Datasets of microarray analysis to identify Gpr137b-dependent interleukin-4-responsive genes in the mouse macrophage cell line RAW264

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Article history:
Received 20 December 2018
Received in revised form 5 January 2019
Accepted 9 January 2019

Abstract

Macrophages are classified mainly into two subtypes, M1 and M2, which exhibit distinct phenotypes, based on their microenvironment. We have recently demonstrated that Gpr137b is abundantly expressed in RAW264 macrophages, “Gpr137b is an orphan G-protein-coupled receptor associated with M2 macrophage polarization” (Islam et al., in press) [1]. Although recent studies have suggested that G-protein-coupled receptors (GPCRs) are associated with M1/M2 macrophage polarization ("G-protein-coupled bile acid receptor 1 (GPBAR1, TGR5) agonists reduce the production of proinflammatory cytokines and stabilize the alternative macrophage phenotype" (Hogenauer et al., 2014) [2], “Leukotriene B4 promotes neovascularization and macrophage recruitment in murine wet-type AMD models” (Sasaki et al., 2018) [3]), available information about GPCR-mediated macrophage polarization is still limited. This prompted us to generate Gpr137b-knockout (KO) RAW264 clones using the CRISPR/Cas9 genome editing system to elucidate the function of Gpr137b in interleukin (IL)-4-induced M2 macrophage polarization (Islam et al., in press) [1].

Here we present the datasets of a microarray analysis to identify Gpr137b-dependent IL-4-responsive genes in RAW264 cells. The raw microarray data are available in the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/) under the

DOI of original article: https://doi.org/10.1016/j.bbrc.2018.12.140
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accession number GSE117578, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117578.
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**Specifications table**

| Subject area       | Biology                                           |
|--------------------|---------------------------------------------------|
| More specific subject area | Transcriptomics                                    |
| Type of data       | Figures, Microarray data                          |
| How data was acquired | Affymetrix Clariom S Assay, mouse                 |
| Data format        | Raw data (CEL files)                              |
| Experimental factors | Gpr137b-knockout and wildtype RAW264 macrophages with or without interleukin-4 treatment |
| Experimental features | Two Gpr137b-knockout and two wildtype clones of RAW264 macrophages were established using the CRISPR/Cas9 genome editing system. These clones were treated with or without interleukin-4 and subjected to microarray-based gene expression analysis. |
| Data source location | Osaka Prefecture University, Sakai, Japan          |
| Data accessibility | The microarray datasets in this article are available through Gene Expression Omnibus database (NCBI) (Accession Number GSE117578). |
| Related research article | Zohirul Islam, Takashi Inui and Osamu Ishibashi. Gpr137b is an orphan G-protein-coupled receptor associated with M2 macrophage polarization. Biochemical and Biophysical Research Communications, in press [1]. |

**Value of the data**

- Signaling pathways involving Gpr137b in IL-4-induced M2 macrophage polarization can be clarified.
- The datasets enrich information about M2 macrophage polarization-associated genes.
- The datasets can provide novel insights into the macrophage-related post-inflammation tissue repair.

1. **Data**

RNA quality was assessed by RNA integrity number equivalent (RINe), a representative index to assess RNA quality. The RINe values of all RNA samples used for the experiment were more than 9.2 (Fig. 1). To visualize differential gene expression among the experimental groups a heatmap was generated from normalized data using the Affymetrix® Transcriptome Analysis Console 4.0 software (Supplementary file 1). Microarray and sample annotation data were deposited in Gene Expression Omnibus under accession number GSE117578. Direct link to the deposited data is available at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117578.

2. **Experimental design, materials and methods**

2.1. **Cell culture**

RAW264 macrophages were obtained from RIKEN Bioresource Center (Tsukuba, Japan) and grown in EMEM culture medium (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum.
(Thermo-Fischer, Waltham, MA, USA) and Antibiotic-Antimycotic (Sigma-Aldrich, St. Louise, MO, USA); it was then incubated in a humidified atmosphere with 5% CO₂ at 37 °C. When the cells were treated with IL-4, the culture medium was replaced with serum-deprived and 0.1% BSA-containing EMEM medium to reduce background.

| Sample Description | RINe |
|--------------------|------|
| Gpr137b-WT1/-IL-4 | 9.6  |
| Gpr137b-WT1/+IL-4 | 9.7  |
| Gpr137b-WT2/-IL-4 | 9.5  |
| Gpr137b-WT2/+IL-4 | 9.5  |
| Gpr137b-KO1/-IL-4 | 9.8  |
| Gpr137b-KO1/+IL-4 | 9.8  |
| Gpr137b-KO2/-IL-4 | 9.2  |
| Gpr137b-KO2/+IL-4 | 9.3  |

**Fig. 1.** RNA integrity number equivalent (RINe) values of RNA samples used for microarray analysis.
2.2. RNA isolation

Total RNA was isolated using RNAiso-Plus reagent (Takara-bio, Kusatsu, Japan) according to the manufacturer's protocol. RINe values were determined using Agilent 2200 TapeStation (Agilent, Santa Clara, CA, USA).

