Data in Brief

Transcriptomic profiling of splenic B lymphomas spontaneously developed in B cell-specific TRAF3-deficient mice

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A B S T R A C T

TRAF3, a critical regulator of B cell survival, was recently recognized as a tumor suppressor gene in B lymphocytes. Specific deletion of TRAF3 from B lymphocytes leads to spontaneous development of marginal zone lymphomas (MZL) or B1 lymphomas in mice. To identify novel oncogenes and tumor suppressive genes involved in malignant transformation of TRAF3-deficient B cells, we performed a microarray analysis to identify genes differentially expressed in TRAF3−/− mouse splenic B lymphomas. We have identified 160 up-regulated genes and 244 down-regulated genes in TRAF3−/− B lymphomas as compared to littermate control splenocytes. Here we describe the samples, quality control assessment, as well as the data analysis methods in detail for the transcriptomic profiling study. Data are archived at NIH GEO with accession number GSE48818.

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Experimental design, materials, and methods

Sample collection and preparation

Spleens were harvested from B cell-specific TRAF3-deficient mice with B lymphomas or tumor-free littermate control mice (Table 1). In the three selected TRAF3−/− splenic B lymphoma samples (mouse ID: 6983-2, 7041-10, and 7060-8), B lymphoma cells are >70% of B cells as assessed by FACS analysis of B cell populations and Southern blot analysis of IgH gene rearrangements [1]. Spleens were separated into single cell suspensions by mechanic dissociation, and red blood cells were depleted using 1X ACK solution as described [2,3]. The resulting splenocytes were collected for total cellular RNA extraction using TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer’s instructions. RNA samples were purified using a RNeasy MinElute Cleanup Kit (QIAGEN, Valencia, CA). RNA concentration and quality were assessed using a NanoDrop spectrometer (NanoDrop Products, Wilmington, DE) (Table 1). RNA integrity was further analyzed on an RNA Nano Chip using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), and the results are shown in Fig. 1.

Gene expression analysis

The mRNA was amplified with a TotalPrep RNA amplification kit with a T7-oligo(dT) primer according to the manufacturer’s instructions.

Direct link to deposited data

Deposited data can be found at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48818.

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Table 1
RNA samples for transcriptome profiling by microarray analysis.

| Sample ID   | Sample Name | Mouse ID     | Genotype              | Tissue     | Concentration | O.D. 260/280 | O.D. 260/230 | Total volume |
|-------------|-------------|--------------|-----------------------|------------|---------------|--------------|--------------|--------------|
| GSM1185225  | XP1         | 6983-2       | TRAF3fl/fox, CD19+/Cre | Spleen     | 200 ng/μl     | 2.09         | 2.01         | 5 μl         |
| GSM1185226  | XP2         | 7041-10      | TRAF3fl/fox, CD19+/Cre | Spleen     | 100 ng/μl     | 2.08         | 2.08         | 10 μl        |
| GSM1185227  | XP3         | 7060-8       | TRAF3fl/fox, CD19+/Cre | Spleen     | 200 ng/μl     | 2.09         | 1.83         | 10 μl        |
| GSM1185228  | XP5         | 6983-6       | TRAF3fl/fox           | Spleen     | 100 ng/μl     | 2.09         | 2.09         | 10 μl        |
| GSM1185229  | XP6         | 7060-5       | TRAF3fl/fox           | Spleen     | 200 ng/μl     | 2.07         | 2.39         | 5 μl         |
| GSM1185230  | XP7         | 7060-6       | TRAF3fl/fox           | Spleen     | 200 ng/μl     | 2.03         | 2.16         | 10 μl        |
| GSM1185231  | XP9         | 7041-9       | TRAF3fl/fox           | Spleen     | 200 ng/μl     | 2.08         | 2.01         | 5 μl         |

Fig. 1. Quality control assay of RNAs used for microarrays. (A) Bioanalyzer output as gel images for all seven samples as identified by the Mouse ID (see Table 1). (B) Bioanalyzer output as traces with RIN (RNA integrity number) shown for each sample. Results are plotted as fluorescence units [FU] over time [s].
(Ambion), and microarray analysis was carried out with the Illumina Sentrix MouseRef-8 24K Array at the Burnham Institute (La Jolla, CA).

