Biochemical and Biophysical Research Communications 428 (2012) 327–332

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Role of RNA-binding protein tristetraprolin in tumor necrosis factor-α mediated gene expression

Xia Chen [a,1], Ziran Wei [b,1], Weimin Wang [b], Ronlin Yan [b], Xudong Xu [a], Qingping Cai [b,*]

[a] Urology Department, Minhang District Central Hospital, Shanghai, China
[b] Department of General Surgery, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, China

ARTICLE INFO

Article history:
Available online 17 September 2012

Keywords:
Tristetraprolin
Microarray
Tumor necrosis factor-α
RNA-binding protein

ABSTRACT

Tumor necrosis factor-α (TNF-α) plays an important role in the pathogenesis of inflammatory diseases. Excessive TNF-α expression induces tristetraprolin (TTP), an RNA-binding protein that regulates mRNA degradation, which in turn downregulates TNF and its downstream genes, thus resulting in anti-inflammatory effects. In order to better understand the TNF-α mediated molecular pathways in inflammatory diseases, embryonic fibroblast (MEF) cell lines derived from TTP-deficient (KO) or wild type (WT) mice were treated with TNF-α and gene expression differences between two cell lines were compared by a microarray essay of 9224 genes. We found that TTP-KO cells had higher expression levels of pro-inflammatory genes than TTP-WT cells, and inflammatory genes were differentially regulated by TNF-α between TTP-KO and TTP-WT cells. Through a study of 2-dimentional gene set matrix analysis, we also found the genes upregulated by TNF-α in TTP KO cells were correlated with the pathologic phenotypes in inflammation, joint, or bone diseases. Our study provided a detailed genetic roadmap for further understanding the regulatory effect of TTP in inflammatory pathways related to human diseases.

1. Introduciton

Tristetraprolin (TTP) is a widely expressed RNA-binding protein that plays a central role in regulating mRNA stability by acting on the AU-rich elements (AREs) identified in the 3′ untranslated region (UTR) of a variety of short-lived mRNAs, including those of cytokines, chemokines and proto-oncogenes. The TTP deficient mice appeared normal at birth, but soon manifest with cachexia, erosive arthritis, and high titers of anti-DNA and antinuclear antibodies [1]. The tumor necrosis factor-α (TNF-α) is one of the major macrophage-derived cytokines present in the rheumatoid joint. It induces the synthesis and secretion of matrix-degrading proteases and prostanoids from synovial fibroblasts. Pathological overexpression of TNF-α resulted in inflammatory arthritis and bowel disease [2]. The pro-inflammatory phenotypes observed in TTP-deficient mice, including inflammatory synovitis, cartilage and bone erosion are similar to those in the disorders where over-abundant TNF-α was seen. Studies demonstrated that injecting antibodies to TNF-α into TTP-deficient mice prevented all aspects of the proinflammatory phenotype [3–5]. Studies also showed that TTP interacted with the ARE of 3′UTR of TNF-α mRNA, limiting inflammation through a negative feedback loop on TNF-mediated activity; and mice lacking TIA-1, an ARE-binding protein, showed higher expression level of TNF-α [3,6–8]. Thus, all lines of evidence point to a critical role of TTP in regulating genes in inflammatory pathway.

The TTP-deficient mice model is a valuable tool of studying autoimmune disorders. Mouse embryonic fibroblasts (MEF) cell lines were derived from TTP knockout (KO) or littermate wild type (WT) mice: Studies using these cell lines have identified novel genomic targets of TTP and further elucidated the role of TTP in regulating TNF-related diseases [9–11]. However, how TTP and TNF-α coordinately affect gene expression in inflammatory pathways is still poorly understood. In the present study, we applied genome-wide methods to examine the dynamics of TNF-α stimulated gene expression in cells derived from TTP KO and WT mice: We expected to gain insight information of the pathological mechanisms of inflammatory pathways in mouse models.

2. Materials and methods

2.1. Cell culture and treatment

TTP KO and WT mouse embryonic fibroblast cell lines were generated from littermate embryos (kindly provided by Drs. W.S. Lai and P.J. Blackshear, National Institute of Environmental Health Sciences, Research Triangle Park, NC). Cells were grown as a monolayer in DMEM (Invitrogen) supplemented with 10% FBS

* Corresponding author.
E-mail addresses: weiziran0010185@163.com (Z. Wei), qingping_cai@yahoo.com.cn (Q. Cai).
[1] Xia Chen and Ziran Wei contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.09.033

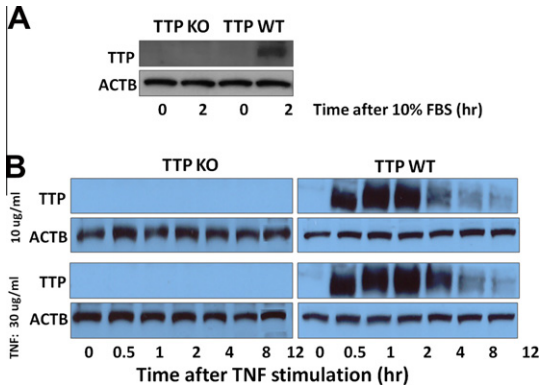

Fig. 1. The Western blot showed the difference in TTP expression in TTP KO and WT cells in response to serum or TNF-α stimulation. (A) Serum-deprived (24 h) MEFs of the TTP KO and WT cells were either untreated (0 h) or treated with 10% FBS for 2 h. Each lane contains 80 μg protein. (B) The TTP KO and WT cells (RPMI 1640 + 10% FBS) were treated with TNF-α (10 μg/mL or 30 μg/mL) for 0, 0.5, 1, 2, 4, 8 and 12 h. Each lane contains 40 μg protein. β-actin (ACTB) was used as loading control.

