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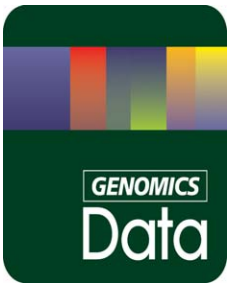

Transcriptomic analyses of primary astrocytes under TNFα treatment

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Specifications

Organism/cell line/tissue	Mus musculus (C57BL/6JOlaHsd)
Sex	Pooled male and female brains
Sequencer or array type	Affymetrix GeneChip Mouse Gene 1.0 ST arrays
Data format	CEL files
Experimental factors	Primary astrocytes were treated with TNFα (50 ng/ml) during 24 h and compared to untreated cells
Experimental features	Total RNA was extracted to study gene expression changes. Three replicates were used for each experimental condition.
Consent	N/A
Sample source location	N/A

1. Direct link to deposited data

Deposited data can be found at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73022.

2. Experimental Design, Materials and Methods

2.1. Cell culture and experimental design

Primary mouse astrocytes cultures were prepared from newborn C57BL/6JOlaHsd mice brains as previously described [8]. After removing meninges and large blood vessels, brains were minced in phosphate-buffered saline solution by mechanical dissociation. Cells were cultivated in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was changed after three days, and cultures reached confluence after 10-14 days. Then, glial cells were separated by a magnetic cell sorting (MACS) method according to the manufacturer's protocol (Miltenyi Biotec, The Netherlands). Briefly, glial cultures were trypsinized and microglia, the CD11b-positive cells present in the astrocyte monolayer, were collected by a positive selection. Simultaneously, astrocytes were negatively sorted as previously described [8, 9]. Astrocyte-enriched cultures were obtained by platting the cells in 75 cm^2 flasks. After 3 days, the culture medium was replaced and after 7 days, when cultures reached confluence, the MACS procedure was repeated in order to reduce the residual microglial contamination in our astrocyte population.

After additional 7 days, cultures of primary mouse astrocytes were treated with TNF (50 ng/ml; R&D Systems, United Kingdom) during 24 h. Total RNA was extracted using RNA NOW reagent (OZYME,

France) according to the manufacturer's instructions.

2.2 Microarrays experiments, quality control and data analysis

To determine the effects of TNFα on primary astrocytes, mRNA samples were analyzed by Affymetrix GeneChip Mouse Gene 1.0 ST arrays. All samples were of high purity and integrity and were assessed by the Agilent 2100 Bioanalyzer and RNA 6000 Nano LabChip kits (Agilent Technologies). Data from three biological replicates were analyzed for each experimental condition.

Microarray gene expression data was normalized using the GC-RMA procedure with default parameters for background correction, quantile normalization, and probe replicate summarization [10]. Differentially expressed genes between control and TNFα conditions were then determined using the empirical Bayes moderated t-statistic (eBayes) [11]. P-value significance scores for these genes were adjusted for multiple hypothesis testing according to the Benjamini–Hochberg procedure [12].

A heat map and dendrogram cluster visualization for the top 150 most significant known genes (Fig. 1) was obtained using standard hierarchical average linkage clustering with a Euclidean distance metric.

A volcano plot for the analysis of differential gene expression between TNFα and control samples was obtained. For each transcript, the negative decadic logarithm of the adjusted p-value significance score was plotted against the logarithm of the fold change. Several genes (green dots) are significantly altered (adjusted $p < 0.05$) and display an absolute log fold change above 1 in expression (Fig. 2).

Alterations in known cellular pathways and processes were identified and visualized by applying the MetaCoreTM GeneGo software onto the differential expression statistics obtained from the eBayes analysis [11]. The genes were pre-filtered using a significance threshold (adjusted p value <0.05) before applying the default GeneGO pathway analysis. Pathway analysis with GeneGO revealed that pathways related to glial differentiation, immune response and apoptosis were modulated (Fig. 3).

