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

M. Bustamante,[1,2,3,4] C. Hernandez-Ferrer,[1,3,4,5] A. Tewari,[6] Y. Sarria,[1,3,4] G.I. Harrison,[6] E. Puigdecanet,[7] L. Nonell,[7] W. Kang,[8] M.R. Friedländer,[8] X. Estivill,[2,3,4,9] J.R. González,[1,3,4] M. Nieuwenhuijsen[1,3,4] and A.R. Young[6]

[1]*ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain*
[2]*Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain*
[3]*Universitat Pompeu Fabra (UPF), Barcelona, Spain*
[4]*CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain*
[5]*Computational Health Informatics Program (CHIP), Boston Children's Hospital, Boston, MA, U.S.A.*
[6]*King's College London, St John's Institute of Dermatology, London, U.K.*
[7]*Servei d'Anàlisi de Microarrays, IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain*
[8]*Science for Life Laboratory, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden*
[9]*Genetics Program, Sidra Medical Center, Al Rayyan Municipality, Qatar*

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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 radiation (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.

Correspondence

Mariona Bustamante and Antony R. Young.
E-mails: mariona.bustamante@isglobal.org and antony.young@kcl.ac.uk

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Conflicts of interest

None to declare.

The authors wish it to be known that, in their opinion, M.B and C.H.-F. should be regarded as joint first authors and M.N. and A.R.Y. should be regarded as joint last authors.

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What does this study add?

- Acute doses of fluorescent solar-simulated radiation (FSSR), of similar magnitude to those received daily in holiday situations, markedly altered the skin's transcriptional profiles.
- The number of differentially expressed genes was FSSR-dose-dependent, reached a peak at 6 h and returned to baseline at 24 h.
- The initial transcriptional response involved apoptosis and keratinization, followed by inflammation and immune modulation. In these conditions, microRNA expression was less affected than gene expression.

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.[3,4,8] 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 effects[10,11] 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. UVR-induced cutaneous gene expression has been investigated,[3,4,6] 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 waveband[14–16] 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]

Material and methods

Study design

The study was conducted according to the Declaration of Helsinki after approval was obtained from the Ethics Committee of St Thomas's Hospital, London, U.K. All participants gave written informed consent. Seven healthy men with similar sun-sensitive skin types II[19] were enrolled (Table 1 and Fig. S1; see Supporting Information). Skin biopsies were collected at 6 h and 24 h after exposure to 3 and 6 standard erythema doses (SED) and a nonirradiated control biopsy was taken at each time point. It should be noted that the number of SED for a minimal erythema dose (MED) varies with skin type, but the MED of a skin type II is typically ~3 SED.[20]

Participants, biological samples and exposure to fluorescent solar-simulated radiation

Previously unexposed buttock skin was exposed to FSSR using Arimed B tubes (Cosmedico, Stuttgart, Germany) in a full-height home phototherapy unit (Waldmann UV 100 L, Waldmann GmbH & Co, Villingen-Schwenningen, Germany). Biopsies were taken at 6 h (0 SED, 3 SED and 6 SED) and at 24 h (0 SED, 3 SED and 6 SED). All skin samples were immediately frozen at −80C and RNA was extracted (Qiagen, Hilden, Germany). Low-quality RNA samples were excluded from the study (Table S1; see Supporting Information).

Gene and microRNA expression (next generation sequencing)

mRNA and miRNA cDNA libraries were prepared with TruSeq Sample Prep Kits (Illumina, San Diego, California, U.S.A.). Libraries were single-end sequenced (100 nt and 50 nt for mRNA and small RNA, respectively) on a HiSeq2000 platform (Illumina, San Diego, California, U.S.A.). For mRNA, reads were mapped against the genome using the R package Rsubread,[21] allowing a maximum of five mismatches and using the hs37d5 as reference. Gene annotation was performed with

Table 1 Anthropometric and dermatological characteristics of the seven male participants[a]