(Invitrogen), 2 mM L-glutamine (Invitrogen) and 100 U/ml penicillin/streptomycin. TTP KO cells were supplemented with 0.3 g/L geneticin (Invitrogen) every five passages as previously described [9].

For experiments of serum stimulation, MEF cells were allowed to grow to 70% confluence, washed once in serum-free DMEM and then incubated in DMEM containing 0.4% FBS for 20 h. 10% FBS (Invitrogen) was then added into growth medium for another 2 h, followed by cell harvest. For experiments of TNF-α stimulation, MEF cells were first grown to 50% confluence: The growth medium was changed to DMEM containing 10% charcoal-stripped FBS (Sigma) and cells were incubated for 24 h. Murine recombinant TNF-α (10 ng/ml or 30 ng/ml, R&D Systems) was then added, and cells were allowed to grow subsequently for 0.5, 1, 2, 4, 8, and 12 h before harvest.

2.2. Western blot analysis

For the detection of TTP and β-actin (ACTB) protein, whole-cell lysates were prepared in lysis buffer containing 10 mM Tris–HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.1% SDS, 1% NP-40, 1% Na-deoxycholate, and supplemented with Proteinase Inhibitor cocktail (added right before use, Roche Applied Biosciences). Protein concentrations were determined by BCA Protein Assay Kit (PIERCE). Forty-five or eighty microgram of total cell lysates were boiled and denatured, followed by gel electrophoresis in MES buffer (Invitrogen). The proteins were electrotransferred onto a polyvinylidene difluoride membrane (Macherey–Nagel). The membrane was blocked in 5% dry milk in TBS-T for 1 h at room temperature and then incubated with primary antibodies at 4 °C overnight. Primary antibody against TTP protein (rabbit polyclonal) was a gift from Dr. P.J. Blackshear (National Institute of Environmental Health Sciences); anti-ACTB monoclonal antibody was purchased

