Oligo GEArray® Identifies Apoptotic Genes Up-Regulated by Chronic Restraint Stress in Splenocytes

Introduction

Gene expression profiling provides a vivid picture of the underlying transcriptional changes behind many biological processes. Oligonucleotide-based DNA microarrays are an indispensable tool for identifying genes of interest in a high throughput fashion. However, researchers are often challenged by the accessibility to a high-density microarray and baffled by the massive amount of generated data. GEArray® products from SuperArray Bioscience offer excellent alternatives to genome-wide arrays. GEArrays® are pathway-focused and low density, thus economical and easy to analyze. Together with quantitative RT² Real-Time™ PCR, they can provide a complete expression analysis for your pathway of interest. For example, in a collaborative effort with a research group at University of Eastern Tennessee (1), we have used both GEArray® microarrays and RT² Real-Time™ PCR primer sets to identify transcriptional changes caused by chronic restraint stress.

Psychological and physical stress can alter the immune system in mammals. It is generally believed that moderate stress, such as exercise, boosts the immune system, while chronic stress, such as depression, decreases immune function and increases the susceptibility to disease (2). Lymphopenia has been observed in humans under psychological stress (3) and in mice under restraint stress (4). In the restraint stress mouse model, the reduction of lymphocytes appears to be due to apoptotic cell death with an up-regulation of Fas expression (4). The cell death receptor Fas is involved in one route that activates caspase-mediated apoptosis, along with other proteins such as FADD and caspase-8. Another activation route originates in the mitochondria and involves Apaf-1 and cytochrome C. To elucidate the cellular machinery for apoptosis in stress-induced lymphopenia, this collaborative effort studied the expression profile of 226 apoptosis-related genes in the restraint stress mouse model system using the Oligo GEArray®, and the results were validated using RT² Real-Time™ PCR.

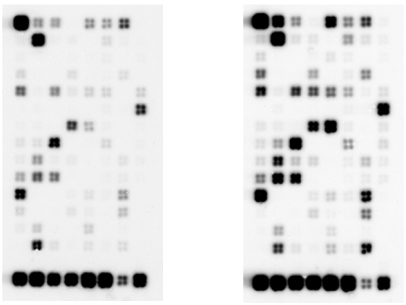

Figure 1: Apoptotic Genes are Up-Regulated in Spleen after Chronic Restraint Stress. Six- to 8-week-old male Balb/c mice were subjected to an established chronic physical restraint protocol (4). Control mice were kept in their original cages and on the same diet schedule as the experimental group. Two days after physical restraint, spleens were dissected and rapidly frozen in liquid nitrogen. Total RNA isolated from spleen was characterized with triplicate Mouse Apoptosis Oligo GEArrays® in the HybTube Format (OMM-012). A representative set of images are displayed.

Apoptosis Microarray

We investigated Fas and other apoptotic genes that may be altered in lymphocytes during chronic restraint stress using the Oligo GEArray® Mouse Apoptosis Microarray (OMM-012). It contains oligo probes for 112 genes involved in apoptosis immobilized on a nylon membrane. (A detailed gene list and layout can be found at www.superarray.com). Microarray experiments were performed in triplicate. A representative image is shown in Figure 1. After normalization and statistical analysis, 23 out of the 112 genes showed significantly increased expression in stressed versus unstressed mice (Table 1).

Table 1: Apoptotic Genes are Up-Regulated in Spleen after Chronic Restraint Stress. Raw signal intensities for individual genes on the microarrays described in Figure 1 were extracted using the GEArray® Expression Analysis Suite software and normalized against signal intensities from housekeeping genes. The genes listed significantly increased their expression under stress conditions relative to the control.

Position	Symbol	Description	Fold Increase
2	Akt1	Thymoma viral proto-oncogene 1	6.8
5	Api5	Apoptosis inhibitor 5	3.5
14	Bax	Bcl2-associated X protein	4.0
25	Bnip3l	BCL2/adenovirus E1B interacting protein 3-like	5.9
28	Birc1e	Baculoviral IAP repeat-containing 1e	4.7
31	Birc2	Baculoviral IAP repeat-containing 2	4.8
35	Bnip2	BCL2/adenovirus E1B interacting protein 1, NIP2	2.4
36	Bnip3	BCL2/adenovirus E1B interacting protein 1, NIP3	16
37	Casp3	Caspase 3	5.1
38	Bok	Bcl-2-related ovarian killer protein	3.2
48	Casp2	Caspase 2	2.4
52	Casp8	Caspase 8	1.8
53	Dsip1	TSC22 domain family 3	12.7
58	Cradd	CASP2 and RIPK1 domain containing adaptor with death domain	4.9
65	Fadd	Fas (TNFRSF6)-associated via death domain	3.6
71	Ltbr	Lymphotoxin B receptor	3.7
74	Nfkb1	Nuclear factor of kappa light chain gene enhancer in B-cells 1, p105	2.9
75	Zc3hc1	Zinc finger, C3HC type 1	2.7
87	Rnf7	Ring finger protein 7	3.3
95	Tnfrsf21	Tumor necrosis factor receptor superfamily, member 21	2.6
98	Fas	Fas (TNF receptor superfamily member)	1.8
109	Trp53	Transformation related protein 53	3.3
111	Trp53inp1	Transformation related protein 53 inducible nuclear protein 1	11.9