		Anthropometrics		Dermatological/pigmentation parameters					
Participant	Age, years	Height, m	Weight, kg	Hair colour	Eye colour	Complexion	Suntan	Sunburn	Freckles
ICE_003	31	1·83	74	Brown	Brown	Pale	Light	Often	+
ICE_004	24	1·78	71	Dark brown	Brown	Pale	Light	Often	+
ICE_005	25	1·73	63	Blond	Blue	Pale	Light	Often	0
ICE_006	22	1·83	79	Blond	Green	Pale	Light	Often	0
ICE_007	20	1·77	72	Dark brown	Blue	Pale	Light	Often	+
ICE_008	20	1·77	67	Brown	Brown	Pale	Faint	Often	0
ICE_009	35	1·88	120	Brown	Blue	Pale	Faint	Always	+

[a]All participants were men with skin type II, according to Fitzpatrick scale.

NCBI hg19 (Entrez Gene) database. The small RNA sequencing data were analysed as previously described.[22]

Validation of the expression levels of candidate genes

Validation of 44 genes was performed using quantitative polymerase chain reaction PCR (qPCR) with the TaqMan Real-Time PCR system (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). The selection criteria are specified in Table S2 (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 A1 and B

Wavelength dependence was investigated from previous data generated by our group.[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) (4#44 K) at 6 h and 24 h from skin biopsies. Intensity signals were background-corrected and normalized using quantile normalization (see Bolstad *et al.*, 2003).[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.[24] mRNA and miRNA differential expression were analysed by fitting negative binomial models using the R package DESeq2 v.1·14·1.[25] 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 $\log_2$ 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 ($^{-}\Delta\Delta$Ct), 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.[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/).[26,27] Enrichment for transcription-factor regulation was assessed with Enrichr (https://amp.pharm.mssm.edu/Enrichr/).[28,29]

microRNA – gene regulatory networks

The regulatory networks of miRNAs and genes were analysed using MAGIA2 (http://gencomp.bio.unipd.it/magia2).[30] This tool combines expression profile analysis with *in 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 Mendeley Data (https://doi.org/10.17632/mhmxn9b2pt.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,[31] FLI1[32] and ZNF384[33]). USF, involved in keratinocyte growth,[34] was only detected at 6 h with both doses; whereas transcription factors related to immune and inflammatory responses (CEBPB, RUNX1, STAT3 and RELA[35]) 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[18] 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).[18]

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[a]), direction of the effect	Genes at 5% FDR, n	Mean Log2FC[b]	Median Log2FC[b]	Min Log2FC[b]	Max Log2FC[b]
6 h					
3 SED vs. 0 SED (7 vs. 5)					
All[c]	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[c]	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[c]	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[c]	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: Log_2 fold change; SED, standard erythemal doses. [a]Samples not included: ICE_003_A, ICE_004_A, ICE_004_E, ICE_004_F; [b]Log2FC for genes differently expressed at 5% FDR; [c]Absolute |Log2FC|.

