



Data in Brief

Transcriptional profiling of dendritic cells matured in different osmolarities

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ABSTRACT

Tissue-specific microenvironments shape the fate of mononuclear phagocytes [1–3]. Interstitial osmolarity is a tissue biophysical parameter which considerably modulates the phenotype and function of dendritic cells [4]. In the present report we provide a detailed description of our experimental workflow and bioinformatic analysis applied to our gene expression dataset (GSE72174), aiming to investigate the influence of different osmolarity conditions on the gene expression signature of bone marrow-derived dendritic cells. We established a cell culture system involving murine bone marrow cells, cultured under different NaCl-induced osmolarity conditions in the presence of the dendritic cell growth factor GM-CSF. Gene expression analysis was applied to mature dendritic cells (day 7) developed in different osmolarities, with and without prior stimulation with the TLR2/4 ligand LPS.

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Specifications	
Organism/cell line/tissue	Bone marrow-derived dendritic cells from C57BL/6 mice
Sex	Male
Sequencer or array type	GeneChip Mouse Gene 1.0 ST Arrays (Affymetrix)
Data format	Raw files (CEL)
Experimental factors	Bone marrow-derived dendritic cells were developed in RPMI medium (Sigma-Aldrich) GM-CSF-enriched (10 ng/mL-R&D systems), supplemented with endotoxin-free, cell culture tested NaCl 4 M (Sigma-Aldrich) to reach final osmolarity of 370 mOsm/L and 450 mOsm/L. Part of the cells was developed in normosmolar medium (290 mOsm/L). Cells matured in different osmolarity were subjected to gene expression profiling, with and without previous TLR 2/4 stimulation with LPS (10 ng/mL- Sigma Aldrich).
Experimental features	Gene expression profiling of unstimulated and LPS-treated bone marrow-derived dendritic cells matured under different salt osmolarity conditions (290/370/450 mOsm/L).
Consent	–
Sample source location	Heidelberg, Germany

1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72174>.

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2. Experimental design, materials and methods

2.1. Generation of bone marrow-derived dendritic cells under different osmolarity conditions

It has been described that local tissue microenvironment modulates the function of macrophages and dendritic cells [1–4]. Dendritic cells (DCs) were developed from the bone marrow of C57BL/6 mice in RPMI medium, enriched with 10 ng/mL of recombinant murine GM-CSF (R&D systems), as previously described [5,6]. On day 3, NaCl solution was added to the medium to reach the final osmolarity of 370 mOsm/L and 450 mOsm/L, while part of the cells was kept in normosmolar medium (290 mOsm/L). The cells were split on day 4 and maintained in different osmolarity until day 7.

2.2. RNA isolation and gene expression profiling

On day 7, mature DCs from the 3 different osmolarity conditions were stimulated for 8 h with LPS (10 ng/mL). Subsequently, total RNA from unstimulated and LPS-challenged DCs was isolated using RNeasy Micro Kit (Qiagen), including on-column DNase digest. Quality and quantity of the isolated RNA was tested using Agilent 2100 Bioanalyzer (Agilent). Gene expression profiling was performed using Affymetrix GeneChip Mouse Gene 1.0 ST Arrays. Biotinylated antisense cRNA was generated with the GeneChip® WT Plus Reagent Kit and the GeneChip® Hybridization, Wash and

Stain Kit (Affymetrix), according to the manufacturer's protocols. The following hybridization on the chip was performed in a GeneChip Hybridization oven 640, then dyed in the GeneChip Fluidics Station 450 and finally scanned with a GeneChip Scanner 3000 (all mentioned equipment was from Affymetrix).

2.3. Microarray preprocessing and analysis

CEL files from Affymetrix Mouse Gene 1.0 ST arrays were rma normalized using the *oligo* Bioconductor package [7] and log₂ transformed. Probes were annotated with Bioconductor annotation

