

Genome Wide Expression Profiling of Paired Cancerous and Normal Breast Tissue



Kelly Li, Irene Lui, and Gary P. Schroth Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404

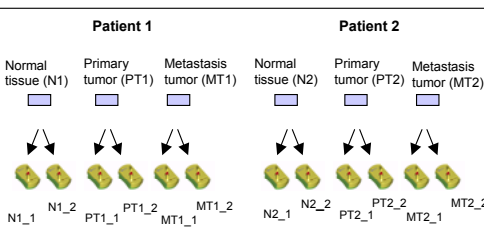
ABSTRACT

In this study we profile over 30,000 genes in paired normal and cancerous breast tissues on the Applied Biosystems Expression Array System. By comparing paired breast tissue specimens from the same patient we can identify genes that are differentially expressed in normal tissue, primary tumor, and metastasis tumor. Both biological replicates and technical replicates have been included in this study.

Using statistical data analysis tools, we have identified numerous genes that are up- or down-regulated in primary tumor and metastasis tumor compared to their normal control. Clear changes in gene expression patterns from normal to primary tumor and to metastasis tumor are observed. Gene expression level changes for genes detected by the Applied Biosystems Expression Array System have been validated using quantitative real-time PCR (TaqMan® probe-based Assays). This study demonstrates the use of microarrays for genome wide screening for gene expression in combination with quantitative real-time PCR for validating and extending results for genes of interest.

EXPERIMENTAL DESIGN

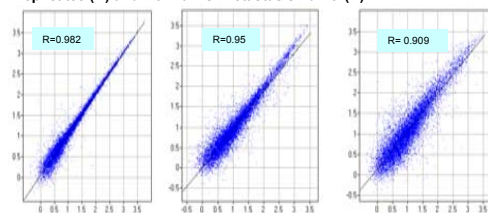
- Two biological replicates
- Three disease status
- Two technical replicates



Total RNA samples from 2 individual patients and 3 conditions (normal, primary and metastasis tumor) were processed into Digoxigenin (DIG) labeled cRNA using the Applied Biosystems RT-IVT Labeling Kit. Dig-labeled cRNAs were hybridized to the Applied Biosystems Human Genome Survey Microarray. Following hybridization a chemiluminescent detection assay was performed and the arrays were read on the 1700 Microarray Analyzer. Two experimental replicates were produced for each condition of both biological replicates.

RESULTS

Figure 1. Scatter Plots of Technical Replicates (A), Biological Replicates (B) and Normal vs. Metastasis Tumor (C)



A) Good reproducibility is seen in technical replicates when comparing expression from the same sample condition and from the same patient: B) Reduced correlation is seen in biological replicates by comparing expression of the same disease condition from 2 patients, suggesting individual differences of gene expression. C) Further reduced correlation is seen when comparing expression from different conditions of the same patient, indicating differential gene expression across different samples.