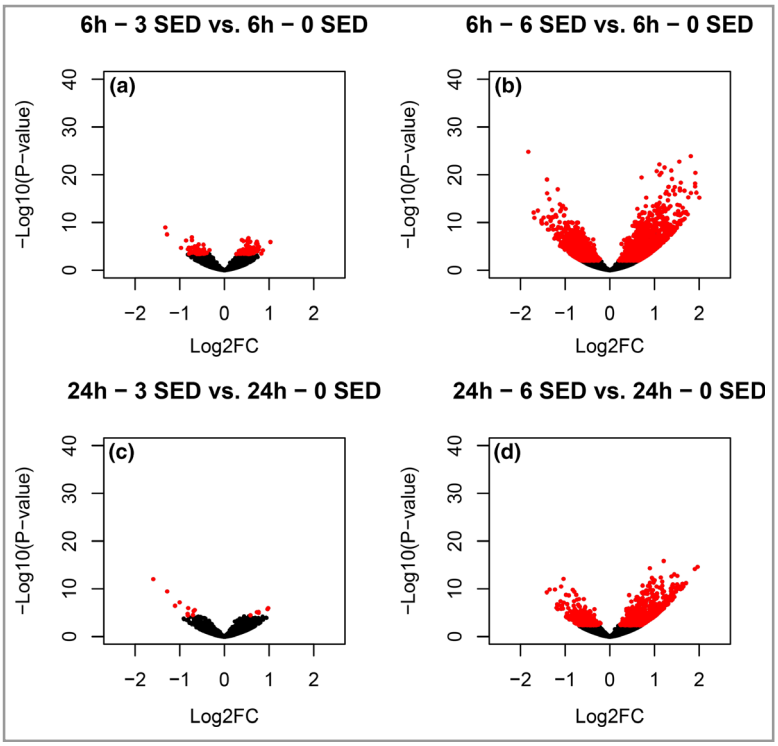

Fig 1. Volcano plots of gene expression in skin after fluorescent solar-simulated radiation exposure (different doses and time points). Plots show effect size $\log_2$ fold change (Log2FC) vs. –log10(P-value). (a) and (b) show dose effects at time 6 h; and (c) and (d) dose effects at 24 h. Genes that reached 5% false discovery rate are in red. The number of differently expressed genes is higher at the higher dose [6 standard erythemal doses (SED)] and earlier time after exposure (6 h). The change in their expression levels is also more pronounced at the higher dose (6 SED) and earlier time (6 h). All plots show rather symmetric patterns. However, at 6 SED, the number of upregulated genes is slightly increased and their Log2FC slightly stronger.

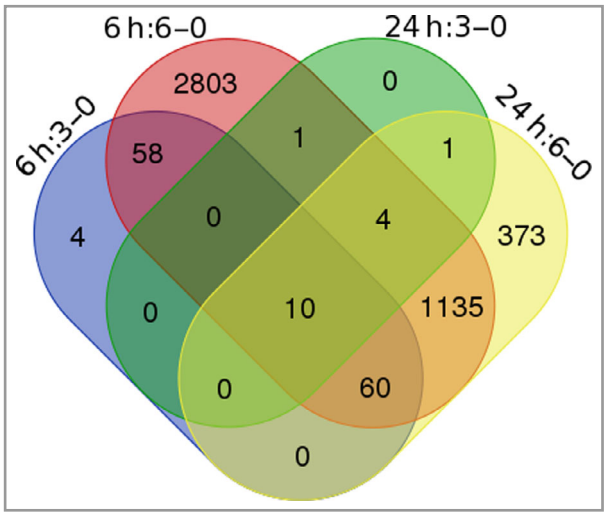

Fig 2. Venn diagram of genes detected at 5% false discovery rate in skin after fluorescent solar-simulated radiation exposure (different doses and time points). Overlap of differently expressed genes under different models: different time (6 h and 24 h) and different dose [3 standard erythemal doses (SED) and 6 SED].

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 mi RNAs, except for hsa-miR-142-5p, showed significant correlations

