Genomics Data 2 (2014) 335–339

Contents lists available at ScienceDirect

Genomics Data

journal homepage: http://www.journals.elsevier.com/genomics-data/

Data in Brief

Gene expression in response to cyclic mechanical stretch in primary human dermal fibroblasts

Maria Reichenbach, Kerstin Reimann, Hendrik Reuter *

Research Skin Care, Beiersdorf AG, Unnastraße 48, 20245 Hamburg, Germany

A R T I C L E   I N F O

Article history:
Received 26 August 2014
Received in revised form 24 September 2014
Accepted 26 September 2014
Available online 16 October 2014

Keywords:
Gene expression profiling
Cyclic mechanical stretch
Mechanotransduction
Dermal skin

A B S T R A C T

The human dermal skin is permanently exposed to mechanical stress, for instance during facial expression, which might cause wrinkles with age. Cyclic mechanical stretching of cells results in cellular and cytoskeleton alignment perpendicular to the stretch direction regulating cellular response. With gene expression profiling it was aimed to identify the differentially expressed genes associated with the regulation of the cytoskeleton to investigate the stretch-induced cell alignment mechanism. Here, the transcription activity of the genome in response to cyclic mechanical stress was measured using DNA microarray technology with Agilent SurePrint G3 Human GE 8x60k Microarrays, based on the overall measurement of the mRNA. Gene expression was measured at the beginning of the alignment process showing first reoriented cells after 5 h stretching and at the end after 24 h, where nearly all cells are aligned. Gene expression data of control vs. stretched primary human dermal fibroblasts after 5 h and 24 h demonstrated the regulation of differentially expressed genes associated with metabolism, differentiation and morphology and were deposited at http://www.ncbi.nlm.nih.gov/geo with the accession number GSE58389.

© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

Specifications	
Organism/cell line/tissue	Primary human dermal fibroblasts isolated from skin biopsies
Sequencer or array type	Agilent SurePrint G3 Human GE 8x60k Microarray (G4858A-028004)
Data format	Normalized signal intensity
Experimental factors	Static vs. stretched cells
Experimental features	Comparison of gene expression profiles of cyclic mechanical stretched primary human dermal fibroblasts with untreated control cells isolated from ten donors after 5 h and 24 h stretching
Consent	Donors provided written consent

Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58389.

Experimental design, materials and methods

Experimental design

Cell alignment is one of the main cellular responses to cyclic mechanical uniaxial stretch [1] and might be associated with mechanically induced wrinkle formation in the skin [2]. The identification of the mechanism leading to alignment may allow analysis and modulation of its role in the formation of mechanically induced wrinkles. To identify changes in gene expression associated with mechanical stretch-induced cell alignment, a whole genome microarray study was performed on primary human dermal fibroblasts (PHDF) subjected to cyclic uniaxial stretching using a Flexer®Cell Tension Plus System. Gene expression was measured at the beginning of the alignment process showing first reoriented cells after 5 h stretching and at the end after 24 h, when nearly all cells are aligned perpendicular to the stretch-direction (Fig. 1). In total, PHDF from ten donors were cultured on BioFlex culture plates and stretched for 5 h and 24 h or left untreated as controls to account for changes occurring during cell culture. This resulted in 4 samples for each of the subjects (control/treated and 5 h/24 h), i.e. 40 samples in total (Table 1).

Materials and methods

Cell culture

Dermal fibroblasts were isolated from skin biopsies obtained from plastic surgery. The biopsies were cut in stripes and incubated in dispase

* Corresponding author at: Laboratory of General Skin Care, Applied Skin Biology, Research and Development, Beiersdorf AG, Unnastraße 48, 20253 Hamburg, Germany. Tel.: +49 4049096250; fax: +49 404909186250.
E-mail address: Hendrik.Reuter@Beiersdorf.com (H. Reuter).

http://dx.doi.org/10.1016/j.gdata.2014.09.010
2213-5960/© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

