Scientific article

RT² Profiler PCR Array application examples

Pathway-focused gene expression profiling in toxicology, oncology, and immunology research

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Abstract: The RT² Profiler PCR Array System is the most reliable and accurate tool for analyzing the expression of a focused panel of genes using SYBR® Green real-time PCR. The RT² Profiler PCR Array System comprise a 96- or 384-well plate containing qPCR primer assays (84 pathway- or disease-focused genes, 5 housekeeping genes, and 3 replicate controls), instrument-specific SYBR Green master mix, and a first strand cDNA synthesis kit. RT² Profiler PCR Arrays can be used for research on signal transduction, cancer, immunology, stem cells, toxicology, biomarker discovery and verification, and analysis of phenotypes.

In this paper, we report on three application-specific studies in the fields of toxicology, cancer, and immunology. In the first study, RT² Profiler PCR Arrays were used to profile gene expression changes due to compound-induced cytotoxicity in liver cells. We identified idiosyncratic patterns of expression changes with three hepatotoxicity drugs, suggesting different mechanisms of action for liver toxicity. In the second study, the expression of cancer-related extracellular matrix and cellular adhesion genes were compared between breast tumors and normal tissue. We discovered a common set of genes with significant gene expression changes associated with two independent breast tumor samples. In the third study, cytokine gene expression between stimulated and unstimulated cells was shown to correlate well with protein level changes.

Introduction

The RT² Profiler PCR Array System is the most reliable and accurate tool for analyzing the expression of a focused panel of genes using SYBR Green real-time PCR. It provides the highly sensitive, specific, and reproducible performance achievable only by real-time PCR. The wide linear dynamic range of the RT² Profiler PCR Array System enables simultaneous and sensitive detection of mRNAs with both high and low abundance. RT² Profiler PCR Arrays offer a complete solution to examine gene expression profiles for any pathway of interest.

Contents

Introduction 1

Materials and methods:

- Example 1: Toxicology 2
- Example 2: Oncology 2
- Example 3: Immunology 3

Results:

- Example 1: Toxicology 4
- Example 2: Oncology 5
- Example 3: Immunology 7

Summary 9

References 9

This system is suitable for many research applications including drug toxicology studies, tumor metastasis and cancer biomarker research, as well as cytokine profiling and inflammatory response studies. The RT² Profiler PCR Array system allows any researcher with access to a real-time PCR instrument to easily examine the changes in gene expression between test and control samples and to quickly identify genes with significant up- or downregulation in response to experimental conditions.

We previously described how the RT² Profiler PCR Array System utilizes optimized SYBR Green master mixes, experimentally verified gene-specific primer assays and a sensitive cDNA synthesis kit to provide a high level of specificity, reproducibility, and precision in gene expression profiling (see www.SABiosciences.com/manuals/pcrarraywhitepaper.pdf).

In this paper, we describe a range of experiments utilizing the RT² Profiler PCR Arrays in toxicology, oncology and immunology research. In each case, the RT² Profiler PCR Array System provides a convenient, reliable, and accurate way to characterize changes in gene expression between experimental groups. Furthermore, the results reported in this paper indicate the tremendous potential of a transcription-based approach via the RT² Profiler PCR Array System for research in toxicology, oncology, immunology, and other biomedical fields.

Materials and Methods:

Toxicology application

The recommended cell propagation and subculture protocols were followed for the hepatocellular carcinoma line HepG2 (ATCC, HB-8065). Troglitazone ("Tro" or T), rosiglitazone ("Rosi" or R) and pioglitazone ("Pio" or P) were purchased from Cayman Chemical. Acetaminophen (APAP) and tetracycline hydrochloride (TC) were purchased from Sigma. At 80% cell confluence, drugs (100 µM) were added with fresh media. DMSO was the vehicle control for the thiazolidinedione (TZD) drugs, while ethanol was the control for the other two drugs. After 24 hours, RNA was prepared using the ArrayGrade™ Total RNA Isolation Kit (SABiosciences) and TURBO DNase™ gDNA cleanup (Ambion). For each RT² Profiler PCR Array, 4 µg total RNA were used to prepare cDNA with the appropriate first strand kit from SABiosciences. The cDNA was characterized on the iCycler® iQ Real-Time PCR System (Bio-Rad Laboratories) using two different RT² Profiler PCR Arrays (SABiosciences): the Human Drug Metabolism PCR Array and the Human Stress and Toxicity PathwayFinder™ PCR Array. The resulting raw data were then analyzed using the PCR Array Data Analysis Template.

Oncology application

Template cDNAs prepared from normal human breast and human breast tumor #1 total RNA (BioChain Institute, Inc., 5.0 µg) were characterized in technical triplicates using the Human Cancer PathwayFinder PCR Array and the RT² SYBR Green/ Fluorescein qPCR Master Mix on the iCycler PCR System.