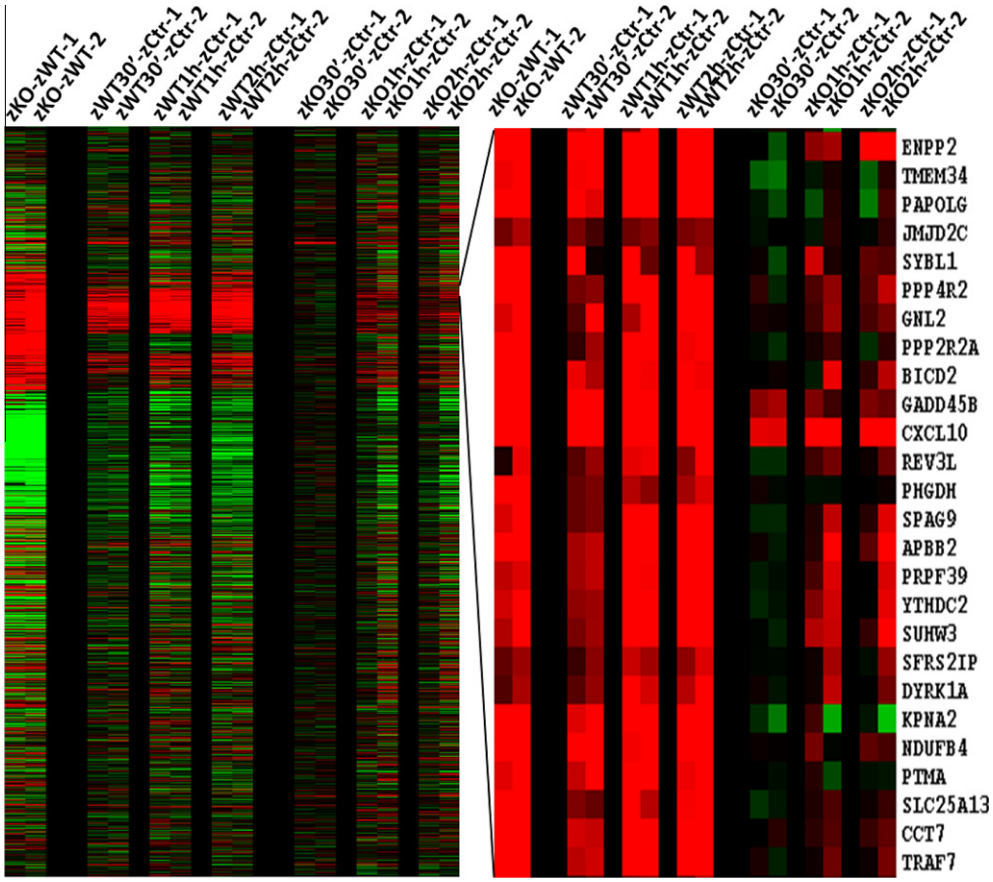

Fig. 2. TNF-α induced gene expression differences in TTP WT and KO cells. TTP WT and KO MEF Cells were either untreated or treated with TNF-α (10 ng/mL) for 30′, 1 h and 2 h. Expression patterns of 9,224 genes were shown in a heat map under these conditions (left, unsupervised clustering). A selected group of genes were also showed in a zoomed view (right). Biological duplicates were shown and gene expression ratios were calculated for each gene in each sample relative to the control expression. The data are colored red for high abundance/upregulation and green for low abundance/downregulation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison of representative immune pathway genes differentially expressed between TTP WT and TTP KO MEFs both at baseline and upon TNF stimulation in a time course.