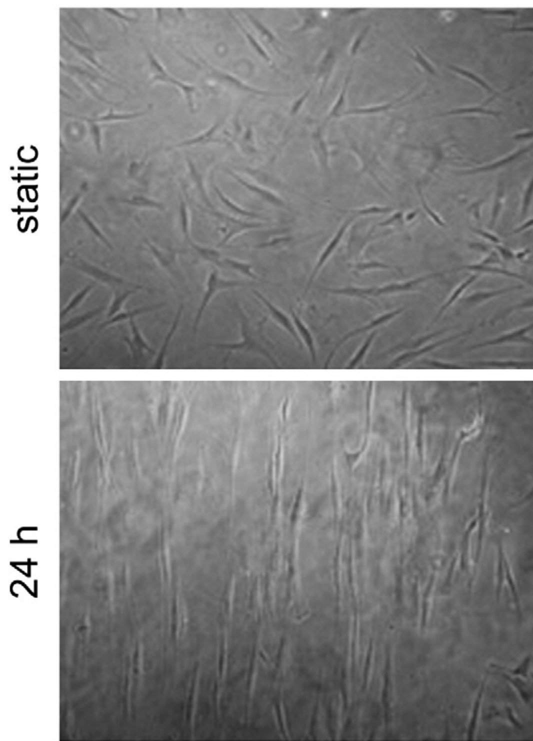

Fig. 1. PHDF were cultured on BioFlex culture plates for 24 h and then stretched with the FX-4000T™ Tension Plus™ System. Cell morphology of PHDF in response to 24 h cyclic mechanical stretching was observed with transmitted light microscopy. Micrographs of static cells show randomized orientated PHDF. In contrary, stretched cells were oriented perpendicular to stretch direction.

II for 3 h at 37 °C. Afterwards the dermis was separated from the epidermis, cut into smaller pieces and digested in collagenase I o/n at 37 °C. The suspension was filtered and the primary human dermal fibroblasts in the filtrate were seeded in cell culture flasks. For long term storage the PHDF were cultured until passage one in cell culture flasks, harvested by centrifugation (5 min, 1000 g) after Trypsin/EDTA digestion for 5 min at 37 °C and resolved in freezing medium. PHDF were thawed with prewarmed DMEM containing 10% FCS, 2 mM GlutaMax™-I and 0.1 mg/ml penicillin/streptomycin, plated in cell culture flasks and incubated at 37 °C in an atmosphere of 5% CO_2 and 90% humidity until confluence. Then cells were subcultured in BioFlex culture plates.

Cyclic mechanical stretching in vitro

Mechanical stretching was performed on flexible silicon membranes using FX-4000T™ Tension Plus™ System. PHDF were seeded on BioFlex culture plates coated with Collagen I and cultured until subconfluence of 70% and cells were serum deprived o/n. For stretching experiments culture plates were mounted on the Baseplate™. Cyclic stretch was applied in the FX-4000T™ Tension Plus™ System with 16% elongation, 0.5 Hz in a half sinus regimen. By application of a vacuum a depression occurs and the silicon membranes with adhering cells were stretched over the loading posts. Cell alignment was microscopically observed at the outer circular region of the well. At 5 h and 24 h cells were harvested for mRNA isolation.

RNA isolation from primary human dermal fibroblasts

PHDF from 10 donors were cultured on BioFlex culture plates for 24 h and stretched for 5 h and 24 h or left untreated. To separate the inhomogeneous stretching areas of the BioFlex culture plates the silicon membranes were punched with a 2 cm diameter punch. Isolation of RNA was done with RNeasy Mini Kit according to manufacturer product information from the outer circular region of the well. DNA and RNA were precipitated with 70% ethanol and bound to a silica membrane. DNA was digested using DNAse I. RNA was eluted with 30 μl RNAse free water and subjected to Experion automated electrophoresis for quality control.