Triplicate total RNA samples prepared from normal human breast and human breast tumor #2 total RNA (BioChain Institute, Inc., 1.0 µg) were converted into template cDNA and then characterized using the Human Extracellular Matrix and Adhesion Molecules RT² Profiler PCR Array and the RT² SYBR Green/Fluorescein qPCR Master Mix on the iCycler PCR System.

Immunology application

Peripheral blood mononuclear cells (PBMC) were treated with or without 50 ng/ml PMA + 1 µg/ml ionomycin for 6 or 24 hours. After each incubation period, total RNA was isolated from each preparation, and first strand cDNAs were prepared from 500 ng total RNA of each sample using the RT² First Strand Kit (SABiosciences). Template cDNAs were characterized in technical triplicates using the Human Common Cytokines RT² Profiler PCR Array with the RT² SYBR Green/ROX qPCR Master Mix on the 7500 FAST® Real-Time PCR System (Applied Biosystems). Fold changes in gene expression between the stimulated and resting PBMC RNA were calculated using the $\Delta\Delta C_T$ method in the PCR Array Data Analysis template.

To verify the results obtained from the RT² Profiler PCR Array, the protein levels of eight selected cytokines secreted by the PBMC (IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IFN-γ, and TNF-α) were measured. Cell supernatants were collected at different time points (0, 6, 24, and 48 hours) and the cytokines were measured by enzyme-linked immunosorbent assay (ELISA) using the Human Th1/Th2 Cytokines Multi-Analyte Profiler ELISArray Kit (SABiosciences). Optical density (OD) readings for each protein analyte from the samples were compared to a standard curve for quantification of the amount of protein in the original samples.

Table 1. Examples of RT² Profiler PCR Arrays for toxicology, oncology, and immunology applications

		Catalog number		
Research area	**PCR Arrays**	**Human**	**Mouse**	**Rat**
Toxicology and drug metabolism	Cancer Drug Resistance	PAHS-004	PAMM-004	PARN-004
	Drug Metabolism	PAHS-002	PAMM-002	PARN-002
	Drug Metabolism: Phase I Enzymes	PAHS-068	PAMM-068	PARN-068
	Drug Transporters	PAHS-070	PAMM-070	PARN-070
	Oxidative Stress	PAHS-065	PAMM-065	PARN-065
	Stress & Toxicity PathwayFinder	PAHS-003	PAMM-003	PARN-003
Oncology	Angiogenesis	PAHS-024	PAMM-024	PARN-024
	Angiogenic Growth Factors	PAHS-072	PAMM-072	PARN-072
	Apoptosis	PAHS-012	PAMM-012	PARN-012
	Estrogen Receptor Signaling	PAHS-005	PAMM-005	PARN-005
	Cancer PathwayFinder	PAHS-033	PAMM-033	PARN-033
	DNA Damage Signaling Pathway	PAHS-029	PAMM-029	PARN-029
	Growth Factors	PAHS-041	PAMM-041	PARN-041
	p53 Signaling Pathway	PAHS-027	PAMM-027	PARN-027
	Tumor Metastasis	PAHS-028	PAMM-028	PARN-028
Immunology	Chemokines & Receptors	PAHS-022	PAMM-022	PARN-022
	Common Cytokines	PAHS-021	PAMM-021	PARN-021
	Inflammatory Cytokines & Receptors	PAHS-011	PAMM-011	PARN-011
	Interferons (IFN) & Receptors	PAHS-064	PAMM-064	PARN-064
	NFκB Signaling Pathway	PAHS-025	PAMM-025	PARN-025
	Th1-Th2-Th3	PAHS-034	PAMM-034	PARN-034
	Th17 for Autoimmunity & Inflammation	PAHS-073	PAMM-073	PARN-073
	Toll-Like Receptor Signaling Pathway	PAHS-018	PAMM-018	PARN-018
	TNF Signaling Pathway	PAHS-063	PAMM-063	PARN-063

For a complete list of PCR Arrays, see www.SABiosciences.com/ArrayList.php

Toxicology application results:

Discovering unique gene expression profiles associated with idiosyncratic liver toxicity

Using the Human Drug Metabolism RT² Profiler PCR Array System and the Human Stress & Toxicity PathwayFinder RT² Profiler PCR Arrays, we examined the toxicity of three diabetes drugs on cultured liver (HepG2) cells. Troglitazone (Tro), a glitazone or thiazolidinedione (TZD) PPARγ agonist, was approved for the treatment of type 2 diabetes mellitus, but was withdrawn from the market due to idiosyncratic liver toxicity. The actual toxicity mechanism has not been completely elucidated1–2. Rosiglitazone (Rosi) and pioglitazone (Pio), two similar drugs, are considered to be safe diabetes treatments. We hypothesized that the in vitro gene expression profile of key drug metabolism and toxicity genes should be different in cultured liver cells treated with the withdrawn drug (Tro) versus those treated with drugs still on the market (Rosi and Pio).