Symbol	Baseline		WT (TNF time point)/WT Oh Ctr						KO (TNF time point) /KO Oh Ctr					
	KO/WT-1	KO/WT-2	T307C-1	T30/C-2	Tlh/C-1	Tlh/C-2	T2h/C-1	T2h/C-2	T30VC-1	T307C-2	Tlh/C-1	Tlh/C-2	T2h/C-1	T2h/C-2
CxdlO	63.49	77.81	16.80	14.96	20.83	23.84	30.79	27.53	1.87	1.82	2.79	2.57	2.61	3.07
ll6	25.78	44.77	14.83	12.29	26.78	40.05	47.63	67.94	2.85	2.00	3.91	2.96	5.70	4.82
Pcna	19.72	17.92	4.43	2.60	4.83	3.30	7.76	5.16	1.03	0.83	1.41	0.89	1.29	1.13
Cxd5	6.71	22.92	3.40	6.12	6.70	11.19	8.11	15.83	1.17	0.89	1.63	1.38	1.65	1.85
Ptgs2	15.88	12.11	27.22	10.16	23.46	9.95	23.33	14.32	2.49	2.43	3.14	4.42	3.29	6.33
ll1rl1	11.95	12.16	1.99	2.08	5.74	5.47	8.15	7.69	1.08	1.17	1.42	1.75	1.41	1.76
Ccl2	13.58	10.52	30.50	21.97	27.30	20.72	32.81	25.31	1.63	1.54	3.00	1.68	2.79	2.39
Cxcl2	9.38	10.64	17.23	13.13	3.54	4.35	2.66	2.50	5.96	4.64	9.34	5.88	12.49	10.16
ll11	9.61	9.42	0.90	1.31	0.81	1.19	0.85	1.12	2.08	1.83	1.95	1.37	2.39	2.16
Hspca	5.25	10.02	2.04	2.56	3.16	3.90	2.28	3.97	0.91	0.95	1.10	0.97	0.98	0.88
Calm2	4.56	10.58	2.28	3.34	2.59	3.74	2.87	4.85	0.89	0.74	1.35	0.69	1.12	0.81
Traf5	5.00	8.70	2.62	2.18	4.39	3.98	4.02	4.48	1.23	0.91	1.16	2.24	1.63	2.98
Cxdl6	4.98	5.67	0.86	0.93	1.12	1.18	1.01	1.22	1.01	1.04	1.48	1.14	2.13	1.82
Egr2	4.25	6.03	44.98	50.92	5.12	5.10	2.44	4.01	6.08	5.98	1.79	2.43	1.26	1.45
Dek	4.00	4.39	1.42	2.08	1.26	1.88	1.57	1.84	0.80	1.14	0.79	0.75	0.99	0.83
Ccl4	5.33	3.01	1.85	1.58	2.38	2.76	3.42	2.97	1.68	1.99	2.51	2.38	2.18	2.30
Ppp2r2a	2.64	5.67	1.19	1.75	2.31	2.66	2.06	3.90	0.97	0.87	1.08	1.27	0.88	0.19
Tebp	3.29	4.77	1.66	2.40	2.39	3.70	2.91	4.69	0.89	0.98	1.10	1.10	1.16	1.22
Sugtl	3.09	4.85	2.68	2.14	2.97	3.08	2.90	3.44	1.04	0.88	1.47	0.95	1.10	0.98
Tnfaip3	3.52	3.61	30.34	31.54	18.70	17.40	11.89	13.70	8.50	8.52	5.16	8.07	4.87	7.42
Icaml	3.21	3.47	2.05	2.62	15.89	13.82	10.27	14.64	1.54	1.67	3.65	6.76	7.51	12.25
Tlr2	3.33	3.33	5.01	3.89	20.28	11.56	13.62	11.02	1.80	1.70	3.25	5.74	3.94	5.82
Blm	3.49	2.94	0.99	1.08	0.95	0.91	0.90	0.97	0.94	1.11	0.97	1.31	0.87	1.29
Mapfcl2	2.72	3.57	0.88	0.86	1.01	1.03	1.01	1.10	0.94	1.04	0.86	0.94	0.83	0.86
Bilnf	2.43	3.10	2.27	1.79	1.90	1.65	2.61	2.08	1.14	0.94	1.41	0.99	0.93	1.04
Birc3	2.59	2.83	2.32	1.72	7.41	3.62	5.45	4.16	1.28	1.10	2.34	2.77	2.22	3.24
Nfkbie	2.20	3.07	1.93	2.18	8.08	6.99	7.14	7.44	1.43	0.92	2.29	2.23	2.82	3.36
Atf4	2.17	3.05	1.87	2.04	1.38	1.57	1.58	1.77	1.35	0.91	1.34	1.06	1.22	1.17
Ccl7	3.01	1.70	15.80	7.49	10.04	6.83	8.92	6.41	1.71	1.69	2.91	1.98	2.95	2.43
Cx3cl1	2.61	1.99	3.11	3.45	10.71	8.50	9.78	8.96	1.23	1.40	1.15	3.09	2.52	3.87
Cxd9	1.74	2.70	0.84	1.01	1.01	1.36	1.39	1.68	1.54	1.32	2.07	1.73	2.13	2.16
Ccrl2	2.30	1.99	0.99	1.17	1.11	1.41	1.31	1.45	1.17	1.37	1.49	2.66	1.75	2.89
Il1f6	2.06	2.18	1.02	0.97	0.87	0.92	0.84	0.87	1.63	1.50	3.56	2.06	4.41	3.53
Irfl	1.76	2.08	14.51	13.10	7.13	5.88	3.48	3.53	4.39	3.70	3.90	4.69	2.75	2.86
Anxal	1.23	2.50	1.90	2.47	2.00	2.77	2.22	3.35	0.93	0.74	1.28	0.72	1.21	0.77
Dscrl	1.75	1.91	2.18	2.09	1.38	1.38	1.00	1.10	2.90	2.90	2.35	2.98	1.73	2.41
Ccl5	2.18	1.42	1.10	1.73	1.79	2.87	12.10	12.05	1.09	1.37	1.12	1.27	2.42	3.31
Ccll7	1.80	1.62	1.08	1.18	1.26	1.60	4.06	4.02	1.19	1.13	1.12	1.06	1.57	1.04
Cebpb	1.62	1.75	3.48	3.29	3.22	3.19	3.32	3.07	1.50	1.56	1.23	1.14	1.19	1.21
Nfil3	1.56	1.80	2.75	2.19	3.04	3.87	1.86	2.81	1.49	1.14	0.92	1.27	0.97	1.35
Csfl	1.52	1.74	2.80	3.34	6.05	5.42	2.73	2.37	1.53	1.47	2.53	3.42	2.17	3.03
E:h2	1.68	1.54	0.80	0.87	0.59	0.61	0.41	0.45	0.87	1.11	0.73	0.72	0.63	0.62
ll10rb	1.17	2.01	1.52	1.79	1.94	2.53	2.19	3.73	1.06	1.02	0.94	0.93	1.06	0.96
Socs3	1.56	1.56	0.93	0.78	3.03	3.22	2.22	2.39	1.33	1.25	0.77	1.19	1.10	1.18
ll23a	1.23	1.42	0.98	0.98	0.97	1.09	0.90	1.09	1.77	1.82	1.92	1.65	1.92	2.16
Tcf7	1.15	1.45	1.01	1.11	1.34	1.29	1.30	1.16	1.17	1.11	1.61	1.58	1.58	1.59
Serpinb2	1.07	1.47	0.90	0.94	1.07	1.25	1.49	1.63	1.65	1.13	2.15	1.68	1.87	1.94
Cdc37	1.12	1.05	1.17	1.16	1.84	1.69	1.68	2.23	1.00	1.22	0.91	1.16	1.30	1.20
Bcl2	0.68	0.86	1.52	1.74	2.15	2.17	1.73	1.94	1.02	1.10	1.05	1.38	0.91	1.18
Srf	0.69	0.67	1.82	2.11	1.83	2.21	1.19	1.30	1.09	1.19	0.87	1.17	0.79	0.90
Traf4	0.50	0.72	1.67	1.63	1.73	1.57	0.85	0.95	1.15	1.09	1.11	1.25	0.93	1.16
Nfkb1	0.54	0.67	0.85	0.82	2.53	1.88	2.86	3.08	1.06	0.84	1.73	2.99	2.58	4.31
Tgfb1	0.36	0.58	1.00	1.28	1.43	1.53	1.69	1.69	1.08	0.90	1.14	0.81	0.89	0.81
Hoxa5	0.32	0.25	0.98	1.16	0.84	1.08	0.91	0.76	0.98	1.35	0.56	0.62	0.66	0.43
Irf5	0.12	0.15	1.03	1.05	3.49	3.46	2.85	3.34	1.07	1.12	0.99	1.26	1.00	1.11

Listed are the folds of indicated genes at baseline or responses induced by TNF in WT and TTP MEFs.

from Sigma. Following secondary antibody incubations, signals were detected by enhanced chemiluminescence and developed using High Performance Chemiluminescence Film (Amersham) or Clear Blue X-ray Film (Thermo Scientific).