2.3. Microarray analysis

Comprehensive gene expression analysis was performed using the Clariom S Assay, mouse (Thermo-Fischer). Gpr137b-wildtype (WT) and -KO RAW264 cells (2 independent clones for each) with or without IL-4 treatment for 48 h were subjected to this analysis. A hundred ng of RNA was used for the following labeling reaction. cRNA and single strand (ss) cDNA were synthesized using Affymetrix® GeneChip® WT Plus Reagent according to the manufacturer's instruction. The ssDNA (5.5 μg) was then fragmented and biotin-labeled using GeneChip® WT Terminal Labeling Kit (Thermo-Fischer) according to the manufacturer's manual. Labeled cRNA was hybridized for 17 h on the microarray using GeneChip Hybridization, Wash, and Stain Kit (Thermo-Fischer). To visualize fluorescence signals the microarray was scanned using the GeneChip® Scanner 3000 7G.

Table 1
Microarray outliers.

| Sample          | GEO sample accession | pm_mean | pos_vs_neg_auc |
|-----------------|----------------------|---------|----------------|
| Gpr137b-WT1/-IL-4 | GSM3304078           | 333.9   | 0.8257         |
| Gpr137b-WT1/+IL-4 | GSM3304079           | 474.5   | 0.8215         |
| Gpr137b-WT2/-IL-4 | GSM3304080           | 437.0   | 0.8050         |
| Gpr137b-WT2/+IL-4 | GSM3304081           | 367.4   | 0.8299         |
| Gpr137b-KO1/-IL-4 | GSM3304082           | 366.3   | 0.8150         |
| Gpr137b-KO1/+IL-4 | GSM3304083           | 366.8   | 0.8256         |
| Gpr137b-KO2/-IL-4 | GSM3304084           | 369.7   | 0.8202         |
| Gpr137b-KO2/+IL-4 | GSM3304085           | 377.5   | 0.8184         |

PM_mean, a probe-level metric, is the mean of perfect match raw intensities prior to data normalization. Pos_vs_neg_auc is the area under the curve (AUC) for a receiver operating characteristic (ROC) plot comparing signal values for the positive controls to the negative controls, and is a robust measure of the global quality of the data.

Fig. 2. Signal box plots of array files after CHP normalization.
2.4. Data processing

The quality of the experiment was assessed based on the values of pos vs neg auc and pm mean, which were calculated using the Affymetrix® Expression Console software (Thermo-Fischer) (Table 1). CEL files were processed for each replicate and experimental condition using the Affymetrix® Transcriptome Analysis Console software 4.0 (Thermo-Fischer). The CEL files were then subjected to normalization using the Signal Space Transformation-Robust Multiarray Analysis (SST-RMA) method [4] to generate CHP files. The comparability of the relative log expression signal across all samples are ensured (Fig. 2).

Acknowledgements

This research was supported by JSPS KAKENHI Grant number 16K08145 (OI).

Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2019.01.017.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2019.01.017.

References

[1] Z. Islam, T. Inu, O. Ishibashi, Gpr137b is an orphan G-protein-coupled receptor associated with M2 macrophage polarization, Biochem. Biophys. Res. Commun. 509 (2019) 657–663 ( PubMed PMID: 30595385).
[2] K. Hogenauer, L. Arista, N. Schmiedeberg, G. Werner, H. Jaksche H, et al., G-protein-coupled bile acid receptor 1 (GPBAR1, TGR5) agonists reduce the production of proinflammatory cytokines and stabilize the alternative macrophage phenotype, J Med Chem. 24 (2014) 10343–10354 (PubMed PMID: 25411721).
[3] F. Sasaki, T. Koga, M. Ohba, K. Saeki, T. Okuno, et al., Leukotriene B4 promotes neovascularization and macrophage recruitment in murine wet-type AMD models, JCI Insights 3 (2018) 96902 (PubMed PMID: 30232269).
[4] R.A. Irizarry, B.M. Bolstad, F. Collin, L.M. Cope, B. Hobbs, et al., Summaries of affymetrix GeneChip probe level data, Nucleic Acids Res. 31 (2003) e15 (PubMed PMID: 12582260).