Using a gene expression fold-change threshold of 2.9 or greater and a p-value threshold of 0.0015 or less, six (6) out of 84 genes represented by the Human Drug Metabolism RT² Profiler PCR Array System were identified as induced in HepG2 cells by treatment with Pio (Table 2). Using the same criteria, the same 6 genes plus 1 extra (MPO) were identified as induced to a similar extent in HepG2 cells upon treatment with Rosi. No genes were found to be significantly downregulated with either of these treatments.

When the same criteria were used to analyze RNA extracted from Tro-treated cells, three downregulated genes were identified (Table 2). Among the four upregulated genes common to all three drug treatments, the degree of induction for two genes (MT2A and CYP1A1, Table 2) was much more dramatic in Tro-treated cells than the other two drug-treated cells. Treatment of the HepG2 cells with Tro did indeed induce a different drug metabolism gene expression profile compared with Rosi or Pio treatment, whereas the latter two drugs induced very similar profiles to one another.

Table 2. Tro induces a different drug metabolism expression profile from Pio or Rosi *

	Pioglitazone (Pio)		Rosiglitazone (Rosi)		Troglitazone (Tro)	
Gene symbol	Fold change	p-value	Fold change	p-value	Fold change	p-value
CYP17A1					–4.4	3.9×10^{-3}
CYP1A1	17.8	2.8×10^{-6}	21.4	4.3×10^{-6}	42.7	1.2×10^{-6}
CYP2B6	5.4	7.7×10^{-5}	3.5	1.5×10^{-3}	4.2	4.5×10^{-4}
GPX2					–9.6	2.8×10^{-3}
GSTP1					7.1	2.8×10^{-3}
CYP3A5	3.0	2.6×10^{-4}	3.3	4.3×10^{-4}		
GCKR	3.1	2.2×10^{-6}	2.9	3.0×10^{-5}		
MPO			3.0	1.7×10^{-3}		
MT2A	18.6	1.2×10^{-5}	8.1	1.4×10^{-3}	297.5	5.5×10^{-7}
NAT2					–4.9	5.3×10^{-4}
NOS3	6.1	9.7×10^{-7}	5.3	3.4×10^{-6}	4.2	6.7×10^{-5}

* The Human Drug Metabolism PCR Array was used to determine relative changes in gene expression between HepG2 cells treated with either Pio, Rosi, Tro, or a DMSO vehicle control. Genes with statistically significant changes in expression upon drug treatment relative to the control are listed.

The results from the Stress & Toxicity PathwayFinder RT² Profiler PCR Array System (Figure 1) indicate that 14 out of 84 stress-responsive genes increase their expression in HepG2 cells upon treatment with Pio, Rosi or Tro. For all 14 genes, treatment with Tro induced a much greater increase in expression than treatment with Pio or Rosi. In contrast, the expression of two housekeeping gene transcripts, 18S rRNA and beta actin (ACTB), did not change upon treatment with any of these drugs (a fold-difference of 1.0). The withdrawn drug (Tro) induced a very different stress- and toxicity-related gene expression profile in HepG2 cells as compared to the drugs remaining on the market (Pio or Rosi).

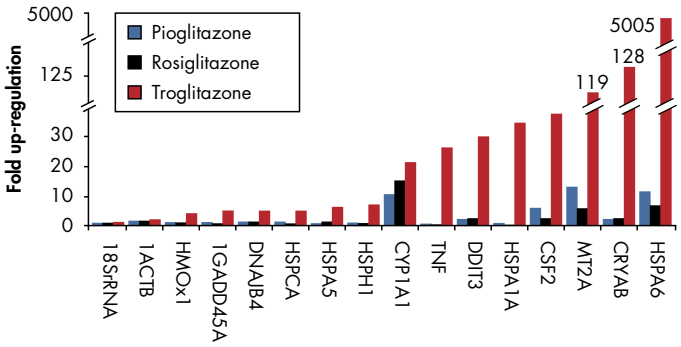

Figure 1. Treatment with Tro stresses HepG2 cells to a greater extent than treatment with Pio or Rosi. The fold upregulation in expression upon treatment with pioglitazone (Pio, blue bars), rosiglitazone (Rosi, black bars), or troglitazone (Tro, red bars) relative to the DMSO vehicle control are plotted for 16 genes (including two housekeeping genes) measured by the Stress and Toxicity PathwayFinder RT² Profiler PCR Array System.

To further demonstrate the application of RT² Profiler PCR Arrays for toxicological screening, we tested two other well-characterized drugs with known mechanisms of liver toxicity. Acetaminophen (APAP) is known to cause hepatic necrosis, while tetracycline (TC) induces steatosis by triglyceride accumulation. As predicted, the gene expression profiles from our RT² Profiler PCR Arrays differed substantially in cells treated with either APAP or TC. Tro treatment induced a third distinct profile in a representative set of four genes (Figure 2), suggesting that it causes liver toxicity by a different mechanism from the well-characterized examples of APAP or TC.