3. Conclusion

Herein we describe the transcriptional analysis of primary astrocytes following a TNFα exposure. These expression data could be useful to describe the effect of the NFκB activation on primary astrocyte cultures devoid of microglia. Taking advantage of the MACS technology, in contrast to the main studies reported in the literature, we were able to characterize pure populations of astrocytes under inflammatory conditions.

We show that TNFα increases the expression of genes associated with the NFκB pathway and induces the re-expression of genes implicated in glial developmental processes.

These data highlight the importance of the NFκB pathway during the conversion of astrocytes into reactive cells and, particularly, its active role in the dedifferentiation process [13].

Conflict of interest

The authors declare that there are no conflicts of interests.

Acknowledgments

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Figures legends

Figure 1. Heat map visualization of the normalized gene expression levels for the top 150 most significant known genes with differential expression between control and TNF samples according to the empirical Bayes moderated *t* statistics.

Figure 2. Volcano plot for the analysis of differential gene expression between TNF and control samples. For each transcript, the negative decadic logarithm of the adjusted p-value significance score is plotted against the logarithm of the fold change. To highlight the transcripts with highest effect size and significance, data points are colored red if the adjusted p-value is below 0.05, orange if the absolute value of the log fold change is greater than 1, and green if both of these criteria are fulfilled.

Figure 3. Cellular pathways enriched in significantly differentially expressed genes between TNF and control sample. These pathways were identified using the GeneGO pathway analysis software.

