Genomics Data 7 (2016) 90–91

Contents lists available at ScienceDirect

Genomics Data

journal homepage: www.elsevier.com/locate/gdata

Data in Brief

Microarray expression analysis of genes involved in innate immune memory in peritoneal macrophages

Keisuke Yoshida [a], Claire Renard-Guillet [b], Kentaro Inoue [c], Katsuhiko Shirahige [b], Mariko Okada-Hatakeyama [c], Shunsuke Ishii [a,*]

[a] Laboratory of Molecular Genetics, CREST Research Project of JST (Japan Science and Technology Agency), RIKEN Tsukuba institute, Tsukuba 305-0074, Japan
[b] Laboratory of Genome Structure and Function, Research Center for Epigenetic Disease, Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 153-8904, Japan
[c] Laboratory for Integrated Cellular Systems, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan

ARTICLE INFO

Article history:
Received 17 November 2015
Accepted 30 November 2015
Available online 1 December 2015

Keywords:
Macrophage
ATF7
Innate immune memory
Microarray

ABSTRACT

Immunological memory has been believed to be a feature of the adaptive immune system for long period, but recent reports suggest that the innate immune system also exhibits memory-like reaction. Although evidence of innate immune memory is accumulating, no *in vivo* experimental data has clearly implicated a molecular mechanism, or even a cell-type, for this phenomenon. In this study of data deposited into Gene Expression Omnibus (GEO) under GSE71111, we analyzed the expression profile of peritoneal macrophages isolated from mice pre-administrated with toll-like receptor (TLR) ligands, mimicking pathogen infection. In these macrophages, increased expression of a group of innate immunity-related genes was sustained over a long period of time, and these genes overlapped with ATF7-regulated genes. We conclude that ATF7 plays an important role in innate immune memory in macrophages.

Specifications	
Organism/cell line/tissue	Wild-type and ATF7$^{-/-}$ mice on the C57BL/6 background peritoneal macrophages
Sex	Male
Sequencer or array type	Affymetrix Mouse Gene 1.0 ST Array
Data format	Raw signal values
Experimental factors	Wild-type vs ATF7$^{-/-}$ or Wild-type administrated by PBS vs LPS, PGN, IMQ or BG
Experimental features	Identification of genes that expressions are regulated by ATF7 or innate immune memory
Consent	Publicly available from NCBI GEO
Sample source location	Mice were maintained in the RIKEN Tsukuba facility

1. Direct link to deposited data[provide URL below]

The data is available at the GEO database under: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE71111.

* Corresponding author.
E-mail address: sishii@rtc.riken.jp (S. Ishii).

2. Experimental design, materials and methods

2.1. Study design

This series contain seven sets of expression array data. Two sets (GSM1826947–GSM1826954) were analyzed to identify ATF7-regulated genes. The remaining five sets (GSM1826955–GSM1826974) were analyzed to identify genes whose expression was potentially linked to innate immune memory. For all sets, we prepared four CEL files generated by four independent biological experiments.

2.2. Materials

- LPS: Sigma, Cat. No. L5293.
- Peptidoglycan (PGN): InvivoGen, Cat. No. tlrl-pgnbs.
- Imiquimod (IMQ): InvivoGen, Cat. No. tlrl-imq.
- β-Glucan from *Candida albicans* (BG): prepared as described [1].
- PBS.
- Macrophage-SFM: Thermo, Cat. No. 12065-074.
- TRIzol: Thermo, Cat. No. 15596018.
- WT expression kit: Thermo, Cat. No. 4411974.
- WT terminal labeling kit: Affymetrix, Cat. No. 901524.
- Mouse Gene 1.0 ST Array: Affymetrix, Cat. No. 901169.

http://dx.doi.org/10.1016/j.gdata.2015.11.028

2.3. Preparation of samples

Peritoneal macrophages were collected from male wild-type and *Atf7*$^{-/-}$ mice by peritoneal washing and cultured in Macrophage-SFM. Before and after overnight incubation, dish surfaces were washed by pipetting with PBS to remove non-adherent cells, such as lymphocytes, leaving behind the peritoneal macrophages.

To analyze the expression of genes involved in innate immune memory, male wild-type mice were injected intravenously with LPS (5 μg), PGN (50 μg), IMQ (250 μg), BG (1 mg), or PBS (as a control). Three weeks after injection, peritoneal macrophages were collected from these mice.