Microarray hybridization and data processing

The obtained RNA was transcribed into cDNA and than subjected to microarray hybridization. 100 ng of each total RNA sample was used for the linear T7-based amplification step. To produce Cy3-labeled cRNA, the RNA samples were amplified and labeled using the Agilent Low Input Quick Amp Labeling Kit (Agilent Technologies) following the manufacturer's protocol. Yields of cRNA and the dye-incorporation rate were measured with ND-1000 Spectrophotometer (NanoDrop Technologies). The hybridization procedure was performed according to the Agilent 60-mer oligo microarray processing protocol using the Agilent Gene Expression Hybridization Kit (Agilent Technologies) (Design ID 028004). Subsequently, 600 ng Cy3-labeled fragmented cRNA in hybridization buffer was hybridized 17 h at 65 °C to Agilent SurePrint G3 Human GE 8x60k Microarrays using Agilent's recommended hybridization chamber and oven. Finally, the microarrays were washed once with the Agilent Gene Expression wash buffer 1 for 1 min at room temperature followed by a second wash with preheated Agilent Gene Expression wash buffer 2 (37 °C) for 1 min. The last washing step was performed with acetonitrile. Fluorescence signals of the hybridized Agilent microarrays were detected using Agilent's Microarray Scanner System (G2505C, Agilent Technologies, Palo Alto, USA). The Agilent Feature Extraction Software (FES 10.7.3.1) was used to obtain and process the microarray image files. The preprocessing started with the conversion of the data after using Agilent Feature Extraction software in txt files suitable for all subsequent analysis steps, which were mainly performed with the R statistical software and its Bioconductor packages. For the preprocessing the agi4x44kpreprocess package was used [3]. To this end an annotation package for the Agilent Whole Genome 8x60k chip has previously been created. The annotation package for the 8x60k Agilent chip was created using Bioconductor

Table 1
Experimental design of microarray-based gene expression profiling. PHDF from ten subjects were cultured on BioFlex culture plates and stretched for 5 h and 24 h or left untreated. RNA was isolated and used for gene expression profiling. Each subject provided the 4 samples with the control/treatment combinations (control; stretched).

	Subject ID	Primary human dermal fibroblasts isolated from 10 subjects			
		Control		Stretched	
		Sample name	Array ID	Sample name	Array ID
5 h	392	Reu_1	co_5h_392	Reu_21	tr_5h_392
5 h	420	Reu_3	co_5h_420	Reu_23	tr_5h_420
5 h	464	Reu_5	co_5h_464	Reu_25	tr_5h_464
5 h	425	Reu_7	co_5h_425	Reu_27	tr_5h_425
5 h	432	Reu_9	co_5h_432	Reu_29	tr_5h_432
5 h	416	Reu_11	co_5h_416	Reu_31	tr_5h_416
5 h	465	Reu_13	co_5h_465	Reu_33	tr_5h_465
5 h	446	Reu_15	co_5h_446	Reu_35	tr_5h_446
5 h	387	Reu_17	co_5h_387	Reu_37	tr_5h_387
5 h	445	Reu_19	co_5h_445	Reu_39	tr_5h_445
24 h	392	Reu_2	co_24h_392	Reu_22	tr_24h_392
24 h	420	Reu_4	co_24h_420	Reu_24	tr_24h_420
24 h	464	Reu_6	co_24h_464	Reu_26	tr_24h_464
24 h	425	Reu_8	co_24h_425	Reu_28	tr_24h_425
24 h	432	Reu_10	co_24h_432	Reu_30	tr_24h_432
24 h	416	Reu_12	co_24h_416	Reu_32	tr_24h_416
24 h	465	Reu_14	co_24h_465	Reu_34	tr_24h_465
24 h	446	Reu_16	co_24h_446	Reu_36	tr_24h_446
24 h	387	Reu_18	co_24h_387	Reu_38	tr_24h_387
24 h	445	Reu_20	co_24h_445	Reu_40	tr_24h_445

Download English Version:

https://daneshyari.com/en/article/2822293

Download Persian Version:

https://daneshyari.com/article/2822293

Daneshyari.com