Published in final edited form as:

Pigment Cell Melanoma Res. 2011 October ; 24(5): 972–974. doi:10.1111/j.1755-148X.2011.00899.x.

Transcriptional signatures of full-spectrum and non-UVB-spectrum solar irradiation in human skin

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Dear Editor,

Despite widespread use of sunscreens that minimize erythema by blocking ultraviolet B (UVB) radiation, melanoma incidence continues to rise. In considering this disparity, we investigated effects of full-spectrum solar-simulated radiation (ssR) and its non-UVB spectrum [ssA: solar-simulated ultraviolet A (UVA)/visible/infrared] on the human skin transcriptome and analyzed gene expression profiles in irradiated sites and adjacent control sites using gene expression microarray and gene set analysis. This study demonstrated that ssR responses were not completely blocked by sunscreen. We also identified ssA-responsive gene sets and showed their utility in detecting ssA responses in situ. Our approach emphasizes the utility of gene sets, rather than individual genes, to sensitively measure the effects of solar irradiation on human skin.

We irradiated focal areas of sun-protected human skin in vivo with full-spectrum ssR at 1 minimum erythema dose (MED; 150–300 J/m²), or with suberythemogenic doses of 100 J/m² or 0.1 MED with or without pretreatment with sunscreen (SPF 15) that absorbed UVB and some UVA (Figure S1). Additional skin areas were irradiated with ssA (the non-UVB spectrum of ssR) that was present in each ssR dose (Figure S2 in Appendix S1). Twenty-four hours later, we biopsied irradiated skin and adjacent control areas for gene expression microarray analysis (Tables S1 and S2; Figures S3 and S4 in Appendix S1). We then identified differentially expressed genes ('treated skin' compared with adjacent 'no-irradiation control skin'; Figure S5 in Appendix S1).

Gene set analysis (Subramanian et al., 2005) demonstrated that 68 of 3272 curated gene sets in the Molecular Signatures Database (Broad Institute) were altered by 1 MED ssR (Table S3). Many of these were associated with the DNA damage response, proteasome pathway, and MYC signaling. Importantly, 36 of the 68 ssR-responsive gene sets were altered also by

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Conflicts of interest

Jonathan C. Vogel and Atsushi Terunuma are named as inventors on a patent application that is related to this study.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary materials and methods, Tables S1–S6 and Figures S1–S5.

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sunscreen + ssR (shaded in Table S3). These included the DNA damage response, and proteasome pathways as well as WNT signaling, antigen presentation and HIV infection gene sets. Gene set analysis indicates that sunscreen application blocked the biological effects of ssR less effectively than suggested by the numbers of differentially expressed genes in single-gene analysis (Figure S5 in Appendix S1). This implies that, even when erythema is absent in sunscreen-protected skin, a modest dose of solar radiation could damage DNA and proteins in skin cells. Irradiation with ssA altered 56 gene sets, eight of which were altered also by sunscreen + ssR, and only one was altered by ssR as well (Table S4). The 36 ssR-responsive gene sets that were not blocked by sunscreen application (shaded in Table S3) and the ssA-responsive gene sets (Table S4) may be particularly useful in designing new sunscreen formulations for better protection.

To develop a specific and sensitive method to detect ssA effects, we created ssA custom gene sets from our microarray data. Sets of genes that were up- or down-regulated by ssA [false discovery rate (FDR) <25%] were termed 'UP_by_A' (49 genes) and 'DOWN_by_A' (23 genes), respectively (Table S5). Two genes involved in pigment biogenesis, tyrosinase (TYR) and P protein (OCA2), were in the UP_by_A gene set while another important pigment-related gene, mast/stem cell growth factor receptor (KIT), was in the DOWN_by_A set. These ssA gene sets were used to assess transcriptional signatures of ssA irradiation in microarray data sets of various skin conditions. The 'ssA signatures' were determined by statistical significance with positive scores for UP_by_A, negative scores for DOWN_by_A, or both. When microarray data of skin diseases including psoriasis, atopic dermatitis, squamous cell carcinoma, actinic keratosis, nevi, and malignant melanoma were analyzed, none of them displayed ssA signatures (large FDR values in Table S6). This observation indicates that the ssA gene sets do not detect nonspecific transcriptional changes that occur in perturbed skin. We then investigated ssA signatures in ssR-irradiated skin that had been pretreated with sunscreen. When analyzing the sunscreen + ssR microarray data, both UP_by_A and DOWN_by_A gene sets detected ssA signatures unambiguously at all doses of ssR: 1 MED, 100 J/m², and 0.1 MED (Table 1), underscoring the sensitivity of the ssA gene sets in detecting ssA signatures. This set of experiments provides a proof of concept for the utility of the ssA custom gene sets and gene set analysis in monitoring biological effects of non-UVB solar radiation, which is not blocked by sunscreen.

