

Cancer-Related Genes: Comparing Expression in Normal Tissues and Tumor Samples using PCR Arrays.

Introduction

The RT² Profiler™ PCR Arrays profile the expression of a panel of genes relevant to a pathway or disease state. To illustrate the utility of these PCR Arrays in cancer research, this application note examines the route taken by two different human breast tumors (#1 and #2) during their oncogenesis. Experiments will compare the relative expression of both cancer- and adhesion- related genes between each tumor sample and a normal breast tissue sample. The study illustrates how researchers may identify a pathway affected by the transformation of a particular tumor type and then further explore the alterations in that pathway using the PCR Arrays.

Cancer PathwayFinder™ PCR Array

Total RNA samples from normal breast tissue and the first unmatched breast tumor were characterized on the RT² Profiler™ Cancer PathwayFinder™ PCR Array. This PCR Array includes representative genes from the following biological pathways involved in oncogenesis: Adhesion, Angiogenesis, Apoptosis, Cell Cycle Control, Cell Senescence, DNA Damage Repair, Invasion, Metastasis, Signal Transduction Molecules, and Transcription Factors.

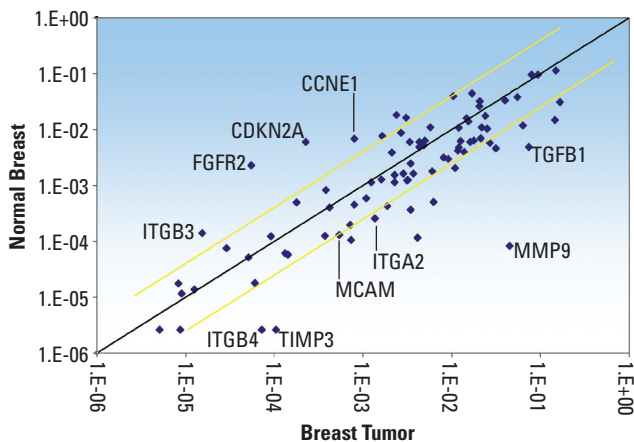


Figure 1: Compare the relative expression of 84 cancer-related genes between normal human breast and human breast tumor #1. Template cDNAs prepared from normal human breast and human breast tumor total RNA (BioChain Institute, Inc., 5.0 µg) were characterized in technical triplicates using the Human Cancer PathwayFinder™ RT² Profiler™ PCR Array (APH-033A) and the RT² Real-Time™ SYBR Green / Fluorescein PCR Master Mix (PA-011) on the Bio-Rad iCycler®. The figure depicts a log transformation plot of the relative expression level of each gene (2^{-ΔC_t}) between breast tumor (x-axis) and normal breast (y-axis). The pink lines indicate a four-fold change in gene expression threshold.

Figure 1 displays a scatter plot report of the results from the PCR Array experiment. Figure 2 in the Featured Pathway section of this edition on Page 3 displays another representation of the same results in the form of a 3D Plot. Both figures indicate the positions of several noteworthy genes based on their large fold-differences in expression between the normal breast and the breast tumor samples. One quarter of the genes in this array demonstrate at least a four-fold difference in gene expression between normal breast tissue and the breast tumor. Seventeen (17) of those genes were up-regulated, and seven (7) genes were down-regulated in the tumor sample, for a total of 24 genes. Table 1 lists all of these genes.

Table 1: Compare the relative expression of 84 cancer-related genes between normal human breast and human breast tumor #1. The table lists genes from the experiment in Figure 1 that exhibit at least a four-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.

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

Six of those 24 genes (ITGA2, ITGA4, ITGB3, MCAM, MMP9, and TIMP3) represent adhesion and extracellular matrix molecules. ITGB3 was down-regulated, while the other five genes were up-regulated. The results suggest that changes in the expression of genes involved in cellular interactions plays 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 on the corresponding PCR Array focused on Extracellular Matrix and Adhesion Molecules.

ECM and Adhesion Molecules

Total RNA samples from normal breast tissue and the second unmatched breast tumor were characterized on the RT² Profiler™ Extracellular Matrix and Adhesion Molecules PCR Array. Genes that have at least a three-fold difference in expression between the samples are listed in Table 2.

On this array, a larger number of genes exhibit differential expression in the second tumor than observed for the first tumor on the Cancer PathwayFinder™ PCR Array. A total of 38 genes have a different level of expression in the breast tumor than in the normal breast tissue, with 27 genes increasing their expression and 11 genes down-regulating their expression. A larger number of genes can therefore be identified by examining a pathway in more detail with a more specific PCR Array.

Four of the same genes that changed expression in the first tumor sample on the Cancer Pathway-Finder™ PCR Array (MMP9, TIMP3, ITGA4, and ITGB3) also changed their expression in the same direction and to a similar extent in the second breast tumor sample on the Extracellular Matrix and Adhesion Molecules 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.

Conclusions

This experiment suggests an important role for changes in the expression of genes related to cellular adhesion in the transformation of breast tissue. Alterations in the expression of these genes could enhance or inhibit metastasis of the tumor from its original location and then invasion into a new tissue or organ. A PCR Array focusing on the Tumor Metastasis pathway is also available and could be used to continue this study.

The overall experiment indicates the usefulness of the pathway-focused PCR Arrays in exploring changes in the expression of genes important in relevant biological pathways. Here, the results from the Cancer PathwayFinder™ PCR Array suggested the use of the Extracellular Matrix and Adhesion Molecules or the Tumor

Metastasis PCR Array. Another example of a PCR Array, the Signal Transduction PathwayFinder™, is designed using a similar strategy by representing the key downstream effectors of 18 different signal transduction pathways. The results from this array can suggest a role for specific pathways in the propagation a biological signal or response. PCR Arrays are then available focusing on each of these signal transduction pathways with a larger more complete list of genes to help further explore their roles.

Table 2: Compare the relative expression of 84 genes encoding extracellular matrix and adhesion molecules between normal human breast and human breast tumor #2. Triplicate total RNA samples prepared from normal human breast and human breast tumor 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 (APH-013A) and the RT² Real-Time™ SYBR Green /Fluorescein PCR Master Mix (PA-011) on a Bio-Rad iCycler®. The table lists genes that exhibit at least a three-fold difference in expression between the normal and tumor breast tissue samples. The raw threshold cycle (C_t) values seen in the two samples are also listed for comparison.

Gene	Fold change	t-Test	Average Raw C _t	
	Tumor/Normal	p value	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
CTNNA1	-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