**Data processing and normalization**

Results were extracted with Illumina GenomeStudio v2011.1 and exported as the sample probe profile format without background correction or normalization. This file, along with a matching control table output from GenomeStudio, was loaded into a limma object in R/Bioconductor [4–6]. Gene probes were tracked using the nulD system [4]. Background correction and quantile normalization was performed using the `lumiExpresso` function. Expression data above detection limits (using the `detectionCall` function) were used to extract RNA group names as factors and assembled into a model matrix. Extracted expression values, the model design, and the array weights were used to model data in the `limma` package [7]. The contrast of “B lymphoma—control” was selected and used to generate contrasts using the `eBayes` function. Finally, gene annotation was added using the `lumiMouseAll.db` and `annotate` packages. Results were selected using the `topTable` function (with `n = Inf` to output all contrasts) and saved as in csv format. This table was reviewed using Excel to select significantly different genes with an adjusted p-value ≤ 0.05 and a log2 fold change ≥ 1 (Fig. 2).

Microarray data are available from NIH GEO Accession GSE48818 and described by Edwards et al. [8].

**Statistics**

Statistical analyses were performed using limma modeling (ANOVA with empirical Bayes moderation of standard errors). Adjusted p-values less than 0.05 with a fold-change greater than 2 are considered significant.

**Discussion**

Results of the microarray analysis have identified 160 up-regulated genes and 244 down-regulated genes in TRAF3−/− B lymphomas as compared to LMC spleens (2-fold up or down fold-change, adjusted p < 0.05) (NCBI GEO accession number: GSE48818). Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2014.10.017.

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**References**

[1] C.R. Moore, Y. Liu, C.S. Shao, L.R. Covey, H.C. Morse III, P. Xie, Specific deletion of TRAF3 in B lymphocytes leads to B lymphoma development in mice. Leukemia 26 (2012) 1122–1127.
[2] L.L. Stunz, L.K. Busch, M.E. Munroe, C.D. Sigmund, L.T. Tygrett, T.J. Waldschmidt, G.A. Bishop, Expression of the cytoplasmic tail of LMP1 in mice induces hyperactivation of B lymphocytes and disordered lymphoid architecture. Immunity 21 (2004) 255–266.
[3] P. Xie, L.L. Stunz, K.D. Larison, B. Yang, G.A. Bishop. Tumor necrosis factor receptor-associated factor 3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. Immunity 27 (2007) 253–267.
[4] P. Du, W.A. Kibbe, S.M. Lin, nulD: a universal naming scheme of oligonucleotides for illumina, affymetrix, and other microarrays. Biol. Direct 2 (2007) 16.
[5] P. Du, W.A. Kibbe, S.M. Lin, lumi: a pipeline for processing illumina microarray. Bioinformatics 24 (2008) 1547–1548.
[6] R.C. Gentleman, V.J. Carey, D.M. Bates, B. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, J. Gentry, K. Hornik, T. Hothorn, W. Huber, S. Iacus, R. Irizarry, F. Leisch, C. Li, M. Maechler, A.J. Rossini, G. Sawitzki, C. Smith, G. Smyth, L. Tierney, J.Y. Yang, J. Zhang, Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5 (2004) R80.
[7] G. Smyth, Limma: linear models for microarray data. Springer, New York, 2005.
[8] S. Edwards, J. Baron, C.R. Moore, Y. Liu, D.H. Perlman, R.P. Hart, P. Xie, Mutated in colorectal cancer (MCC) is a novel oncogene in B lymphocytes. J. Hematol. Oncol. 7 (2014) 56.