chronically UVR-treated mouse skin.58

The study has some limitations. Firstly, the small sample limits its detection power, which is of particular importance for miRNAs, whose FSSR-induced expression seems to be subtle. Secondly, the study investigates the effects of an acute FSSR exposure. It is not possible to comment on the chronic effects of FSSR exposure on the skin.

The study also has several strengths. Firstly, the effects of FSSR on transcription were investigated in vivo in biopsies obtained from the skin of healthy sun-sensitive volunteers, in contrast to the more artificial in vitro cellular models.59 Secondly, the transcriptional profile was investigated comprehensively, including miRNAs. Thirdly, results were validated by qPCR and wavelength dependence was explored in independent settings, that gave broadly similar results. This is of crucial importance given that millions of people intentionally expose themselves to high doses of UVR. For instance, the Danish population of 5–5.5 million takes 1–2 million holidays annually to sunny destinations, of which half are estimated to be for sunbathing.60 The highest FSSR dose in our study is lower than the mean daily erythemal dose received by Danish holiday makers during a 6-day holiday in Tenerife in March, when at least 50% of their body surface was exposed to 9.4 ± 7.0 SED per day.60 However, it would be important to do field studies because reciprocity may not hold when a given dose is delivered over a longer period of time. In conclusion, UVR doses that are easily achieved during intentional solar exposure have a marked effect on the skin transcriptome.

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References

1 Young AR. Chromophores in human skin. Phys Med Biol 1997; 42:789–802.
2 Damiani E, Ulrich SE. Understanding the connection between platelet-activating factor, a UV-induced lipid mediator of inflammation, immune suppression and skin cancer. Png Lipid Res 2016; 63:14–27.
3 Ramasamy K, Shannugam M, Balupillai A et al. Ultraviolet radiation-induced carcinogenesis: mechanisms and experimental models. J Radiat Cancer Res 2017; 8:4.
4 Schuch AP, Moreno NC, Schuch NJ et al. Sunlight damage to cellular DNA: focus on oxidatively generated lesions. Free Radic Biol Med 2017; 107:110–24.
5 Young AR, Chadwick CA, Harrison GI et al. The similarity of action spectra for thymine dimers in human epithidium and erythema suggests that DNA is the chromophore for erythema. J Invest Dermatol 1998; 111:982–8.
6 D’Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. Int J Mol Sci 2013; 14:12222–48.
7 Kruitmann J. Ultraviolet A radiation-induced biological effects in human skin: relevance for photoaging and photodermatosis. J Dermatol Sci 2000; 23 (Suppl. 1):S2–6.
8 Tewari A, Sarkany RP, Young AR. UVA1 induces cyclobutane pyrimidine dimers but not 6–4 photoproducts in human skin in vivo. J Invest Dermatol 2012; 132:394–400.
9 Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. Photochem Photobiol 2008; 84:539–49.
10 Greinert R, Boguhn O, Harder D et al. The dose dependence of cyclobutane dimer induction and repair in UVB-irradiated human keratinocytes. Photochem Photobiol 2000; 72:701–8.
11 Ridley AJ, Whiteside JR, McMillan TJ, Allinson SL. Cellular and sub-cellular responses to UVA in relation to carcinogenesis. Int J Radiat Biol 2009; 85:177–95.

12 Sheehan JM, Potkin CS, Young AR. Tanning in human skin types II and III offers modest photoprotection against erythema. Photochem Photobiol 1998; 68:588–92.

13 Syed DN, Khan MI, Shabbir M, Mukhtar H. MicroRNAs in skin response to UV radiation. Curr Drug Targets 2013; 14:1128–34.

14 Enk CD, Shahar I, Amargiolgi N et al. Gene expression profiling of in vivo UVB-irradiated human epidermis. Photodermatol Photoimmunol Photomed 2004; 20:129–37.

15 Kennedy Crispin M, Fuentes-Duculan J, Gulati N et al. Gene profiling of narrowband UVB- induced skin injury defines cellular and molecular innate immune responses. J Invest Dermatol 2013; 133:692–701.

16 Daves JM, Antunes-Martins A, Perkins JR et al. Genome-wide transcriptional profiling of skin and dorsal ganglia after ultraviolet-B-induced inflammation. PLOS ONE 2014; 9:e93338.

17 Choi W, Miyamura Y, Wolber R et al. Regulation of human skin pigmentation in situ by repetitive UV exposure: molecular characterization of responses to UVA and/or UVB. J Invest Dermatol 2010; 130:1685–96.

18 Tewari A, Grys K, Kollet J et al. Upregulation of MMP12 and its activity by UVA1 in Human skin: potential implications for photaging. J Invest Dermatol 2014; 134:2598–609.