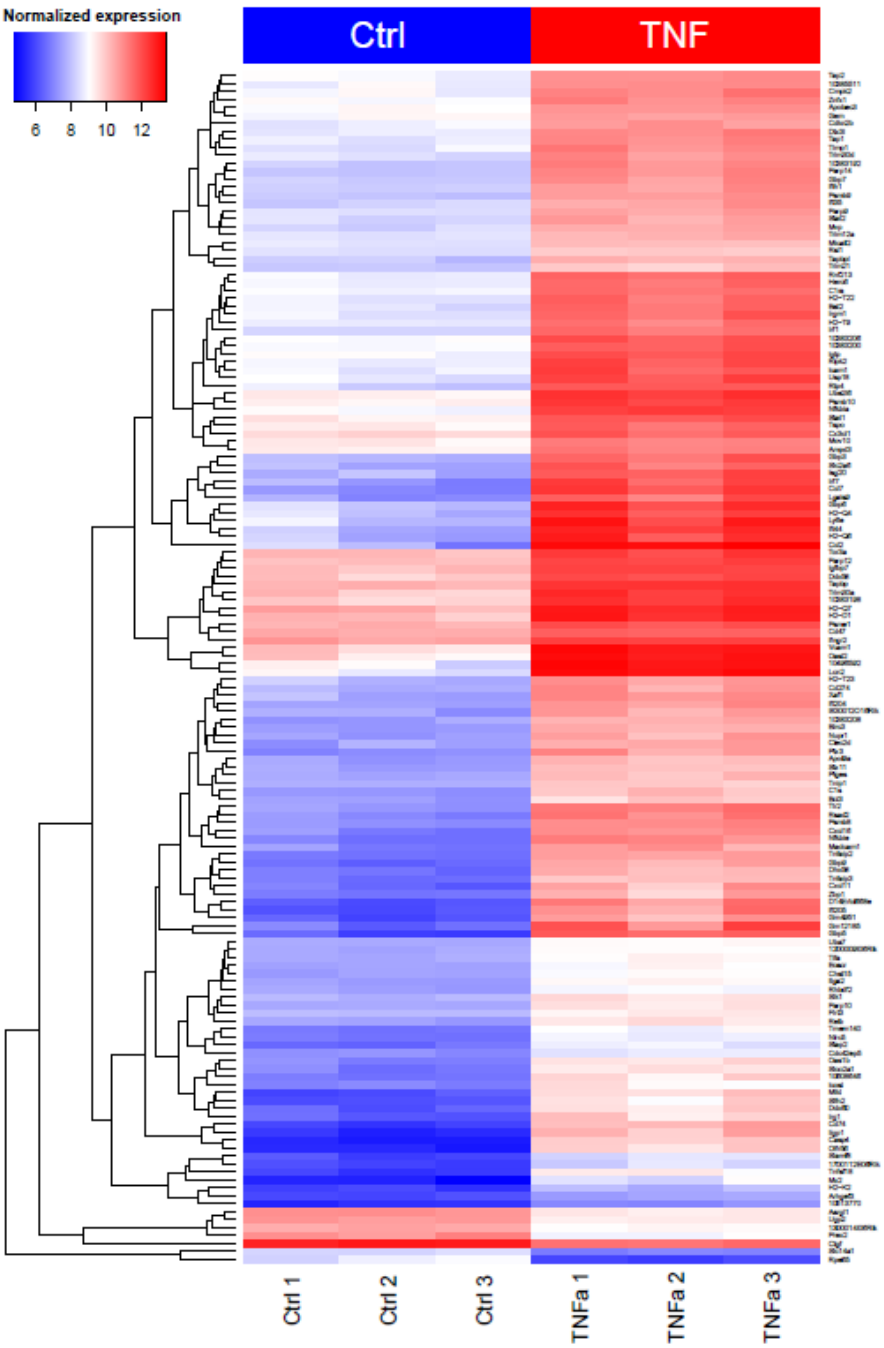

Fig. 1

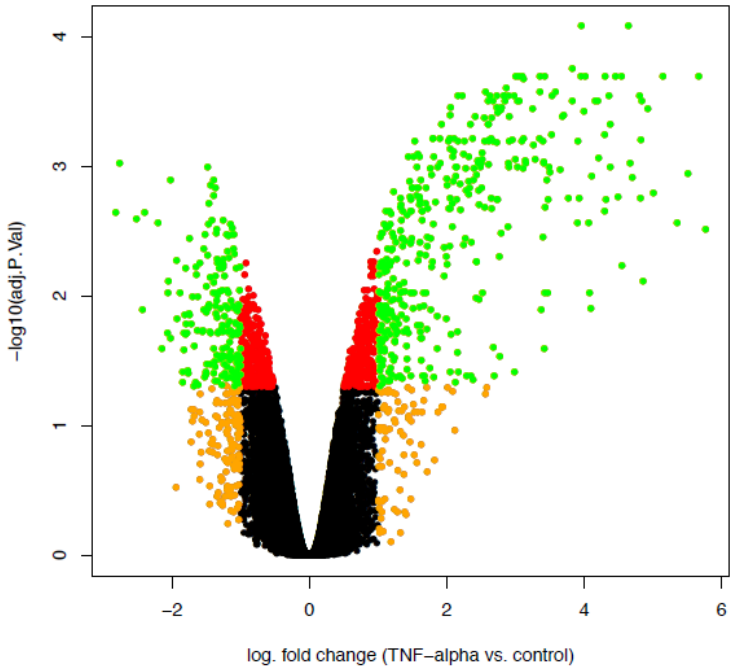

Fig. 2

Pathways (GeneGO)	Total	pValue	Min FDR	p-value	FDR	In Data	Pathway members
Apoptosis and survival_Role of PKR in stress-induced apoptosis	53	7.928E-11	1.810E-08	7.928E-11	1.810E-08	17	NFKBIB, I-kB, PP2A regulatory, Caspase-7, IFN-gamma receptor, NF-kB, IRF1, NF-kB p50/p65, PKR, p53, TLR3, Tyk2, ATF-3, TRAF2, NFKBIA, FasR(CD95), PPP2R5A
Signal transduction_NF-kB activation pathways	51	4.198E-10	5.751E-08	4.198E-10	5.751E-08	16	I-kB, c-IAP2, NF-kB2 (p100), RelA (p65 NF-kB subunit), NF-kB, RelB (NF-kB subunit), NF-kB1 (p105), NF-kB1 (p50), NF-kB p50/p65, MyD88, NF-kB2 (p52), TRAF2, c-IAP1, NF-kB p52/RelB, TLR2, BAFF(TNFSF13B)
Immune response_IL-10 signaling pathway	62	1.242E-09	1.418E-07	1.242E-09	1.418E-07	17	STAT3, IL-6, IKBZ, STAT5, MHC class II, NF-kB, iNOS, Bcl-3, NF-kB1 (p50), NF-kB p50/p65, ICAM1, IL10RB, Tyk2, microRNA 155, TIMP1, STAT1, miR-155-5p
Immune response_TNF-R2 signaling pathways	45	5.732E-08	4.363E-06	5.732E-08	4.363E-06	13	I-kB, TNF-R2, c-IAP2, NF-kB2 (p100), VEGFR-2, RelA (p65 NF-kB subunit), NF-kB, NF-kB p50/p65, NF-kB2 (p52), TRAF2, c-IAP1, p38 MAPK, NF-kB p52/RelB
Development_PEDF signaling	49	1.745E-07	9.963E-06	1.745E-07	9.963E-06	13	NF-kB p50/p50, IL-6, c-IAP2, VEGFR-2, RelA (p65 NF-kB subunit), NF-kB, NF-kB1 (p50), NF-kB p50/p65, JunB, SOD2, c-IAP1, Fra-2, NFKBIA
Immune response_IL-17 signaling pathways	60	2.173E-06	9.303E-05	2.173E-06	9.303E-05	13	IL-6, I-kB, IL-17RC, NF-kB, iNOS, ICAM1, CCL2, Stromelysin-1, C/EBPbeta, NGAL, p38 MAPK, CCL7, C/EBPdelta
Apoptosis and survival_TNFR1 signaling pathway	43	2.331E-06	9.305E-05	2.331E-06	9.305E-05	11	I-kB, HtrA2, c-IAP2, jBid, Caspase-7, NF-kB, TRAF2, c-IAP1, tBid, ARTS-1, Bid
Immune response_Alternative complement pathway	39	6.613E-06	1.893E-04	6.613E-06	1.893E-04	10	iC3b, C3dg, Factor B, C3a, C3, C5 convertase (C3bBb), Factor Ba, C3b, Factor Bb, C3c
Immune response_C3a signaling	48	7.453E-06	1.964E-04	7.453E-06	1.964E-04	11	CCL5, IL-6, MHC class II, RelA (p65 NF-kB subunit), NF-kB p50/p65, CCL2, KLF5, C3a, PI3K reg class IB (p101), p38 MAPK, NFKBIA