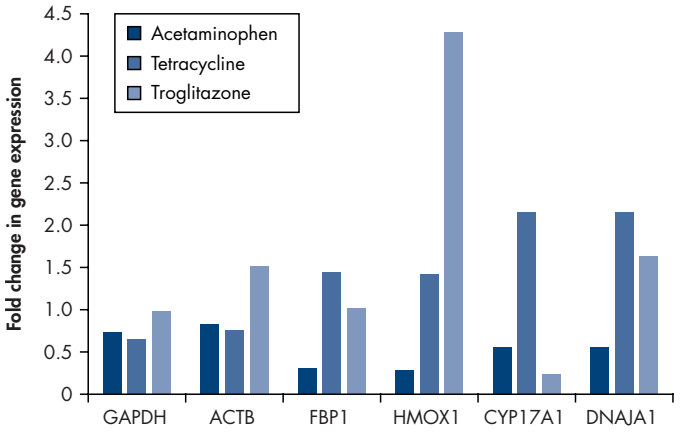

Figure 2. Expression profiling suggests different mechanisms of drug-mediated toxicity. Fold changes in gene expression for four representative genes in HepG2 cells caused by treatment with tetracycline, acetaminophen, or troglitazone are displayed. Consistent expression of two housekeeping genes (GAPD and ACTB) is also presented. Each of the three drugs demonstrates a different gene expression profile, suggesting different causes for their toxicity.

Gene expression profiling is a convenient and reliable way to characterize drugs that have a common target, but have differing toxic side-effects. For example, the RT² Profiler PCR Array System results correctly predicted the differences in cellular stress and toxicity induced by the thiazolidinediones (TZDs) used in this study. Furthermore, the distinct set of genes that differentiate the cellular response to Tro from responses to Rosi or Pio (as well as APAP and TC) may hold the key to explaining the molecular mechanisms of the observed idiosyncratic liver toxicity.

The composition of relevant genes organized in pathway-focused format can aid molecular mechanistic studies (3–4) of the toxicity of new and existing drugs through gene expression analysis. The pathway-profiling capabilities of the RT² Profiler PCR Array System format provide toxicity researchers a powerful systems biology tool to discover new toxicity biomarkers.

Oncology application results:

Identifying and monitoring oncogenic pathways

Gene expression profiling is important for discovering and verifying tumor biomarkers and therapeutic targets. Using the Cancer PathwayFinder RT² Profiler PCR Array System and the Human Extracellular Matrix and Adhesion Molecules RT² Profiler PCR Array System, we examined the gene expression profiles exhibited by two different human breast tumors relative to normal tissues. The study compared the relative expression of both tumorigenesis- and adhesion-related genes between each tumor sample and a normal breast tissue sample. This study provides an example of the identification of a pathway affected by the transformation of a particular tumor type.

Total RNA samples from normal breast tissue and the first of two unmatched breast tumor were analyzed using the Cancer PathwayFinder RT² Profiler PCR Array System. This array includes representative genes from the following biological pathways involved in tumorigenesis: adhesion, angiogenesis, apoptosis, cell cycle control, cell senescence, DNA damage repair, invasion, metastasis, signal transduction molecules, and transcription factors.

Figure 3 displays a scatter plot report of the results from the Cancer PathwayFinder RT² Profiler PCR Array experiment, indicating the positions of several noteworthy genes based on large fold-differences in expression between the normal breast and the breast tumor samples. Of the 84 cancer pathway-focused genes in this array, 24 genes demonstrated at least a 3-fold difference in gene expression between normal breast tissue and the breast tumor. Upregulation was observed in 17 genes, while 7 genes appeared to be downregulated in the tumor samples, for a total of 24 differentially regulated genes (Table 3).

A subset of six of the 24 genes (ITGA2, ITGA4, ITGB3, MCAM, MMP9, and TIMP3) represents adhesion and extracellular matrix molecules. ITGB3 was downregulated, while the other five genes were upregulated. The results suggest that changes in the expression of genes involved in cellular interactions played an important role in the transformation of this and perhaps other breast tumors. To further test this hypothesis and to analyze the expression of other adhesion-related genes, a second breast tumor sample was characterized using a cellular adhesion-focused RT² Profiler PCR Array.