19 Bustamante M, Hernandez-Ferrer C, Sarria Y et al. The acute effects of ultraviolet radiation on the blood transcriptome are independent of plasma 25OHD3. Eur J Clin Invest 2017; 47:239–48.

20 Harrison GI, Young AR. Ultraviolet radiation-induced erythema in human skin. Method 2002; 28:114–9.

21 Liao Y, Smyth GK, Shi W. The subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucl Acids Res 2013; 41:e108.

22 Lappalainen T, Sammeth M, Friedlander MR et al. Transcriptome and genome sequencing uncovers functional variation in humans. Nature 2015; 501:506–11.

23 Bollstad BM, Irizarry RA, Astrand M et al. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003; 19:185–93.

24 R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing; 2008.

25 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014; 15:550.

26 Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4:44–57.

27 Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009; 37:1–13.

28 Chen EY, Tan CM, Kou Y et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics 2013; 14:128.

29 Kuleshov MV, Jones MR, Rouillard AD et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016; 44:W90–7.

30 Bisognin A, Sales G, Coppe A et al. MAGIA2: from miRNA and genes expression data integrative analysis to microRNA-transcription factor mixed regulatory circuits (2012 update). Nucleic Acids Res 2012; 40:13–21.

31 Segre JA, Bauer C, Fuchs E. KLF4 is a transcription factor required for establishing the barrier function of the skin. Nat Genet 1999; 22:356–60.

32 Kopecki Z, Yang GN, Arkell RM et al. Flughless I over-expression impairs skin barrier development, function and recovery following skin blistering. J Pathol 2014; 232:541–52.

33 Torrungruangk A, Alvarez M, Shah R et al. DNA binding and gene activation properties of the Nmp4 nuclear matrix transcription factor. J Biol Chem 2002; 277:16153–9.

34 Qi L, Higgins CE, Higgins SP et al. The basic helix-loop-helix-Leucine zipper transcription factor USF2 integrates serum-induced PAI-1 expression and keratinocyte growth. J Cell Biochem 2014; 115:1840–7.

35 Thys R, Viroille V, Imbert V et al. NF-kB/Egr-1/Gadd45 are sequentially activated upon UVB irradiation to mediate epidermal cell death. EMBO J 2005; 24:128–37.

36 Dorfho A, Iannaccone M, Farinacci M et al. MicroRNA-223 controls susceptibility to tuberculosis by regulating lung neutrophil recruitment. J Clin Invest 2013; 123:4836–48.

37 He H, Jazdzewski K, Li W et al. The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A 2005; 102:19075–80.

38 Pillai MM, Gillen AE, Yamamoto TM et al. HITS-CLIP reveals key regulators of nuclear receptor signaling in breast cancer. Breast Cancer Res Treat 2014; 146:85–97.

39 Siskonen H, Törönen K, Kummel T et al. Chronic UVR causes increased immunostaining of CD44 and accumulation of hyaluronan in mouse epidermis. J Histochem Cytochem 2011; 59:908–17.

40 Bito T, Sumita N, Masaki T et al. Ultraviolet light induces Stat3 activation in human keratinocytes and fibroblasts through reactive oxygen species and DNA damage. Exp Dermatol 2010; 19:654–60.

41 Choi WJ, Youn SH, Back JH et al. The role of KLF4 in UVB-induced murine skin tumor development and its correlation with cyclin D1, p53, and p21Waf1/Cip1 in epithelial tumors of the human skin. Arch Dermatol Res 2011; 303:191–200.

42 Hazar-Rethinam M, Endo-Munoz L, Gannon O, Saunders N. The role of the E2F transcription factor family in UV-induced apoptosis. Int J Mol Sci 2011; 12:8947–60.

43 Young AR, Chadwick CA, Harrison GI et al. The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. J Invest Dermatol 1998; 111:982–8.

44 Norval M, Björn LO, De Grujil FR. Is the action spectrum for the UV-induced production of previtamin D 3 in human skin correct? Photosch Photobiol Sci 2010; 9:11–7.

45 Webb AR, Decosta BR, Holick MF. Sunlight regulates the cutaneous production of vitamin D3 by causing its photodegradation. J Clin Endocrinol Metab 1998; 68:882–7.

46 Mason RS, Dixon KM, Sequeira VB, Gordon-Thomson C. Sunlight protection by vitamin D compounds. In: Vitamin D: Vol. 2: Health, Disease and Therapeutics, 4th edn. London: Academic Press; 2018; 1055–75.

47 Tagashira H, Miyamoto A, Kitamura SI et al. UVB stimulates the expression of endothelin B receptor in human melanocytes via a sequential activation of the p38/MSK1/CREB/MITF pathway which can be interrupted by a French maritime pine bark extract through a direct inactivation of MSK1. PLOS ONE 2015; 10:1–17.