2.3. RNA isolation and microarray assay

Total RNA was extracted and purified using an RNeasy Mini Kit plus DNase I treatment (QIAGEN). The quality of total RNA samples was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies). For microarray analysis, 500 ng of RNA from each sample was used to generate complementary RNA (cRNA) using a TotalPrep RNA Amplification and Purification Kit (Illumina). PCR producent of 2 μg biotin-labeled cRNA was then used for microarray hybridization (Sentrix BeadChip Array For Gene Expression MouseRef-8, Illumina; 24,044 genes/probes per array). Biological duplicates were performed.

2.4. Microarray data analysis

Bead-Studio software (Illumina) was used for initial analysis of the scanned data. It yielded a set of data including single intensity value and a trimmed mean average for each gene of each probe type. The returned information includes the bead measurements

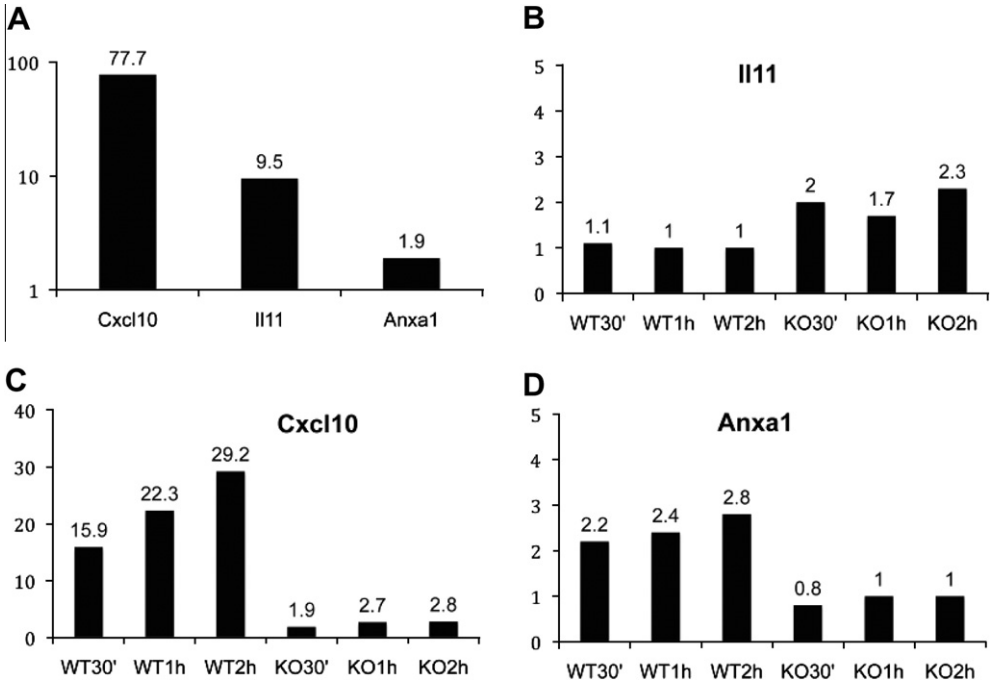

Fig. 3. Inflammatory genes were differentially regulated by TNFα between TTP KO and WT cells. (A) Under control condition, three genes related to inflammatory pathway, Cxcl10, Il11 and Anxa1 were shown as the fold changes of gene expression levels in TTP KO cells compared to the expression levels in WT cells. (B–D) TTP WT and KO cells were treated with TNFα (10 ng/mL) for 30′, 1 h and 2 h. The fold changes of gene expression levels of Il11, Cxcl10 and Anxa1, compared to the expression level in control condition, were shown for TTP WT and KO cells.

of all probes (or genes) and a detection call based on the comparison between the intensity of a single probe and the intensities of a large pool of negative controls (D = percent above negative/100, i.e., 1 = perfect, the intensity value of a gene is greater than all the intensities for every negative control tested). Any gene with its D value consistently below 0.98 was eliminated from further analysis.

Global normalization of original data was done by calculating the mean or median of the signal intensities of each individual experimental dataset and then the means of all intensities. Each individual data set was then mathematically adjusted such that the mean of that dataset equaled the calculated grand mean. In our study, under the conditions of not applying TNF-α, the averaged intensities for WT cells were termed as control. Under the conditions of applying TNF-α for various time periods (0.5, 1 or 2 h), the averaged intensities at time 0 were termed as controls. Z score transformation normalization was calculated according to the following formula: $\text{Zscore} = (\text{intensity}_{G_1} - \text{mean intensity}_{G_1 \ldots G_n})/\text{SD}_{G_1 \ldots G_n}$, where G is any gene on the microarray and $G_1 \ldots G_n$ represents the aggregate measure of all the genes [12]. Patterns of gene enrichment were analyzed by clustering (unsupervised, single linkage, hierarchical clustering with uncentered Pearson correlations, Eisen Laboratory software, Stanford University). Gene Set Matrix Analysis (GSMA) was conduted according to previous study [13].