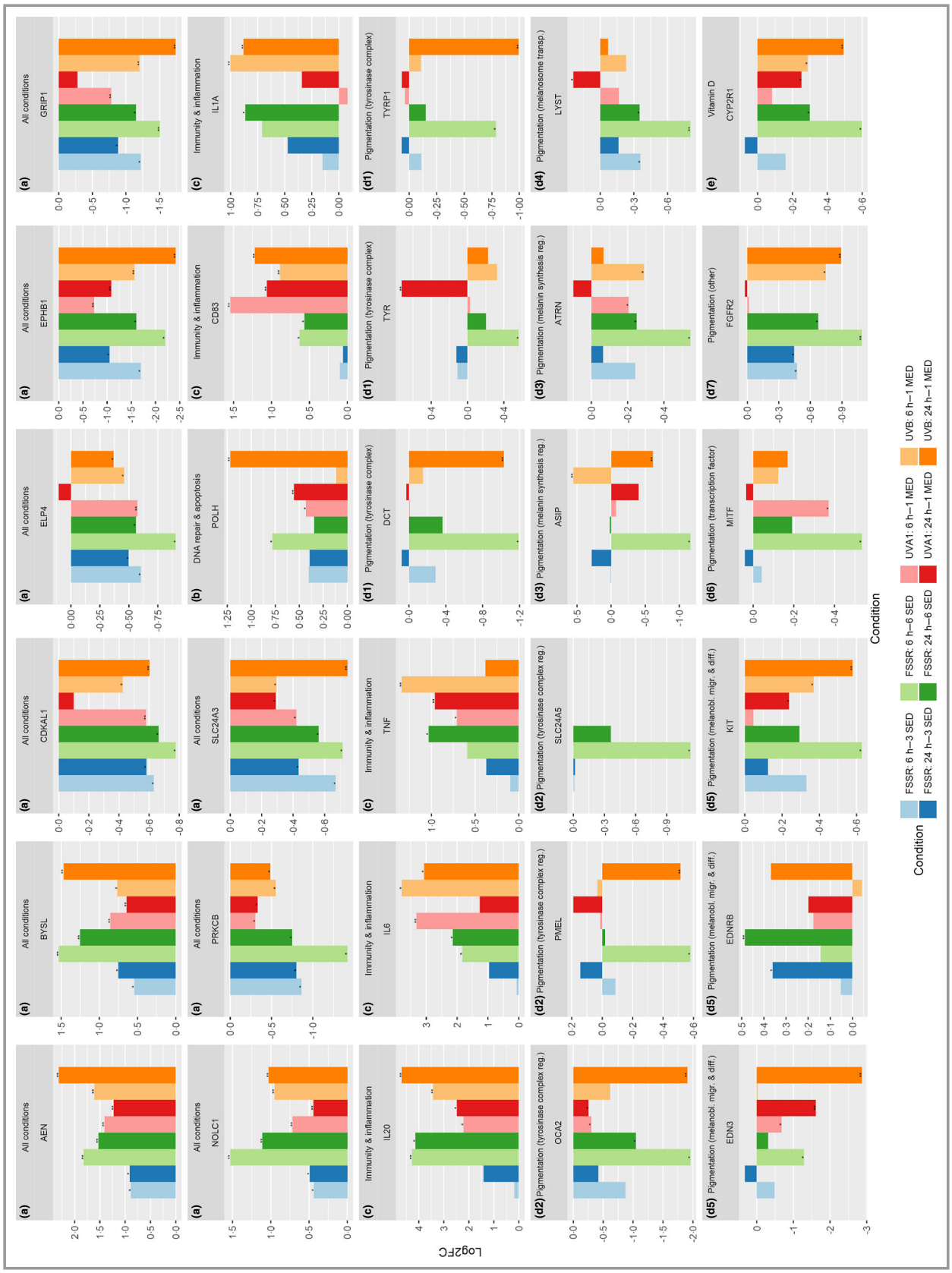
Log2FC
Condition
FSSR: 6 h−3 SED
FSSR: 24 h−3 SED
FSSR: 6 h−6 SED
FSSR: 24 h−6 SED
UVA1: 6 h−1 MED
UVA1: 24 h−1 MED
UVB: 6 h−1 MED
UVB: 24 h−1 MED
(a) All conditions AEN
(a) All conditions BYSL
(a) All conditions CDKAL1
(a) All conditions ELP4
(a) All conditions EPHB1
(a) All conditions GRIP1
(a) All conditions NOLC1
(a) All conditions PRKCB
(a) All conditions SLC24A3
(b) DNA repair & apoptosis POLH
(c) Immunity & inflammation CD83
(c) Immunity & inflammation IL1A
(c) Immunity & inflammation IL20
(c) Immunity & inflammation IL6
(c) Immunity & inflammation TNF
(d1) Pigmentation (tyrosinase complex) DCT
(d1) Pigmentation (tyrosinase complex) TYR
(d1) Pigmentation (tyrosinase complex) TYRP1
(d2) Pigmentation (tyrosinase complex reg.) OCA2
(d2) Pigmentation (tyrosinase complex reg.) PMEL
(d2) Pigmentation (tyrosinase complex reg.) SLC24A5
(d3) Pigmentation (melanin synthesis reg.) ASIP
(d3) Pigmentation (melanin synthesis reg.) ATRN
(d4) Pigmentation (melanosome transp.) LYST
(d5) Pigmentation (melanobl. migr. & diff.) EDN3
(d5) Pigmentation (melanobl. migr. & diff.) EDNRB
(d5) Pigmentation (melanobl. migr. & diff.) KIT
(d6) Pigmentation (transcription factor) MITF
(d7) Pigmentation (other) FGFR2
(e) Vitamin D CYP2R1