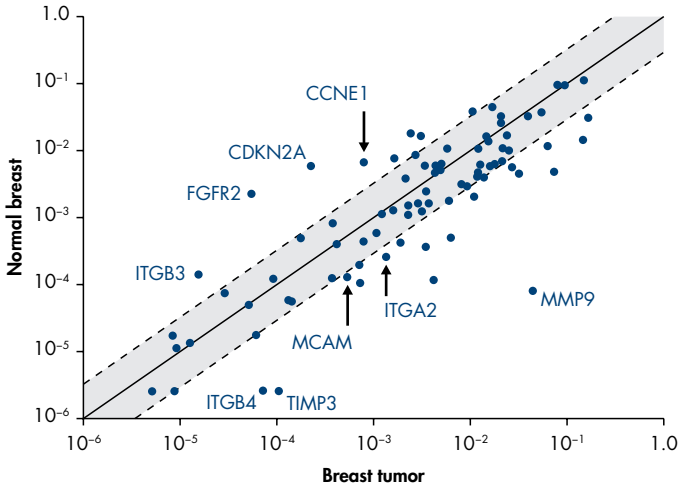

Figure 3. Relative expression comparison for 84 cancer-related genes between normal human breast and human breast tumor #1. The figure depicts a log transformation plot of the relative expression level of each gene ($2^{-\Delta C_T}$) between breast tumor (y-axis) and normal breast (x-axis). The dotted lines indicate a four-fold change in gene expression threshold.

Table 3. Changes in expression for cancer-related genes between normal human breast and human breast tumor #1 *

Gene	Fold change Tumor/Normal	t-Test p value	Average raw C_T	
			Tumor	Normal
MMP9	542.45	0.0000	21.8	30.0
TIMP3	39.85	0.0000	30.5	35.0
TNF	35.51	0.0000	25.2	29.5
ITGA4	27.54	0.0001	31.1	35.0
TGFB1	15.10	0.0000	21.1	24.1
BCL2	12.27	0.0012	24.6	27.4
FOS	9.74	0.0003	20.1	22.5
GZMA	9.30	0.0003	25.5	27.9
TEK	6.88	0.0003	27.7	29.7
JUN	6.88	0.0008	22.3	24.2
APAF1	5.34	0.0018	23.8	25.4
ATM	5.34	0.0001	19.9	21.5
ITGA2	5.34	0.0042	26.8	28.4
PIK3R1	5.34	0.0001	21.3	22.9
SYK	4.65	0.0003	22.5	23.9
PLAUR	4.44	0.0007	26.4	27.7
MCAM	4.14	0.0000	28.2	29.4
PLAU	3.61	0.0132	27.8	28.8
ETS2	3.44	0.0015	23.5	24.4
ANGPT1	3.36	0.0028	31.3	32.2
FAS	3.36	0.0031	24.7	25.6
TERT	3.29	0.0314	34.1	35.0
NFKB1	3.07	0.0068	22.9	23.6
NME4	3.07	0.0019	24.1	24.9
ERBB2	-3.29	0.0000	25.9	23.3
ITGA3	-3.78	0.0000	23.9	21.1
UCC1	-4.65	0.0003	26.6	23.5
MYC	-5.34	0.0004	25.7	22.4
SNCG	-7.73	0.0000	26.0	22.2
CCNE1	-8.48	0.0000	27.6	23.7
ITGB3	-9.08	0.0026	33.3	29.3
CDKN2A	-26.91	0.0000	29.4	23.8
FGFR2	-41.74	0.0007	31.5	25.2

* Genes from the experiment in Figure 3 that exhibit a three-fold or greater change in expression between normal and tumor breast tissue are listed.

Total RNA samples from normal breast tissue and the second of the two unmatched breast tumors were characterized on the Extracellular Matrix and Adhesion Molecules RT² Profiler PCR Array System. Genes that displayed at least a 3-fold difference in expression between the samples are listed in Table 4. On this array, a larger number of genes exhibited differential expression in the second tumor than was observed for the first tumor on the Cancer PathwayFinder RT² Profiler PCR Array. A total of 38 genes had a different level of expression in the breast tumor than in the normal breast tissue, with 27 genes showing upregulation and 11 genes showing downregulation. The first and second breast tumor sample displayed concordant results for four genes (MMP9, TIMP3, ITGA4, and ITGB3) that changed expression in the same direction on the Cancer PathwayFinder RT² Profiler PCR Array and the Extracellular Matrix and Adhesion Molecules RT² Profiler PCR Array. These results not only further verify that cellular adhesion genes changed their expression in these two particular breast cancer tumors, but also suggest a more general role for these genes in breast tissue transformation.