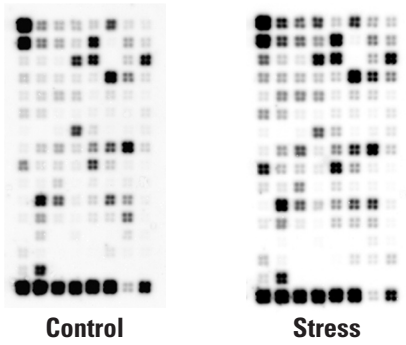

Figure 2: p53 Signaling Pathway Genes are also Up-Regulated in Spleen after Chronic Restraint Stress. RNA isolated as described in Figure 1 was also characterized with triplicate Mouse p53 Signaling Pathway Oligo GEArrays® in the HybTube Format (OMM-027). A representative set of images are displayed.

p53 Signaling Pathway Microarray

Because of its apparent role in apoptosis, we also investigated the p53-mediated signaling pathway in lymphocytes during restraint stress using the Mouse p53 Signaling Pathway Microarray. A representative image is shown in Figure 2. After normalization and statistical analysis, 26 out of the 113 genes was significant up-regulated and one (1) gene was down-regulated in stressed versus unstressed mice (Table 2).

Table 2: Genes in the p53 Signaling Pathway are Up-Regulated in Spleen after Chronic Restraint Stress. Raw signal intensity for individual genes on the microarrays described in Figure 2 was extracted using the web-based GEArray® Expression Analysis Suite software and normalized against signal intensities from housekeeping genes. The genes listed significantly changed their expression under stress conditions relative to the control.

Position	Symbol	Description	Fold Increase
3	Apaf1	Apoptotic peptidase activating factor 1	3.1
4	Apex1	Apurinic/apyrimidinic endonuclease 1	4.8
7	Aurkb	Aurora kinase B	3.8
8	Wdr39	WD repeat domain 39	3.7
10	Bak1	BCL2-antagonist/killer 1	2.2
11	Bap1	Brca1 associated protein 1	2.6
12	Bax	Bcl2-associated X protein	3.8
15	Bid	BH3 interacting domain death agonist	2.1
16	Birc5	Baculoviral IAP repeat-containing 5	3.1
17	Bnip3	BCL2/adenovirus E1B interacting protein 1, NIP3	7.5
18	Brca1	Breast cancer 1	3.8
19	Brca2	Breast cancer 2	4.7
20	Btg2	B-cell translocation gene 2, anti-proliferative	2
25	Ccng2	Cyclin G2	2.5
26	Ccnh	Cyclin H	3.4
27	Cdc25a	Cell division cycle 25 homolog A	3.7
28	Cdc25c	Cell division cycle 25 homolog C	3.4
29	Cdc2a	Cell division cycle 2 homolog A	3.6
31	Cdk7	Cyclin-dependent kinase 7	2.6
32	Cdkn1a	Cyclin-dependent kinase inhibitor 1A (P21)	5.3
34	Chek1	Checkpoint kinase 1 homolog	4.8
35	Chek2	CHK2 checkpoint homolog	3
36	Cradd	CASP2 and RIPK1 domain containing adaptor with death domain	3.3
39	Cyr61	Cysteine rich protein 61	3.5
41	Daxx	Fas death domain-associated protein	2.7
53	Gadd45a	Growth arrest and DNA-damage-inducible 45 alpha	3
95	Stat1	Signal transducer and activator of transcription 1	0.5

RT² Real-Time™ PCR Validation

To further validate the gene expression changes observed in the microarray experiments, we chose to quantitatively measure mRNA levels of four up-regulated genes of interest in the spleen of stressed mice. Real-time PCR experiments confirmed the significantly increased expressions of Fas, FADD, p53 and p21 (Figure 3).

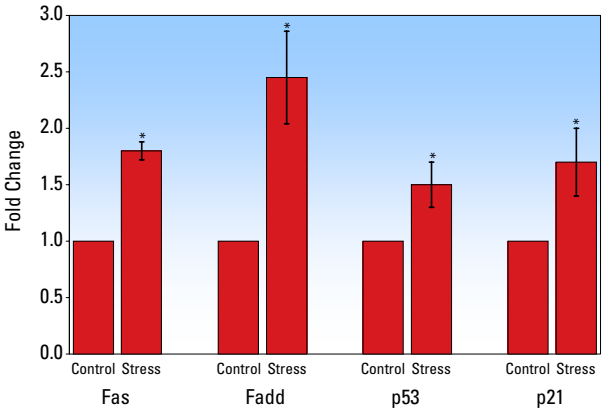

Figure 3: Real-Time PCR Validation of Microarray Results. The expression levels of Fas, Fadd, p53, and p21 as well as Actb and Gapd in both stressed and control splenocytes were determined. RNA was first converted into cDNA using the ReactionReady™ First Strand cDNA Synthesis Kit (C-01) followed by real-time PCR characterization using the appropriate RT² Real-Time™ PCR Primer Sets and the RT² Real–Time™ SYBR Green / Fluorescein PCR Master Mix (PA-011) on the Bio-Rad iCycler®. Fold changes in gene expression were calculated using the $\Delta\Delta C_t$ method. The statistical significance was determined by one-way ANOVA and Bonferroni tests (*, $p<0.05$).

Conclusions

Using Oligo GEArray® technology and RT² Real–Time™ PCR, we have identified numerous genes in the apoptotic and p53 signaling pathways that are significantly altered in lymphocytes using the restraint stress mouse model. The majority of these genes (except one) are up-regulated in response to restraint stress, including Fas, FADD, p53 and p21. This provides further evidence that apoptotic machinery is the underlying cause of lymphopenia during stress. The exact roles and functionality of these genes require further investigation. This study also showcases the feasibility and convenience of combining a GEArray® microarray analysis with RT² Real–Time™ PCR primer sets and master mixes in pathway-focused gene expression profiling.

References

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