Fig 3. Comparison of the effect size of candidate genes after exposure to fluorescent solar-simulated radiation (FSSR), ultraviolet (UV)A1 and UVB. (a) Genes detected under all conditions of FSSR exposure: *AEN*, *BYSL*, *CDKAL1*, *ELP4*, *EPHB1*, *GRIP1*, *NOLC1*, *PRKCB*, *SLC24A3*. (b) DNA repair and apoptosis genes: *POLH*. (c) Immunity and inflammation genes: *CD83*, *IL1A*, *IL20*, *IL6*, *TNF*. (d) Pigmentation genes. (d1) Tyrosinase complex: *DCT*, *TYR*, *TYRP1*; (d2) tyrosinase complex regulation (reg.): *OCA2*, *PMEL*; *SCL24A5*; (d3) melanin synthesis regulation: *ASIP*, *ATRN*; (d4) melanosome transport (trans.): *LYST*; (d5) melanoblast (melanobl.) migration (migr.) and differentiation (diff.): *EDN3*, *EDNRB*, *KIT*; (d6) transcription factor: *MITF*; (d7) other: *FGFR2*. (e) vitamin D genes: *CYP2R1*. *y*-axis represents $\log_2$ fold change (Log2FC), scale adapted to each gene; *x*-axis represents different exposure conditions: different wavelength [FSSR, UVA1 (340–400 nm) or UVB (300 nm)], dose [3 standard erythemal doses (SED), 6 SED or 1 minimal erythemal dose (MED) (~2 SED, ranging from 1·6 to 2·6 SED)], time after exposure (6 h or 24 h). For FSSR, Log2FC ($^{-}\Delta\Delta$Ct) obtained in the quantitative polymerase chain reaction experiment are shown. *P < 0·05 compared with unexposed samples (0 SED); **P < 2·8E-04 compared with unexposed samples (0 SED).

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

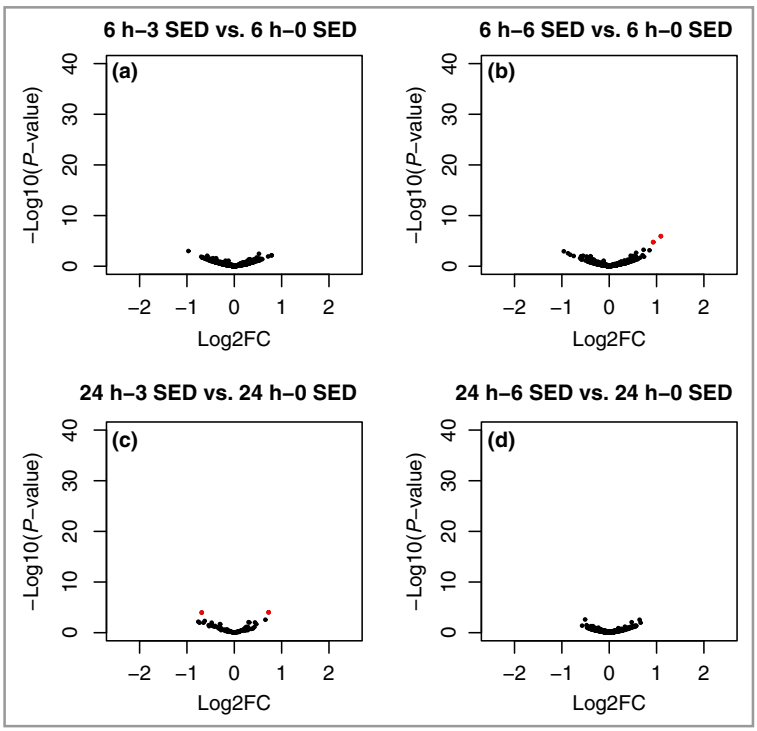

Fig 4. Volcano plots of microRNA (miRNA) expression in skin after fluorescent solar-simulated radiation (FSSR) exposure (different doses and time points). Plots show effect size $\log_2$ fold change (Log2FC) vs. –log10(P-value). (a) and (b) show dose effects at time 6 h; and (c) and (d) dose effects at 24 h. Only four miRNAs survived multiple testing correction (shown in red). All plots show symmetric patterns. The size of the effects of FSSR on miRNA expression was smaller than on gene expression.