Table 4. Changes in relative expression for genes encoding ECM and adhesion molecules between normal human breast and human breast tumor #2 *

Gene	Fold change Tumor/Normal	t-Test p value	Average raw C_T	
			Tumor	Normal
CTNND2	229.39	0.0000	23.8	31.6
TIMP3	104.57	0.0000	28.4	35.0
SELE	43.46	0.0000	26.3	31.7
MMP1	36.97	0.0000	27.9	33.0
MMP3	34.50	0.0000	29.9	35.0
KAL1	31.45	0.0000	23.1	28.0
MMP13	21.73	0.0000	26.9	31.2
MMP10	16.47	0.0000	31.0	35.0
MMP16	16.09	0.0000	25.3	29.2
FN1	11.92	0.0512	29.9	33.4
CD44	11.92	0.0046	23.5	27.0
TNC	10.87	0.0000	22.9	26.2
MMP9	10.62	0.0001	27.1	30.4
SELP	9.46	0.0001	26.1	29.2
MMP11	7.51	0.0000	25.0	27.9
COL7A1	7.00	0.0057	30.9	33.7
CSPG2	6.39	0.0000	24.0	26.6
COL4A2	5.56	0.0009	23.9	26.3
TNA	5.43	0.0001	26.9	29.3
COL11A1	5.31	0.0017	30.7	33.0
THBS1	4.84	0.0185	24.1	26.3
SELL	4.21	0.0002	24.7	26.7
HAS1	3.93	0.0010	27.5	29.4
CTNND1	3.84	0.0007	30.4	32.2
ITGA4	3.34	0.0000	25.4	27.1
ITGA7	3.34	0.0003	27.6	29.3
THBS2	3.19	0.0058	26.1	27.7
SPP1	-3.08	0.0000	23.6	21.9
ITGB5	-3.31	0.0000	23.2	21.4
CTNNB1	-3.31	0.0003	21.2	19.4
ITGAV	-4.57	0.0072	26.5	24.2
CNTN1	-5.25	0.0001	28.8	26.3
MMP7	-5.37	0.0000	25.7	23.2
ITGB3	-7.25	0.0094	32.1	29.2
ADAMTS1	-9.35	0.0003	25.5	22.2
LAMA3	-10.26	0.0000	24.7	21.2
NCAM1	-23.02	0.0000	30.9	26.3
ITGB4	-30.38	0.0000	26.6	21.6

* The table lists genes that exhibit at least a three-fold difference in expression in the breast tumor sample when compared to the normal breast tissue. The raw threshold cycle (C^T) values seen in the two samples are also listed for comparison.

These types of studies provide a new and convenient way to investigate the mechanisms underlying oncogenesis of specific tumors on a pathway-focused basis. The data shown here is consistent with known principles, that changes in the expression of genes related to cellular adhesion play a role in the transformation of breast tissue (5–6). Alterations in the expression of these genes enhance or inhibit metastasis of the tumor from its original location and may aid tumor invasion into a new tissue or organ. A RT² Profiler PCR Array focusing on human tumor metastasis is available and could be used to continue this study.

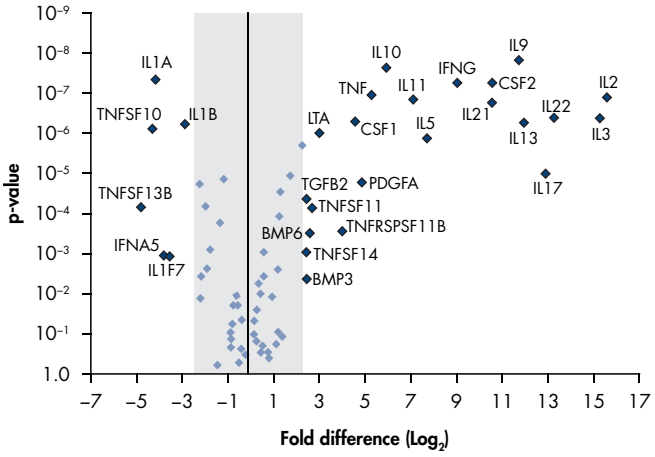

Figure 4. RNA isolated from resting PBMC or PBMC stimulated with PMA ionomycin for 6 or 24 hours were characterized on the Human Common Cytokines RT² Profiler PCR Array. Log_2 fold changes in gene expression between PBMC stimulated with PMA ionomycin and resting PBMC are plotted against t-test p-values to produce a "volcano plot". The higher the position, the more significant the gene's fold-change. Genes plotted farther from the central axis have larger changes in gene expression. Thresholds for fold change (vertical lines, 5-fold) and significant difference (horizontal line, p<0.005) were used in this display.

Immunology application results:

Monitoring cytokine expression levels

Cytokine quantification is an important element in studies of inflammation and immune responses. Quantitative RT-PCR, a rapid and sensitive assay, is the preferred method to quantify cytokine mRNA levels because they are often expressed at low levels. The RT² Profiler PCR Array System offers a simple, reliable and sensitive tool for multiple cytokine profiling. Using the Human Common Cytokines RT² Profiler PCR Array, we have monitored the mRNA levels of 84 different cytokines in stimulated versus and untreated human peripheral blood mononuclear cells (PBMC).