Table 1. Partial list of genes shown in Figure 2B

Gene	GeneName	MT2/N2	ANOVA P	Panther	MolecularFunction
BRCA1	BRCA1	1.11	0.000	BRCA1	BRCA1
BRCA2	BRCA2	1.11	0.000	BRCA2	BRCA2
BRCA3	BRCA3	1.11	0.000	BRCA3	BRCA3
BRCA4	BRCA4	1.11	0.000	BRCA4	BRCA4
BRCA5	BRCA5	1.11	0.000	BRCA5	BRCA5
BRCA6	BRCA6	1.11	0.000	BRCA6	BRCA6
BRCA7	BRCA7	1.11	0.000	BRCA7	BRCA7
BRCA8	BRCA8	1.11	0.000	BRCA8	BRCA8
BRCA9	BRCA9	1.11	0.000	BRCA9	BRCA9
BRCA10	BRCA10	1.11	0.000	BRCA10	BRCA10
BRCA11	BRCA11	1.11	0.000	BRCA11	BRCA11
BRCA12	BRCA12	1.11	0.000	BRCA12	BRCA12
BRCA13	BRCA13	1.11	0.000	BRCA13	BRCA13
BRCA14	BRCA14	1.11	0.000	BRCA14	BRCA14
BRCA15	BRCA15	1.11	0.000	BRCA15	BRCA15
BRCA16	BRCA16	1.11	0.000	BRCA16	BRCA16
BRCA17	BRCA17	1.11	0.000	BRCA17	BRCA17
BRCA18	BRCA18	1.11	0.000	BRCA18	BRCA18
BRCA19	BRCA19	1.11	0.000	BRCA19	BRCA19
BRCA20	BRCA20	1.11	0.000	BRCA20	BRCA20
BRCA21	BRCA21	1.11	0.000	BRCA21	BRCA21
BRCA22	BRCA22	1.11	0.000	BRCA22	BRCA22
BRCA23	BRCA23	1.11	0.000	BRCA23	BRCA23
BRCA24	BRCA24	1.11	0.000	BRCA24	BRCA24
BRCA25	BRCA25	1.11	0.000	BRCA25	BRCA25
BRCA26	BRCA26	1.11	0.000	BRCA26	BRCA26
BRCA27	BRCA27	1.11	0.000	BRCA27	BRCA27
BRCA28	BRCA28	1.11	0.000	BRCA28	BRCA28
BRCA29	BRCA29	1.11	0.000	BRCA29	BRCA29
BRCA30	BRCA30	1.11	0.000	BRCA30	BRCA30
BRCA31	BRCA31	1.11	0.000	BRCA31	BRCA31
BRCA32	BRCA32	1.11	0.000	BRCA32	BRCA32
BRCA33	BRCA33	1.11	0.000	BRCA33	BRCA33
BRCA34	BRCA34	1.11	0.000	BRCA34	BRCA34
BRCA35	BRCA35	1.11	0.000	BRCA35	BRCA35
BRCA36	BRCA36	1.11	0.000	BRCA36	BRCA36
BRCA37	BRCA37	1.11	0.000	BRCA37	BRCA37
BRCA38	BRCA38	1.11	0.000	BRCA38	BRCA38
BRCA39	BRCA39	1.11	0.000	BRCA39	BRCA39
BRCA40	BRCA40	1.11	0.000	BRCA40	BRCA40
BRCA41	BRCA41	1.11	0.000	BRCA41	BRCA41
BRCA42	BRCA42	1.11	0.000	BRCA42	BRCA42
BRCA43	BRCA43	1.11	0.000	BRCA43	BRCA43
BRCA44	BRCA44	1.11	0.000	BRCA44	BRCA44
BRCA45	BRCA45	1.11	0.000	BRCA45	BRCA45
BRCA46	BRCA46	1.11	0.000	BRCA46	BRCA46
BRCA47	BRCA47	1.11	0.000	BRCA47	BRCA47
BRCA48	BRCA48	1.11	0.000	BRCA48	BRCA48
BRCA49	BRCA49	1.11	0.000	BRCA49	BRCA49
BRCA50	BRCA50	1.11	0.000	BRCA50	BRCA50
BRCA51	BRCA51	1.11	0.000	BRCA51	BRCA51
BRCA52	BRCA52	1.11	0.000	BRCA52	BRCA52
BRCA53	BRCA53	1.11	0.000	BRCA53	BRCA53
BRCA54	BRCA54	1.11	0.000	BRCA54	BRCA54
BRCA55	BRCA55	1.11	0.000	BRCA55	BRCA55
BRCA56	BRCA56	1.11	0.000	BRCA56	BRCA56
BRCA57	BRCA57	1.11	0.000	BRCA57	BRCA57
BRCA58	BRCA58	1.11	0.000	BRCA58	BRCA58
BRCA59	BRCA59	1.11	0.000	BRCA59	BRCA59
BRCA60	BRCA60	1.11	0.000	BRCA60	BRCA60
BRCA61	BRCA61	1.11	0.000	BRCA61	BRCA61
BRCA62	BRCA62	1.11	0.000	BRCA62	BRCA62
BRCA63	BRCA63	1.11	0.000	BRCA63	BRCA63
BRCA64	BRCA64	1.11	0.000	BRCA64	BRCA64
BRCA65	BRCA65	1.11	0.000	BRCA65	BRCA65
BRCA66	BRCA66	1.11	0.000	BRCA66	BRCA66
BRCA67	BRCA67	1.11	0.000	BRCA67	BRCA67
BRCA68	BRCA68	1.11	0.000	BRCA68	BRCA68
BRCA69	BRCA69	1.11	0.000	BRCA69	BRCA69
BRCA70	BRCA70	1.11	0.000	BRCA70	BRCA70
BRCA71	BRCA71	1.11	0.000	BRCA71	BRCA71
BRCA72	BRCA72	1.11	0.000	BRCA72	BRCA72
BRCA73	BRCA73	1.11	0.000	BRCA73	BRCA73
BRCA74	BRCA74	1.11	0.000	BRCA74	BRCA74
BRCA75	BRCA75	1.11	0.000	BRCA75	BRCA75
BRCA76	BRCA76	1.11	0.000	BRCA76	BRCA76
BRCA77	BRCA77	1.11	0.000	BRCA77	BRCA77
BRCA78	BRCA78	1.11	0.000	BRCA78	BRCA78
BRCA79	BRCA79	1.11	0.000	BRCA79	BRCA79
BRCA80	BRCA80	1.11	0.000	BRCA80	BRCA80
BRCA81	BRCA81	1.11	0.000	BRCA81	BRCA81
BRCA82	BRCA82	1.11	0.000	BRCA82	BRCA82
BRCA83	BRCA83	1.11	0.000	BRCA83	BRCA83
BRCA84	BRCA84	1.11	0.000	BRCA84	BRCA84
BRCA85	BRCA85	1.11	0.000	BRCA85	BRCA85
BRCA86	BRCA86	1.11	0.000	BRCA86	BRCA86
BRCA87	BRCA87	1.11	0.000	BRCA87	BRCA87
BRCA88	BRCA88	1.11	0.000	BRCA88	BRCA88
BRCA89	BRCA89	1.11	0.000	BRCA89	BRCA89
BRCA90	BRCA90	1.11	0.000	BRCA90	BRCA90
BRCA91	BRCA91	1.11	0.000	BRCA91	BRCA91
BRCA92	BRCA92	1.11	0.000	BRCA92	BRCA92
BRCA93	BRCA93	1.11	0.000	BRCA93	BRCA93
BRCA94	BRCA94	1.11	0.000	BRCA94	BRCA94
BRCA95	BRCA95	1.11	0.000	BRCA95	BRCA95
BRCA96	BRCA96	1.11	0.000	BRCA96	BRCA96
BRCA97	BRCA97	1.11	0.000	BRCA97	BRCA97
BRCA98	BRCA98	1.11	0.000	BRCA98	BRCA98
BRCA99	BRCA99	1.11	0.000	BRCA99	BRCA99
BRCA100	BRCA100	1.11	0.000	BRCA100	BRCA100