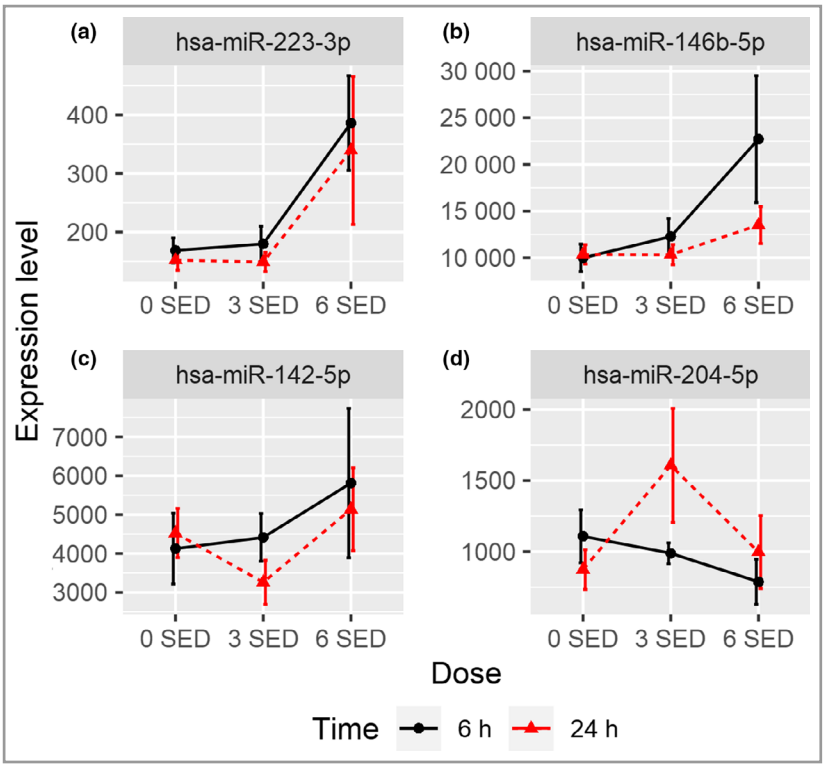

Fig 5. microRNA (miRNA) expression in skin after fluorescent solar-simulated radiation (FSSR) exposure (different doses and time points). Normalized miRNA expression levels and mean standard error (*y*-axis) by FSRR dose (*x*-axis) and time (6 h in black and 24 h in red). (a) hsa-miR-223-3p [6 h–0 standard erythemal doses (SED) vs. 6 h–6 SED: P = 1·17E-06] and (b) hsa-miR-146b-5p (6 h–0 SED vs. 6 h–6 SED: P = 1·71E-05); (c) hsa-miR-142-5p (24 h 0 SED vs. 24 h 3 SED: P = 1·08E-04) and (d) hsa-miR-204-5p (24 h 0 SED vs. 24 h 3 SED: P = 9·52E-05).

observed an enrichment of USF transcription factors, which promote keratinocyte growth;[33] whereas at 24 h, transcription factors related to immune and inflammatory responses were found (CEBPB, RUNX1, STAT3 and RELA[35]). Some of the transcription factors detected in the enrichment analysis had been previously associated with UVR: STAT3,[40] KLF4[31,41] and E2F.[42]

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 5·3% UVB, which accounts for 79·6% 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,[43] 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 SED terms (3–6 SED for FSSR and 1·6–2·6 SED 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 calcaemic activity.[44–46] 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.[47] In general, these effects were observed with FSSR, UVA1 and/or UVB. The exceptions were *LYST*, that participates in melanosome transport, and *TYR*, 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*, *TYRP1* 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] *GRIP1* (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 miRNA 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 miRNA 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 *SLC6A4*, a serotonin transporter, as a potential target of hsa-miR-146b-5p. Activation of the serotonin pathway has been suggested to mediate UVB-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 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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Supporting 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–3 standard erythemal dose (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 [6h–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 [24h–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].

Table S14 Transcription-factor 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 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.