3. Results

3.1. Characterization of TTP KO and WT cell lines and TTP induction by TNF-α stimulation

TTP WT and KO MEF cells were characterized by serum stimulation [9]. After 2 h of stimulation of 10% FBS, significant induction of TTP protein was detected by Western blot in TTP WT cells, whereas no TTP protein was detected in KO cells (Fig. 1A). To investigate the effect of TNF-α stimulation on TTP induction, TTP KO and WT MEF cells were treated with two doses of TNF-α (10 ng/mL or 30 ng/mL). In TTP WT cells, TTP protein could be detected by Western blotting after 30 min of TNF-α stimulation at lower concentration (10 ng/mL). The protein expression level saturated by 2 h of TNF-α stimulation (Fig. 1B, top panel, lane 2–4). Longer periods of TNF-α stimulation seemed to eventually downregulate TTP (Fig. 1B, WT, top panel, lane 5–7). There were no significant changes in TTP protein expression levels while higher concentration of TNF-α was applied (30 ng/mL). In TTP KO cells, no TTP could be detected at any time points after TNF-α stimulation (Fig. 1B, left panels).

3.2. Differential regulation of gene expression by TNF-α in TTP KO and WT cells

TTP-KO and TTP-WT cells were initially cultured in growth medium containing 10% charcoal-stripped FBS for 24 h to eliminate the effect of any steroids in regular FBS, followed by TNF-α stimulations for 30′, 1 h or 2 h. The genomic expressions in TTP KO and WT cells were analyzed and compared with biological duplicates. Through an unsupervised clustering of 9224 genes, distinctly expression differences were detected between the TTP KO and WT cells under the control condition without TNF-α stimulation (Fig. 2, lane 1–2, red color for high abundance and green color for low abundance). We identified a large number of genes that expressed at significantly higher levels (>10 folds) in WT cells than in KO cells (Fig. 2, lane 3–14, and zoomed view). Further characterization demonstrated that the majority of these genes were involved in immune and inflammatory pathways (Table 1). Among them, proinflammatory genes of Cxcl10 and Il11 were expressed at higher levels in TTP KO cells than in WT cells (Fig. 3A). Interestingly, gene of Anxa1, known to be anti-inflammatory with full-length translation of protein but pro-inflammatory after proteolytical cleavage [14], was also highly expressed in KO cells (Fig. 3A).

In response to TNF-α stimulation, genes were differentially regulated in TTP KO and WT cells. Gene of Il11 was upregulated in KO cells but little changed in WT cells by TNF-α stimulation. On the