Development_PDGF signaling via STATs and NF-kB	32	8.440E-06	2.141E-04	8.440E-06	2.141E-04	9	STAT3, I-kB, STAT5, RelA (p65 NF-kB subunit), NF-kB, PKR, Tyk2, STAT1, NF-kB p65/p65
Development_Oligodendrocyte differentiation from adult stem cells	51	1.389E-05	3.171E-04	1.389E-05	3.171E-04	11	NOTCH1 (NICD), MCT8, SMAD9 (SMAD8), Noggin, FGFR3, NOTCH1 receptor, OATP-A, p38 MAPK, BMP2, FGFR1, TR-beta1
Signal transduction_PTMs in BAFF-induced canonical NF-kB signaling	43	1.663E-05	3.742E-04	1.663E-05	3.742E-04	10	NF-kB2 (p100), NF-kB1 (p105), NF-kB1 (p50), NF-kB p50/p65, c-Rel (NF-kB subunit), MyD88, TRAF2, NF-kB p50/c-Rel, NFKBIA, BAFF(TNFSF13B)
Immune response_IL-13 signaling via JAK-STAT	44	2.104E-05	4.239E-04	2.104E-05	4.239E-04	10	STAT3, STAT5, CCL17, iNOS, IL4RA, IL13RA1, CCL2, Tyk2, IL-4R type II, STAT1
Immune response_TLR5, TLR7, TLR8 and TLR9 signaling pathways	48	4.710E-05	7.943E-04	4.710E-05	7.943E-04	10	IRF7, IL-6, I-kB, TPL2(MAP3K8), NF-kB, NF-kB1 (p105), IRF1, MyD88, p38 MAPK, IRF5
Signal transduction_PTMs (ubiquitination and phosphorylation) in TNF-alpha-induced NF-kB signaling	39	4.801E-05	7.943E-04	4.801E-05	7.943E-04	9	c-IAP2, RelA (p65 NF-kB subunit), Zibra, NF-kB1 (p105), NF-kB1 (p50), NF-kB p50/p65, TRAF2, c-IAP1, NFKBIA
Immune response_IL-15 signaling via JAK-STAT cascade	23	4.986E-05	7.943E-04	4.986E-05	7.943E-04	7	STAT3, STAT5A, STAT5, sIL-15RA, STAT2, IL-15RA, Tyk2
Apoptosis and survival_Role of IAP-proteins in apoptosis	31	5.372E-05	8.363E-04	5.372E-05	8.363E-04	8	HtrA2, c-IAP2, Caspase-7, HSP70, c-IAP1, tBid, FasR(CD95), Bid
Development_Astrocyte differentiation from adult stem cells	40	5.950E-05	8.671E-04	5.950E-05	8.671E-04	9	STAT3, SMAD9 (SMAD8), OLIG1, Nestin, CD44, ID4, SHP-2, BMP2, LIF
Immune response_Classical complement pathway	52	9.679E-05	1.353E-03	9.679E-05	1.353E-03	10	C2, iC3b, C3dg, C2b, C3a, C3, C3b, C1s, C2a, C3c
Signal transduction_PTMs in BAFF-induced non-canonical NF-kB signaling	34	1.096E-04	1.473E-03	1.096E-04	1.473E-03	8	c-IAP2, NF-kB2 (p100), RelB (NF-kB subunit), NF-kB2 (p52), TRAF2, c-IAP1, NF-kB p52/RelB, BAFF(TNFSF13B)