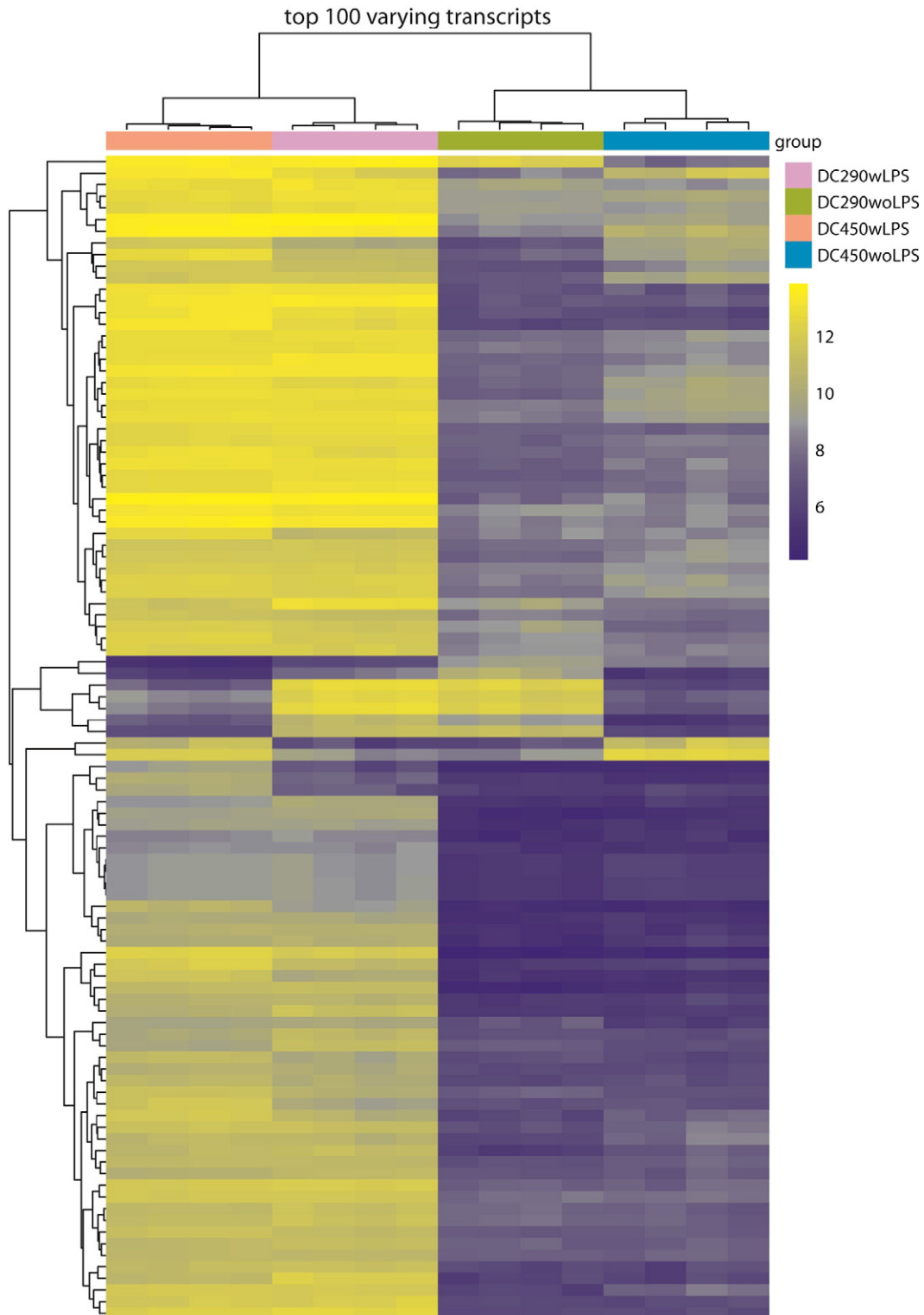


Fig. 1. Hierarchical clustering and heat map of the 100 most varying transcripts. Gene expression is illustrated with a color gradient: yellow illustrates higher expression, whereas blue indicates lower expression. (n = 4).

package *mogene10sttranscriptcluster.db*. Control probes were excluded and only probes with valid gene symbol were kept for downstream analysis. Reactome [8] pathways from the Molecular Signatures Database (MSigDB) v4.0 [9] were used for gene set testing after mapping all pathway genes to mouse orthologues as provided by the WEHI group (<http://bioinf.wehi.edu.au/software/MSigDB/>). Hierarchical clustering was performed using euclidean distance and complete linkage (Fig. 1). Differentially expressed probes were identified using the empirical Bayes approach based on moderated t-statistics [10] as implemented in the Bioconductor package *limma* [11]. Linear models were fitted with a random mouse effect. In case multiple probes mapped to the same gene, the probe with the largest absolute test statistics based on *limma* analysis was used for pathway analysis. The camera test [12] with default settings was used to competitively test pathways for regulation between conditions. Enrichment maps [13] were used to visualize results, adapting R code from Bioconductor package *HTSanalyzeR* [14]. All p-values were adjusted for multiple testing using Benjamini-Hochberg correction in order to control the false discovery rate. All p-values were two-sided. Analysis was carried out with statistical software R 3.1 [15].

Disclosure

All the authors declared no competing interests.

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