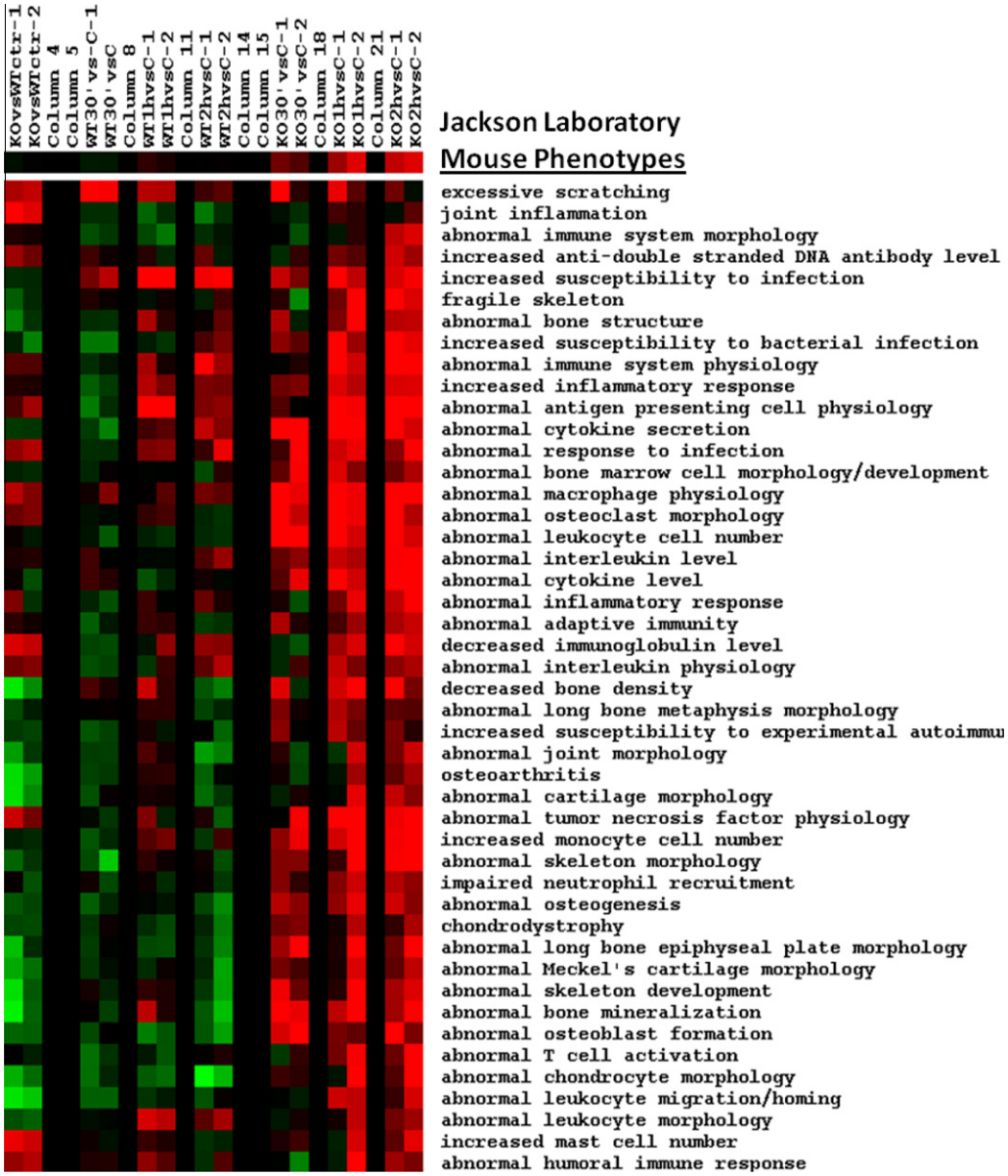

Fig. 4. 2-D GSMA analysis. Z differences under control condition (KO vs. WT) and under the conditions of TNF-α stimulation for 30 min, 1 h and 2 h (treated vs. untreated, both WT and KO) were mapped with Jackson Lab Mouse Phenotype Database. Results were shown as connections between predicted mouse disease phenotypes and the duplicates of microarray data.

other hand, Anxa1 was oppositely regulated by TNF-α, upregulated in WT cells but little changed in KO cells (Fig. 3B, D). Gene of Cxcl10 was upregulated by TNF-α in both WT and KO cells but the upregulation was more significant in WT cells (Fig. 3C).

3.3. 2-D GSMA analysis of mapping mouse disease phenotypes

Finally, we integrated our microarray datasets with the Jackson Laboratory Mouse Phenotype Database by 2-dimentional GSMA analysis. The results showed that gene expression patterns in TTP KO cells, after TNF-α stimulation, were strongly correlated to the phenotypes of inflammation or joint/bone disease (Fig. 4).

4. Discussion

In an attempt to characterize the genetic interactions between TTP and TNF-α in the development of rheumatoid arthritis, we chose to use a microarray method to analyze the gene expression patterns in the model of MEF cell lines, including both TTP WT and TTP KO cells, in response to the stimulation of TNF-α. TTP participates in a negative feedback loop to limit the magnitude or duration of a burst expression of cytokines or chemokines, including TNF-α [15]. In the present study, we first tested the dynamics of TTP protein expressions by stimulating both TTP WT and KO cells with TNF-α. IN KO cells, no TTP protein expression was detected. In WT cells, TTP protein was significantly induced by 30 min of TNF-α stimulation, saturated by 2 h stimulation. However, longer time of stimulation diminished the expression of TTP. The effect of transient TTP induction by TNF-α is not unexpected as TNF-α was a negatively regulated downstream targets of TTP [9].

The results of our microarray essay revealed for the first time, there was a significant difference in the gene expression levels between TTP KO and WT cells. We found that genes involved in immune and inflammatory pathways were at markedly higher

expression levels in TTP KO cells compared to the levels in WT cells, such as genes of cytokines Il6, Il11, Il1f6, Il23a and chemokines Ccl2, Ccl5, Ccl7, Ccl17, Cxcl5, Cxcl9, Cxcl10, Cxcl12. It is known that TTP is a key mediator in promoting mRNA decay in many proinflammatory genes [16,17]. The fact that higher expression levels of those genes were detected in TTP KO cells is in line with previous studies showing that pathologic and inflammatory symptoms were observed in TTP deficient mice:

Further comparison of genomic expression differences between TTP KO and WT cells in response to TNF-α stimulation confirmed that TTP played an important role in TNF-α mediated gene expression. We observed that a large group of the genes involved in autoimmune or pro-inflammatory pathways were up-regulated in TTP WT cells, but little changed in TTP KO cells, in response to TNF-α stimulation. This is consistent with the pathological reactions to TNF-α during inflammatory response in other animal models. It may also be explained by that genes with high abundance in TTP KO cells were less responsive to TNF-α treatment than genes with low abundance in TTP WT cells. Nevertheless, we observed a large number of genes were differentially regulated by TNF-α in TTP KO and TTP WT cells.