48 Kawase T, Ichikawa H, Ohta T et al. NF-kB-induced inflammation is a nuclear exonuclease required for p53-dependent apoptosis. Oncogene 2008; 27:3797–810.

49 Suravaska H, Ma PC, Salgia R. The role of ephrins and Eph receptors in cancer. Cytokine Growth Factor Rev 2004; 15:419–33.

50 Lin S, Wang B, Getsios S. Eph/ephrin signaling in epidermal differentiation and disease. Semin Cell Dev Biol 2012; 23:92–101.

51 Bladé T, Tafuri A, Gelkop S et al. Epidermolysis bullosa and embryonic lethality in mice lacking the multi-PDZ domain protein GRIP1. Proc Natl Acad Sci U S A 2002; 99:6816–21.
Ultraviolet radiation and in vivo human skin transcriptome, M. Bustamante et al.

Supplemental Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

**Files S1** Supplementary materials and methods.

**Fig S1.** Study design, library preparation and bioinformatic analysis.

**Fig S2.** Volcano plots of gene expression in skin after fluorescent solar-simulated radiation exposure (different doses and time points) for the subset of five volunteers with complete samples at all conditions.

**Fig S3.** Interactions between microRNA, genes and transcription factors based on gene expression data and in silico target predictions (all samples).

**Table S1** RNA samples used in the study per participant and condition.

**Table S2** List of selected genes for validation by quantitative polymerase chain reaction and wavelength-dependence analysis.

**Table S3** Anthropometric and dermatological characteristics of the nine participants in the wavelength-dependence analysis.

**Table S4** Discovery phase (mRNAseq): genes differently expressed at 5% false discovery rate after fluorescent solar-simulated radiation exposure [6 h–6 standard erythemal doses (SED) vs. 6 h–0 SED].

**Table S5** Discovery phase (mRNAseq): genes differently expressed at 5% false discovery rate after fluorescent solar-simulated radiation exposure [6 h–6 standard erythemal doses (SED) vs. 6 h–0 SED].

**Table S6** Discovery phase (mRNAseq): genes differently expressed at 5% false discovery rate after fluorescent solar-simulated radiation exposure [24 h–3 standard erythemal doses (SED) vs. 24 h–0 SED].

**Table S7** Discovery phase (mRNAseq): genes differently expressed at 5% false discovery rate after fluorescent solar-simulated radiation exposure [24 h–6 standard erythemal doses (SED) vs. 24 h–0 SED].

**Table S8** Summary of the number of statistically significant genes at 5% false discovery rate and their effect size after different fluorescent solar-simulated radiation dose and time post-exposure, for the subset of participants with complete samples in all the conditions.

**Table S9** Gene-set cluster enrichment analysis of genes differently expressed after fluorescent solar-simulated radiation exposure [6 h–6 standard erythemal doses (SED) vs. 6 h–0 SED].

**Table S10** Gene-set cluster enrichment analysis of genes differently expressed after fluorescent solar-simulated radiation exposure [24 h–6 standard erythemal doses (SED) vs. 24 h–0 SED].

**Table S11** Gene-set cluster enrichment analysis of genes differently expressed after fluorescent solar-simulated radiation exposure [6 h–3 standard erythemal doses (SED) vs. 6 h–0 SED].

**Table S12** Gene-set enrichment analysis of genes differently expressed after fluorescent solar-simulated radiation exposure [24 h–3 standard erythemal doses (SED) vs. 24 h–0 SED].

**Table S13** Transcription-factor enrichment analysis of genes differently expressed after fluorescent solar-simulated radiation exposure [6 h–3 standard erythemal doses (SED) vs. 6 h–0 SED].

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**Table S15** Transcription-factor enrichment analysis of genes differently expressed after fluorescent solar-simulated radiation exposure [24 h–3 standard erythemal doses (SED) vs. 24 h–0 SED].

**Table S16** Transcription-factor enrichment analysis of genes differently expressed after fluorescent solar-simulated radiation exposure [24 h–6 standard erythemal doses (SED) vs. 24 h–0 SED].

**Table S17** Validation of the expression of candidate genes by quantitative polymerase chain reaction.

**Table S18** Function of genes differently expressed after fluorescent solar-simulated radiation exposure in all conditions.

**Tables S19** Discovery phase (microRNAseq): microRNAs differently expressed at any comparison.

**Table S20** List of experimentally validated microRNA – targeted gene interactions from miRWalk2.

**Table S21** Number of reads per sample in the mRNAseq experiment.

**Table S22** Number of reads per sample in the small RNAseq experiment.