Choi et al. (2010) repeatedly irradiated human lower back skin with suberythemogenic doses of ssR and observed increased expression of TYR as well as paracrine receptors for melanocytic growth factors including KIT, endothelin B receptor (EDNRB), and melanocyte-stimulating hormone receptor (MC1R). In our study, 24 hours after a single dose of 1 MED ssR, we also observed increased expression of TYR (1.60X, FDR = 0%) and EDNRB (1.50X, FDR = 0%), while KIT expression decreased (0.53X, FDR = 0%). MC1R expression increased only marginally (1.39X, FDR = 26%). This observation suggests that a modest dose of solar radiation can affect melanocytes, directly or indirectly, even when skin is pretreated with a standard SPF-15 sunscreen. We also applied our custom ssA gene sets and gene set analysis to Choi's data of repeated irradiation. Interestingly, the UP_by_A gene set, which includes two pigment biogenesis genes (TYR and OCA2) detected the ssA signature in skin irradiated with repeated doses of ssR or ssA, but not UVB, indicating that the UP_by_A signature is common for acute and repeated ssR irradiation (Table 1). In contrast, the DOWN_by_A signature was ambiguous in skin with repeated irradiation. This is consistent with differential responses of certain genes to repeated versus acute ssR irradiation; e.g. KIT expression was up-regulated by repeated doses of ssA and down-regulated by a single dose.

Gene set analysis offers a new in vivo strategy to understand biological effects of UVB and ssA. Additionally, the use of the ssA custom gene sets in gene set analysis can provide a

sensitive and quantitative approach to measure ssA effects on human skin in vivo and may circumvent shortcomings of conventional UVA protection assays that rely on persistent pigment darkening or on in vitro measurements of spectral absorption (Gasparro, 2000; Lowe, 2006; Rosenstein et al., 1999). This strategy could facilitate the development of more effective means of skin protection from full-spectrum solar radiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Authors thank Drs Stephen I. Katz, Vincent J. Hearing, Thomas J. Hornyak, Edward W. Cowen and Heidi H. Kong for helpful discussions and comments on the manuscript. Authors also thank Drs Paul Meltzer and Sean Davis for valuable advice. This manuscript was finalized without the involvement of one of the major contributors, Dr Jonathan C. Vogel, now deceased. Dr Vogel was integrally involved in the design and implementation of this study, in analysis of the data that are reported, and also in development of initial drafts of the manuscript. Products and manufacturers mentioned in this manuscript do not imply FDA endorsement. The manuscript was reviewed by the CCR Fellows Editorial Board. This study was supported by the Intramural Research Program of the NIH, NCI, CCR.

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Table 1

Gene set analysis with ssA custom gene sets

Skin conditions	UP_by_A		Down_by_A	
	Score ^a	FDR ^b (%)	Score ^a	FDR ^b (%)
Sunscreen + ssR, 1 MED	2.67	0	-2.15	0
Sunscreen + ssR, 100 J/m ²	1.86	0	-1.22	0
Sunscreen + ssR, 0.1 MED	1.04	0	-1.41	0
Repetitive ssR, 0.4 MED X5 + 0.5 MED X5 in 2 weeks ^c	0.77	0	0.02	55
Repetitive ssA, 2.3 times the ssA dose in the repetitive ssR irradiation ^c	0.56	0	0.00	100
Repetitive UVB, 1.1 times the UVB dose in the repetitive ssR irradiation ^c	0.02	60	0.24	85

Gene sets with significant changes are shaded.

^aScore indicates the level of consistency of all genes in each gene set for up- or down-regulation. A positive score indicates consistent up-regulation; a negative score indicates consistent down-regulation.

^bFDR: false discovery rate.

^cThese data were obtained from Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>).