Total RNA was isolated from the macrophages using TRIzol following the manufacturer's protocol. cDNA was generated from the RNA sample using the WT expression kit and was labeled using the WT terminal labeling kit. These samples were hybridized to the Mouse Gene 1.0 ST Array according to the manufacturer's instructions.

2.4. Statistical analysis of the microarray data sets

The raw data (CEL file) were first normalized using the Robust Multi-array Average (RMA) method (RMA-sketch workflow in the Expression Console software [Affymetrix]). Comparison of the expression levels was then performed using the R package 'limma' [3]. Briefly, after a log2 transformation of the signal, a linear model was fitted to each sample to assess variability, and comparisons of groups of interest were made using the empirical Bayes test. The resulting *P* values were corrected for multiple comparisons tests according to the Benjamini-Hochberg method [2]. Differentially expressed genes (DEGs) that were upregulated were defined as a fold change >1.3 and $P < 0.05$ in *Atf7*$^{-/-}$ mice relative to wild-type mice, or in TLR ligand-injected mice relative to PBS-injected mice.

2.5. Network analysis

We predicted the transcription factors responsible for the regulation of the DEGs and their mechanistic networks. To explore upstream regulatory molecules of the upregulated DEGs, we performed "upstream analysis" using Ingenuity Pathway Analysis (IPA) software [4], and transcription factors responsible for regulation of the DEGs and their mechanistic networks were predicted. The candidates for the upstream transcription factors were confirmed by changes in their own expression.

To assess the functions of DEGs, enrichment analysis was performed based on the functional categories of the KEGG database [5](Kyoto Encyclopedia of Genes and Genomes). Enriched functional categories were identified by Fisher's exact test using R version 2.15 and visualized using FuncTree [6] (http://bioviz.tokyo/functree/), an online visualization tool for KEGG pathways.

2.6. Results

To find ATF7-regulated genes, we compared the expression profiles of macrophages from wild-type and *Atf7*$^{-/-}$ mice, finding 2225 upregulated DEGs. At 3 weeks after injection, 117, 49, 72, and 602 DEGs were detected in LPS, PGN, IMQ, or BG-injected mice, respectively, using PBS-injected mice as a control. A significant fraction of the LPS-induced DEGs was regulated by ATF7 (43.6%, 51/117, chi-square test, $P = 9.9 \times 10^{-158}$). PGN- and BG-induced DEGs had a similar tendency, but not the IMQ-induced DEGs. Network analysis revealed that LPS- and BG-induced DEGs included genes regulated directly or indirectly by STAT1, which expression is regulated by ATF7 (ref. [7]).

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Sports, Science, and Technology (#22125005).

References

[1] K. Ishibashi, N.N. Miura, Y. Adachi, N. Ogura, H. Tamura, S. Tanaka, N. Ohno, Relationship between the physical properties of *Candida albicans* cell well beta-glucan and activation of leukocytes in vitro. Int. Immunopharmacol. 2 (2002) 1109–1122.
[2] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. 57 (1995) 289–300.
[3] G.K. Smyth, Limma: linear models for microarray data. Bioinformatics and Computational Biology Solutions Using R and Bioconductor 2005, pp. 397–420.
[4] A. Krämer, J. Green, J. Pollard Jr., S. Tugendreich, Causal analysis approaches in ingenuity pathway analysis. Bioinformatics 30 (2014) p523–p530.
[5] M. Kanehisa, S. Goto, Y. Sato, M. Kawashima, M. Furumichi, M. Tanabe, Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res. 42 (2014) D199–D205.
[6] T. Uchiyama, M. Irie, H. Mori, K. Kurokawa, T. Yamada, FuncTree: functional analysis and visualization for large-scale omics data. PLoS One 10 (5) (2015).
[7] K. Yoshida, T. Maekawa, Y. Zhu, C. Renard-Guillet, B. Chatton, K. Inoue, T. Uchiyama, K. Ishibashi, T. Yamada, N. Ohno, K. Shirahige, M. Okada-Hatakeyama, S. Ishii, The transcription factor ATF7 mediates lipopolysaccharide-induced epigenetic changes in macrophages involved in innate immunological memory. Nat. Immunol. 16 (2015) 1034–1043.