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Data in Brief

Expression profiling of human melanocytes in response to UV-B irradiation

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ABSTRACT

A comprehensive gene expression analysis of human melanocytes was performed assessing the transcriptional profile of dark melanocytes (DM) and light melanocytes (LM) at basal conditions and after UV-B irradiation at different time points (6, 12 and 24 h), and in culture with different keratinocyte-conditioned media (KCM+ and KCM−). The data, previously published in [1], have been deposited in NCBI's Gene Expression Omnibus (GEO accession number: GSE70280).

Specifications	
Organism/cell line/tissue	*Homo sapiens*/epidermal melanocytes/skin
Sex	Male
Sequencer or array type	Agilent-028004 SurePrint G3 Human GE 8 × 60K Microarrays
Data format	Raw data: TXT; normalized data: SOFT, MINiML and TXT
Experimental factors	Skin pigmentation (light vs dark pigmented), time after UV-B irradiation (0, 6, 12 and 24 h) and keratinocyte-conditioned media (KCM+ vs KCM−)
Experimental features	Gene expression analysis of dark melanocytes (DM) and light melanocytes (LM) at basal conditions and after UV-B at different time points (6, 12 and 24 h), and in culture with different keratinocyte-conditioned media (KCM+ and KCM−)
Consent	Data is publicly available and open for re-use given appropriate citation
Sample source location	Cells were purchased from Cascade Biologics (Life Technologies) and Innoprot (see Table 1)

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70280.

2. Experimental design, materials and methods

2.1. Culture of human epidermal keratinocytes

Human epidermal keratinocytes (HEK) were purchased from Cascade Biologics. HEKs were maintained in EpiLife Medium supplemented with human keratinocyte growth supplement (HKGS) consisting of 0.2 ng ml^{-1} human recombinant epidermal growth factor (EGF), 0.18 μg ml^{-1} hydrocortisone, 5 μg ml^{-1} insulin, 5 μg ml^{-1} transferrin, and 0.2% (*v*/*v*) bovine pituitary extract. Cell cultures were maintained in an incubator under an atmosphere of 5% CO_2 at 37 °C. Media were refreshed every two days and cells from the third to fifth passage were used. Subconfluent cultures were then irradiated at 75 mJ/cm^2 UV-B in an ICH2 photoreactor (LuzChem, Canada) at 37 °C. Prior to irradiation, the culture medium was replaced with phosphate buffer saline (PBS) and immediately after irradiation PBS was replaced again with culture medium. 24 h after irradiation supernatant (KCM+), was collected. The non-irradiated keratinocytes' supernatant was also harvested as a control (KCM-) (Fig. 1).

2.2. Culture of human skin melanocytes

Human epidermal melanocytes (HEM) were purchased from Cascade Biologics and Innoprot (Table 1): six lines isolated from lightly pigmented neonatal foreskin (LM) and six lines from darkly pigmented neonatal foreskin (DM). LM and DM were cultivated in Medium 254 supplemented with 1% human melanocyte growth supplement (HMGS), consisting of 10 ng ml^{-1} phorbol 12-myristate 13-acetate (PMA), 3 ng ml^{-1} human recombinant basic fibroblast growth factor, 3 μg ml^{-1} heparin, 500 nM hydrocortisone, 5 μg ml^{-1} insulin, 5 μg ml^{-1} transferrin, 0.2% (*v*/*v*) bovine pituitary extract, and 0.5% (*v*/*v*) fetal bovine serum. Cell lines were maintained under an atmosphere of 5% CO_2 at 37 °C. Media were refreshed every two days and cells from the third to fifth passage were used. Next, subconfluent melanocyte cultures were transferred to KCM conditioned medium (Medium 254 supplemented with HMGS and KCM+ or KCM− medium in the proportion 1:1). 24 h later, the culture medium was replaced with

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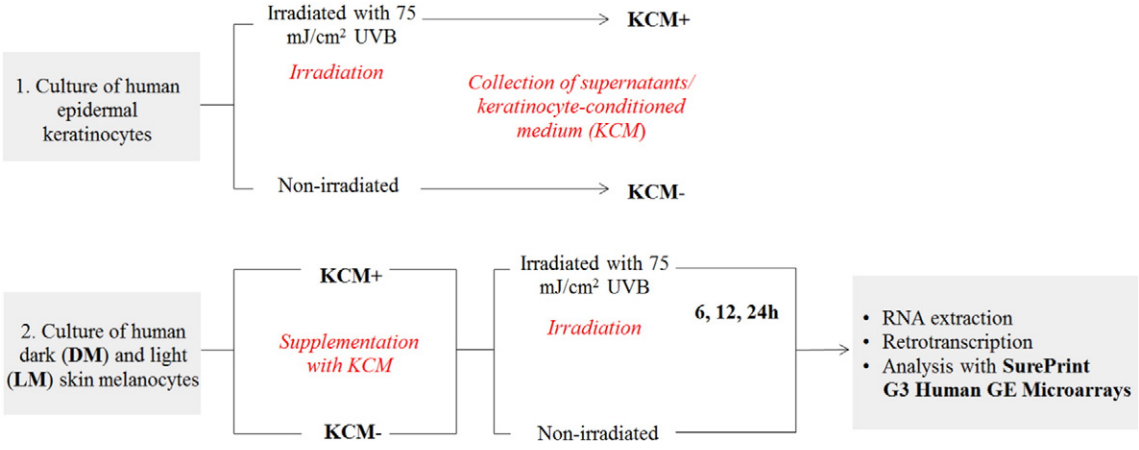