The gene expression results identify 23 upregulated and 6 downregulated genes (with >5 fold-change and p<0.005) upon 6 hours of stimulation. At 24 hours, the effects of PMA-ionomycin on genes such as BMP's, CSF's, IFNγ, IL1β, IL6, IL11, TGFβ and TNF are continuously observed, while the effect on other genes such as interleukins 2, 3, 5, 9, 10, 13, 17 and 22 diminishes 24 hours after stimulation (Figure 4 and Table 5). To verify these results, the protein levels of 8 selected cytokines secreted by the PBMC were measured using a multiplex ELISA array (Figure 5). The effects of these mRNA expression changes were observed in the changes in cytokine production induced by PMA ionomycin at 6 hours after stimulation. The induction in cytokine production by PMA-ionomycin was sustained up to 48 hours after stimulation, despite subdued mRNA expression for some cytokines at 24 hours after stimulation.

Table 5. List of cytokines induced or downregulated in phorbol myristate acetate ionomycin-stimulated peripheral blood mononuclear cells (PBMC) versus resting PBMC *

	6 hours after stimulation				24 hours after stimulation			
	Average raw C_T value		Stimulated/Resting		Average raw C_T value		Stimulated/Resting	
Gene	Stimulated	Resting	Fold change	t-test p-value	Stimulated	Resting	Fold change	t-test p-value
IL2	14.64	29.99	47820.23	0.0000	13.54	26.91	11190.60	0.0000
IL3	19.53	34.56	38218.94	0.0000	18.46	30.35	4020.99	0.0000
IL22	21.08	34.14	9823.35	0.0000	24.26	30.62	87.02	0.0000
IL17	21.51	34.21	7601.14	0.0000	20.63	32.26	3365.64	0.0000
IL13	21.05	32.80	3961.96	0.0000	23.65	30.74	144.67	0.0000
IL9	23.49	35.00	3339.31	0.0000	22.22	31.15	516.75	0.0000
IL21	19.76	30.13	1522.26	0.0000	20.00	30.09	1152.06	0.0000
CSF2	16.80	27.15	1494.38	0.0000	15.53	26.86	2714.87	0.0000
IFNG	13.57	22.41	525.91	0.0000	13.94	24.19	1287.18	0.0000
IL5	21.89	29.40	208.71	0.0000	25.77	29.35	12.70	0.0000
IL11	24.22	31.12	136.74	0.0000	25.35	34.35	542.45	0.0000
IL10	21.43	27.21	62.77	0.0000	26.37	24.33	-3.87	0.0015
TNF	17.91	23.04	40.00	0.0000	18.69	23.72	34.54	0.0000
PDGFA	24.17	28.84	29.22	0.0000	23.27	28.05	29.11	0.0000
CSF1	21.27	25.64	23.73	0.0000	20.64	23.85	9.78	0.0000
TNFRSF11B	30.39	34.25	16.63	0.0003	30.63	32.16	3.06	0.0060
LTA	22.19	25.06	8.39	0.0000	20.26	24.76	23.92	0.0000
TNFSF11	26.61	29.10	6.40	0.0001	27.28	29.61	5.30	0.0001
BMP6	26.37	28.79	6.14	0.0003	26.40	29.28	7.84	0.0000
BMP3	31.45	33.71	5.50	0.0041	35.00	34.71	-1.16	0.1996
FASLG	20.90	23.16	5.46	0.0000	21.54	24.16	6.48	0.0000
TGFB2	28.98	31.23	5.43	0.0000	30.88	33.36	5.91	0.0029
TNFSF14	32.77	35.00	5.37	0.0009	33.51	35.00	2.98	0.0003
TNFSF8	20.16	22.27	4.92	0.0000	19.94	24.17	19.88	0.0000
TNFSF13	29.20	30.38	2.60	0.0000	31.80	26.02	-52.10	0.0000
BMP4	32.11	33.29	2.58	0.0935	28.99	32.54	12.38	0.0003
IL6	18.77	19.88	2.47	0.0002	19.92	22.49	6.29	0.0000
GDF10	33.11	34.08	2.23	0.1166	32.95	29.13	-13.30	0.0006
IL20	31.75	32.56	2.00	0.0117	32.27	35.00	7.03	0.0001
IL4	32.00	32.31	1.42	0.3010	33.36	32.22	-2.08	0.0025
TNFSF12	26.05	26.25	1.32	0.0057	29.28	23.84	-41.16	0.0000
IL12A	27.19	27.19	1.14	0.0971	27.18	27.18	1.06	0.3060
IL1F6	30.28	29.72	-1.29	0.2311	33.34	30.17	-8.48	0.0046
IL18	29.14	28.53	-1.33	0.0449	33.32	28.83	-21.26	0.0000
LTB	22.22	21.47	-1.48	0.0120	27.18	20.42	-102.54	0.0000
IL17C	28.78	27.95	-1.55	0.0213	31.86	27.66	-17.31	0.0001
IFNK	29.27	28.40	-1.60	0.0206	29.73	27.14	-5.71	0.0011
IL16	23.52	22.25	-2.11	0.0000	24.75	20.97	-12.91	0.0000
TNFSF4	28.43	26.89	-2.54	0.0002	27.96	25.45	-5.38	0.0000
IL1F9	29.69	28.07	-2.68	0.6977	26.92	22.81	-16.34	0.0000
IL15	29.46	27.55	-3.28	0.0007	28.79	26.32	-5.23	0.0000
IFNB1	31.11	29.07	-3.58	0.0022	34.37	30.03	-19.03	0.0015
BMP8B	29.36	27.25	-3.76	0.0001	31.35	28.51	-6.74	0.0018
IL12B	35.00	32.72	-4.25	0.0132	31.24	29.86	-2.46	0.0049
TGFA	29.29	26.92	-4.49	0.0000	27.96	24.06	-14.06	0.0000
IL1B	18.66	15.64	-7.12	0.0000	20.12	16.46	-11.93	0.0000
IL1F7	34.52	30.84	-11.19	0.0012	35.00	30.85	-16.76	0.0000
IFNA5	33.53	29.65	-12.89	0.0011	31.19	29.13	-3.93	0.0002
IL1A	24.27	20.02	-16.62	0.0000	25.48	23.24	-4.46	0.0000
TNFSF10	26.16	21.70	-19.22	0.0000	25.41	20.73	-24.20	0.0000
TNFSF13B	29.68	24.75	-26.62	0.0001	31.27	22.50	-411.10	0.0001