Fig. 3

Abstract

Astrocytes, the most abundant glial cell population in the central nervous system, have important functional roles in the brain as blood brain barrier maintenance, synaptic transmission or intercellular communications [1, 2]. Numerous studies suggested that astrocytes exhibit a functional and morphological high degree of plasticity. For example, following any brain injury, astrocytes become reactive and hypertrophic. This phenomenon, also called reactive gliosis, is characterized by a set of progressive gene expression and cellular changes [3]. Interestingly, in this context, astrocytes can re-acquire neurogenic properties. It has been shown that astrocytes can undergo dedifferentiation upon injury and inflammation, and may re-acquire the potentiality of neural progenitors [4, 5, 6, 7].

To assess the effect of inflammation on astrocytes, primary mouse astrocytes were treated with tumor necrosis factor α (TNFα), one of the main pro-inflammatory cytokines. The strength of this study is that pure primary astrocytes were used. As microglia are highly reactive immune cells, we used a magnetic cell sorting separation (MACS) method to further obtain highly pure astrocyte cultures devoid of microglia.

Here, we provide details of the microarray data, which have been deposited in the Gene Expression Omnibus (GEO) under the series accession number GSE73022. The analysis and interpretation of these data are included in Gabel *et al.* (2015). Analysis of gene expression indicated that the NF□B pathway-associated genes were induced after a TNFα treatment. We have shown that primary astrocytes devoid of microglia can respond to a TNFα treatment with the re-expression of genes implicated in the glial cell development.

Data in Brief

Title: Transcriptomic analyses of primary astrocytes under TNFα treatment

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