In the light of microarray data, we sought to compare the TNF-α regulated gene expression patterns in cell lines with the phenotypes of various mouse diseases to further understand the regulatory role of TTP in inflammatory pathways. GSMA is a simple and straightforward method for such task in which genes are tested by groups across multiple datasets for patterns of expression resemblance [13]. Our GSMA analysis, comparing microarray datasets with 452 pathways, showed that the gene regulatory patterns in TTP KO cells were strongly correlated to the phenotypes of cell cycle and joint/bone mouse diseases. Though there is still a long way to go to fully understand the molecular mechanisms of TTP and its roles in regulating inflammatory responses, our study here provided a detailed genetic roadmap for further elucidating the molecular pathways and selecting pharmacological candidates in rheumatoid arthritis and other inflammatory diseases.

References

[1] G.A. Taylor, E. Carballo, D.M. Lee, W.S. Lai, M.J. Thompson, D.D. Patel, D.I. Schenkman, G.S. Gilkeson, H.E. Broxmeyer, B.F. Haynes, P.J. Blackshear, A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency, Immunity 4 (1996) 445–454.
[2] J.C. Shanahan, W. St Clair, Tumor necrosis factor-alpha blockade: a novel therapy for rheumatic disease, Clin. Immunol. 103 (2002) 231–242.
[3] E. Carballo, W.S. Lai, P.J. Blackshear, Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin, Science 281 (1998) 1001–1005.
[4] E. Douni, K. Akassoglou, L. Alexopoulou, S. Georgopoulos, S. Haralambous, S. Hill, G. Kassiotis, D. Kontoyiannis, M. Pasparakis, D. Plows, L. Probert, G. Kollias, Transgenic and knockout analyses of the role of TNF in immune regulation and disease pathogenesis, J. Inflamm. 47 (1995) 27–38.
[5] J. Keffer, L. Probert, H. Cazlaris, S. Georgopoulos, E. Kaslaris, D. Kioussis, G. Kollias, Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis, EMBO J. 10 (1991) 4025–4031.
[6] W.S. Lai, E. Carballo, J.R. Strum, E.A. Kennington, R.S. Phillips, P.J. Blackshear, Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA, Mol. Cell Biol. 19 (1999) 4311–4323.
[7] K. Phillips, N. Kedersha, L. Shen, P.J. Blackshear, P. Anderson, Arthritis suppressor genes TIA-1 and TTP dampen the expression of tumor necrosis factor alpha, cyclooxygenase 2, and inflammatory arthritis, Proc. Natl. Acad. Sci. USA 101 (2004) 2011–2016.
[8] K.M. Deleault, S.J. Skinner, S.A. Brooks, Tristetraprolin regulates TNF TNF-alpha mRNA stability via a proteasome dependent mechanism involving the combined action of the ERK and p38 pathways, Mol. Immunol. 45 (2008) 13–24.
[9] W.S. Lai, J.S. Parker, S.F. Grissom, D.J. Stumpo, P.J. Blackshear, Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts, Mol. Cell Biol. 26 (2006) 9196–9208.
[10] S. Ghosh, M.J. Hoenerhoff, N. Clayton, P. Myers, D.J. Stumpo, R.R. Maronpot, P.J. Blackshear, Left-sided cardiac valvulitis in tristetraprolin-deficient mice: the role of tumor necrosis factor alpha, Am. J. Pathol. 176 (2010) 1484–1493.
[11] T.J. Horner, W.S. Lai, D.J. Stumpo, P.J. Blackshear, Stimulation of polo-like kinase 3 mRNA decay by tristetraprolin, Mol. Cell Biol. 29 (2009) 1999–2010.
[12] C. Cheadle, M.P. Vawter, W.J. Freed, K.G. Becker, Analysis of microarray data using Z score transformation, J. Mol. Diagn. 5 (2003) 73–81.
[13] C. Cheadle, T. Watkins, J. Fan, M.A. Williams, S. Georas, J. Hall, A. Rosen, K.C. Barnes, GSMA: gene set matrix analysis an automated method for rapid hypothesis testing of gene expression data, Bioinform. Biol. Insights 1 (2007) 49–62.
[14] S.L. Williams, I.R. Milne, C.J. Bagley, J.R. Gamble, M.A. Vadas, S.M. Pitson, Y. Khew-Goodall, A proinflammatory role for proteolytically cleaved annexin A1 in neutrophil transendothelial migration, J. Immunol. 185 (2010) 3057–3063.
[15] A. Clark, Post-transcriptional regulation of pro-inflammatory gene expression, Arthritis Res. 2 (2000) 172–174.
[16] S. Datta, R. Biswas, M. Novotny, P.G. Pavicic Jr., T. Herjan, P. Mandal, T.A. Hamilton, Tristetraprolin regulates Cxcl1 (KC) mRNA stability, J. Immunol. 180 (2008) 2545–2552.
[17] F.T. Ishmael, X. Fang, M.R. Galdiero, U. Atasoy, W.F. Rigby, M. Gorospe, C. Cheadle, C. Stellato, Role of the RNA-binding protein tristetraprolin in glucocorticoid-mediated gene regulation, J. Immunol. 180 (2008) 8342–8353.