* The significance of the change in gene expression between the two samples was evaluated by unpaired Student's t-test for each gene. The level of statistical significance is set at <0.005. Genes that show at least a five-fold difference in expression between the two samples are listed in the table. After six hours of stimulation, a total of 29 genes show at least a 5-fold change in expression between the stimulated and resting PBMC, with 23 genes having increased expression and six genes having decreased expression in stimulated PBMC.

Using the Human Common Cytokines RT² Profiler PCR Array, we identified 29 genes that exhibited at least a five-fold change in gene expression between resting and PMA-ionomycin stimulated peripheral blood mononuclear cells at 6 hours after stimulation. Our data show that changes in cytokine mRNA levels detected by RT² Profiler PCR Arrays accurately predict changes in protein levels measured by ELISA. Hence, the RT² Profiler PCR Array System offers a simple, reliable and sensitive tool for multiple cytokine profiling.

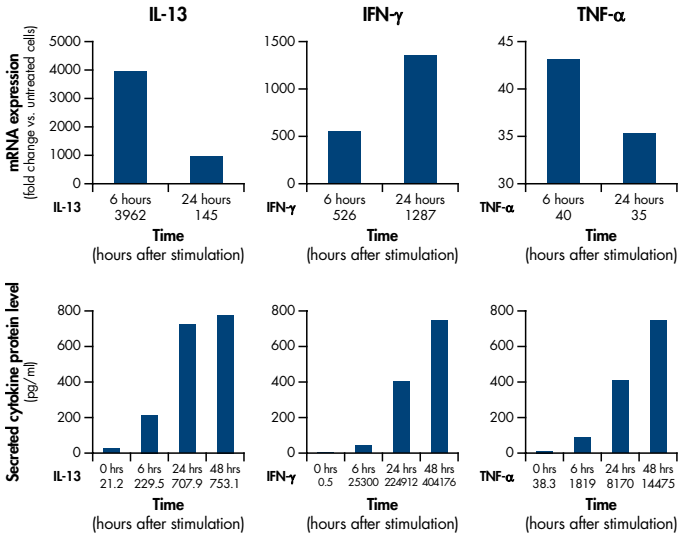

Figure 5. The effects of PMA-ionomycin on the secretion of the eight selected cytokines were assessed by multiplex cytokine ELISA. As shown in the above graphs, in parallel with the RT² Profiler PCR Array System results (upper panel), a marked increase in cytokine release (lower panel) was seen for IL-13, and IFN-γ and TNF-α. The induction in cytokine secretion by PMA-ionomycin were sustained for up to 48 hours of stimulation, despite subdued mRNA expression for some cytokines such as IL-13 and TNF-α after 24 hours of stimulation.

Summary:

Powerful and practical research applications

In this paper, we have described several experiments in the fields of toxicology, oncology, and immunology where the RT² Profiler PCR Array System demonstrated practical applicability and congruity with previously published results. The RT² Profiler PCR Array System provides a unique, pathway-focused approach to gene expression profiling experiments. The SYBR-Green RT² Profiler PCR Array System is both affordable and widely applicable to most real-time instruments. With a combination of the sensitivity, specificity, and reproducibility of real-time PCR, pathway-focused multiplexing capability, and guaranteed performance, the RT² Profiler PCR Array System provides a powerful new tool for many areas of biomedical research.

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