Fig. 1. Experimental design.

PBS, melanocytes were irradiated with 75 mJ/cm^2 of UV-B, and PBS was replaced again with conditioned medium. Cells were harvested at different time points (6, 12 and 24 h) after irradiation to assess both the early and the late response to this factor. In all cases non-irradiated melanocytes were used as controls. These were subject to the same procedure as irradiated melanocytes, but covered with aluminum foil during irradiation (Fig. 1).

2.3. Gene expression microarrays

Total RNA from melanocytes was isolated using the RNAqueous Kit (Ambion, Thermo Fisher). Quantification and integrity check were performed using a UV/VIS NanoDrop 8000 (Thermo Fisher) and the Agilent 2100 Bioanalyzer (eukaryote total RNA nano assay), respectively. In all the samples the RNA integrity number (RIN) was between 9.9 and 10 and the 28S/18S ribosomal RNA ratio was in the range of 1.9–2.5.

Samples were analyzed using SurePrint G3 Human GE Microarrays (Agilent), which contain features for 27,958 annotated genes and 7419 long intergenic non-coding RNAs (lincRNAs). The protocol for the labeling and the hybridization of the samples are described in [1]. Image processing of the microarrays was performed by using Agilent Feature Extraction software v10.7.3.1, which also performs different evaluation parameters (QC-Metrics) to check the quality of the microarrays. This revealed 2 outlier arrays that did not satisfy the quality parameters: L_5.6K- (LM; replicate 5; 6 h; medium KCM−) and L_4.24K- (LM; replicate 4; 24 h; medium KCM−) and were therefore removed from subsequent analyses.

The variance of the raw data was stabilized by performing a DDHF (Data-Driven Haar-Fisz) transformation of the data with the R package DDHFm [2]. Data were transformed to log base 2 and normalized following the quantile method. Those probes in which more than 50% of the samples in any of the following 7 conditions: 0 h, 6 h KCM−, 12 h KCM−, 24 h KCM, 6 h KCM+, 12 h KCM+ and 24 h KCM+, had non-detected or compromised flags were removed for the analysis, thus leaving a total of 26,493 probes for further analyses. Additionally, we performed a principal component analysis (PCA) of the transformed and normalized probe signals with the software Unscrambler X v10.3 (CAMO A/S, Trondheim, Norway). As shown in [1], no additional outliers were identified in the dataset.

Table 1
Melanocyte cell lines used in the study.

Sample_ID	Source of melanocytes	Provider	Lot number
DM_1	Dark pigmented donor	Cascade Biologics	716538
DM_2	Dark pigmented donor	Cascade Biologics	792326
DM_3	Dark pigmented donor	Cascade Biologics	765194
DM_4	Dark pigmented donor	Cascade Biologics	6C = 474
DM_5	Dark pigmented donor	Cascade Biologics	814729
DM_6	Dark pigmented donor	Cascade Biologics	200707-980
LM_1	Light pigmented donor	Cascade Biologics	200706-893
LM_2	Light pigmented donor	Cascade Biologics	200708-556
LM_3	Light pigmented donor	Cascade Biologics	423339
LM_4	Light pigmented donor	Innoprot	3207
LM_5	Light pigmented donor	Innoprot	7253
LM_6	Light pigmented donor	Innoprot	2842

2.4. Statistical analyses

The expression profiles of melanocytes were compared with SAM (Significance Analysis of Microarrays) [3] using two class non-pairwise comparisons and 500 permutations in each test. We performed the following comparisons: dark vs light melanocytes at different time points after irradiation, irradiated vs non-irradiated melanocytes, and melanocytes growing with KCM+ or KCM−. Significance was assessed by adjusted False Discovery Rate ($p < 0.05$) [4].

Pathway enrichment analysis was performed using Web-based Gene Set Analysis Toolkit (WebGestalt) (http://bioinfo.vanderbilt.edu/-webgestalt/option.php), using as the reference list all the probes analyzed in the microarray. The significance analysis was performed using the Hypergeometric test. The minimum number of genes for enrichment was set at 5, and the significance level at Bonferroni adjusted — $p < 0.01$.

Acknowledgments

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