Using the Panther classification system, we identify 200 genes that are involved in various signal transduction pathways. These genes are among the 2,508 genes which have p-values <0.01.

Table 2. Identification of genes that are absent in normal tissue, but are expressed in breast cancers.

Gene Symbol	Gene Name	ANOVA P Value	PANTHER Molecular Function
CCNE1	cyclin E 1	4.9E-11	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE2	cyclin E 2	1.77E-05	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE3	cyclin E 3	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE4	cyclin E 4	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE5	cyclin E 5	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE6	cyclin E 6	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE7	cyclin E 7	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE8	cyclin E 8	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE9	cyclin E 9	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE10	cyclin E 10	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE11	cyclin E 11	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE12	cyclin E 12	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE13	cyclin E 13	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE14	cyclin E 14	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE15	cyclin E 15	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE16	cyclin E 16	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE17	cyclin E 17	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE18	cyclin E 18	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE19	cyclin E 19	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE20	cyclin E 20	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE21	cyclin E 21	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE22	cyclin E 22	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE23	cyclin E 23	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE24	cyclin E 24	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE25	cyclin E 25	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE26	cyclin E 26	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE27	cyclin E 27	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE28	cyclin E 28	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE29	cyclin E 29	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE30	cyclin E 30	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE31	cyclin E 31	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE32	cyclin E 32	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE33	cyclin E 33	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE34	cyclin E 34	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE35	cyclin E 35	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE36	cyclin E 36	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE37	cyclin E 37	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE38	cyclin E 38	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE39	cyclin E 39	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE40	cyclin E 40	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE41	cyclin E 41	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE42	cyclin E 42	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE43	cyclin E 43	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE44	cyclin E 44	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE45	cyclin E 45	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE46	cyclin E 46	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE47	cyclin E 47	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE48	cyclin E 48	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE49	cyclin E 49	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE50	cyclin E 50	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE51	cyclin E 51	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE52	cyclin E 52	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE53	cyclin E 53	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE54	cyclin E 54	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE55	cyclin E 55	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE56	cyclin E 56	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE57	cyclin E 57	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE58	cyclin E 58	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE59	cyclin E 59	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE60	cyclin E 60	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE61	cyclin E 61	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE62	cyclin E 62	0.0000	Signaling molecule -> Cyclinase -> Other cyclinase
CCNE63	cyclin E 63	0.0000	Signaling molecule