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Award Number: DAMD17-02-1-0283

TITLE: Can Gene Expression Pattern Analysis Predict Recurrence
in Node-Negative Breast Cancer

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REPORT DATE: June 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20031104 040

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE June 2003	3. REPORT TYPE AND DATES COVERED Annual Summary (6 May 2002 - 5 May 2003)	
4. TITLE AND SUBTITLE Can Gene Expression Pattern Analysis Predict Recurrence in Node-Negative Breast Cancer			5. FUNDING NUMBERS DAMD17-02-1-0283	
6. AUTHOR(S) Anand Immaneni, Ph.D. Peter O'Connell, Ph.D				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Baylor College of Medicine Houston, Texas 77030 E-Mail: immaneni@breastcenter.tmc.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES Original contains color plates: All DTIC reproductions will be in black and white.				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) Although a majority of women with node-negative breast cancers have a good prognosis, 30% experience recurrence and death from metastatic disease. As result, systemic therapies are routinely administered to nearly all of these node-negative patients. Markers that better predict recurrence risk would more effectively target adjuvant therapies to the patients most likely to benefit from them. Our goal is to identify the genetic markers that 1) are differentially expressed in good versus bad outcome node-negative primary breast cancers, 2) help dichotomize node-negative patients into low and high-risk categories so that adjuvant treatment could be more effectively utilized, 3) identify genetic pathways associated with the metastatic phenotype. cDNA micro-arrays were used to analyze 30 untreated primary node-negative breast tumors from patients who were either completely cured by surgery alone (good outcome) and those who experienced metastatic recurrences (Bad outcome). At the p =0.05 level of significance, 137 genes involved in cell cycle, apoptosis samples DNA repair, cell adhesion, cytoskeleton and signal transduction were found to be differentially expressed between the good versus bad outcome tumors by Wilcoxon tests. Tree-view analyses generated dendrograms showing that the two categories of tumors mostly, but not completely, formed outcome-related clusters. We are currently validating the array data using semi-quantitative RT-PCR. Preliminary assays showed that the tested genes are indeed differentially expressed in the tumor samples. More candidate genes are currently being assayed. We have just started immunohistochemistry analysis on tissue arrays of archival specimens to assess the prognostic significance of some of these interesting candidate metastasis markers for which antibodies are commercially available.				
14. SUBJECT TERMS Breast cancer, metastasis, differential gene-expression, node-negative			15. NUMBER OF PAGES 7	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

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Introduction:

We compared gene expression profiles of untreated, node-negative, primary breast tumors from patients who either relapsed within 28 months (Bad outcome), or were cancer-free >10 years (Good outcome). The samples selected for this study were carefully matched for age, menopausal status, ER/PR status, tumor size and grade and other pathological parameters. Preliminary analysis was done by individual Wilcoxon tests for each gene across the arrays. Some of the microarray signal data that did not pass our normalization criteria were discarded from the study. The normalized data for the available samples was further subjected to clustering analysis using the Cluster/Tree View programs.

Some of the sorted genes from the clustering analysis are currently being validated by semi-quantitative RT-PCR. We are using rigorous and exhaustive criteria to validate the micro-array gene expression data. Firstly, the primer sequences are BLAST searched to ensure uniqueness. Secondly, the primers are tested on cDNAs generated from a mixture of cell line RNA and a trial set of tumor RNA to confirm a single product. Each primer pair is also optimized for the appropriate PCR cycles required for the gene amplification. An 18s rRNA control primer set is used as an endogenous control to normalize the target PCR product across the samples tested.

Key research accomplishments:

- 1) The preliminary data demonstrates that gene expression profiling of archived frozen tumors is indeed feasible and could distinguish heterogeneous tumor types based on gene expression differences. This signature patterns will be invaluable for clinicians to target adjuvant treatments only to those patients who are at-risk for recurrence.
- 2) Some of the genes could serve as novel bio-markers for metastatic recurrence, when further confirmed by Immunohistochemistry on Tumor Tissue Arrays. Further more, a few of the biologically relevant genes would be studied in detail for their role in metastatic pathways, in the final phase of this study.

Reportable Outcomes:

- 1) Clustering of the normalized gene expression data yielded better sorting of the genes, dichotomizing the two groups of tumors forming outcome based clusters.
- 2) Semi-Quantitative RT-PCR analyses is being used in validating the gene expression differences obtained from the micro arrays. We have thus far tested about 5 genes in an identical cohort of 3 of each good and bad outcome tumor RNA. More genes (15 more) are currently being assayed to validate their gene expression patterns and correlate with the micro-array data. As we await the completion of the validation assays, we expect to validate at least 50% of the selected genes based on initial assays (not shown).

3) A manuscript is currently under preparation based on this study.

Conclusions:

- 1) Cluster analyses proved to be a very useful tool to analyze complex expression array data that does not follow Normal distribution.
- 2) Wilcoxon analyses while useful to perform group comparisons, it obscures differences between heterogeneous tumor samples.
- 3) We have identified genes, some of which have been implicated in the metastasis of cancers. More recently, CyclinE has independently been shown to be a prognosticator in breast cancers, which has also panned out in our clustering data. We are currently evaluating other interesting genes as well. Once completed, the RT-PCR data will be correlated to micro-array data, to ascertain the up or down regulation of sorted genes.
- 4) We have also started the confirmation studies of the protein expression of some of these candidate markers on tumor tissue-arrays. Since not all proteins have commercially available and or reliable antibodies, we are currently in the process of developing immunohistochemistry (IHC) assays for the available antibodies on test tissue arrays.

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Cheng Li and Wing Hung Wong. Model based analysis of oligo-nucleotide arrays: Expression index computation and outlier detection. PNAS. USA 98, No.1, 31-36, 2001.

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Appendices:

The cluster diagrams on the following pages demonstrate the quality of the profile data. Although, there is some interspersions of good and bad outcome cases, the majority of these tend to cluster together.

Figure 1: Clustering
of a set of 137 genes sel-
ected by Wilcoxon
analysis ($p=0.05$) show
visual differences bet-
ween the tumor types.

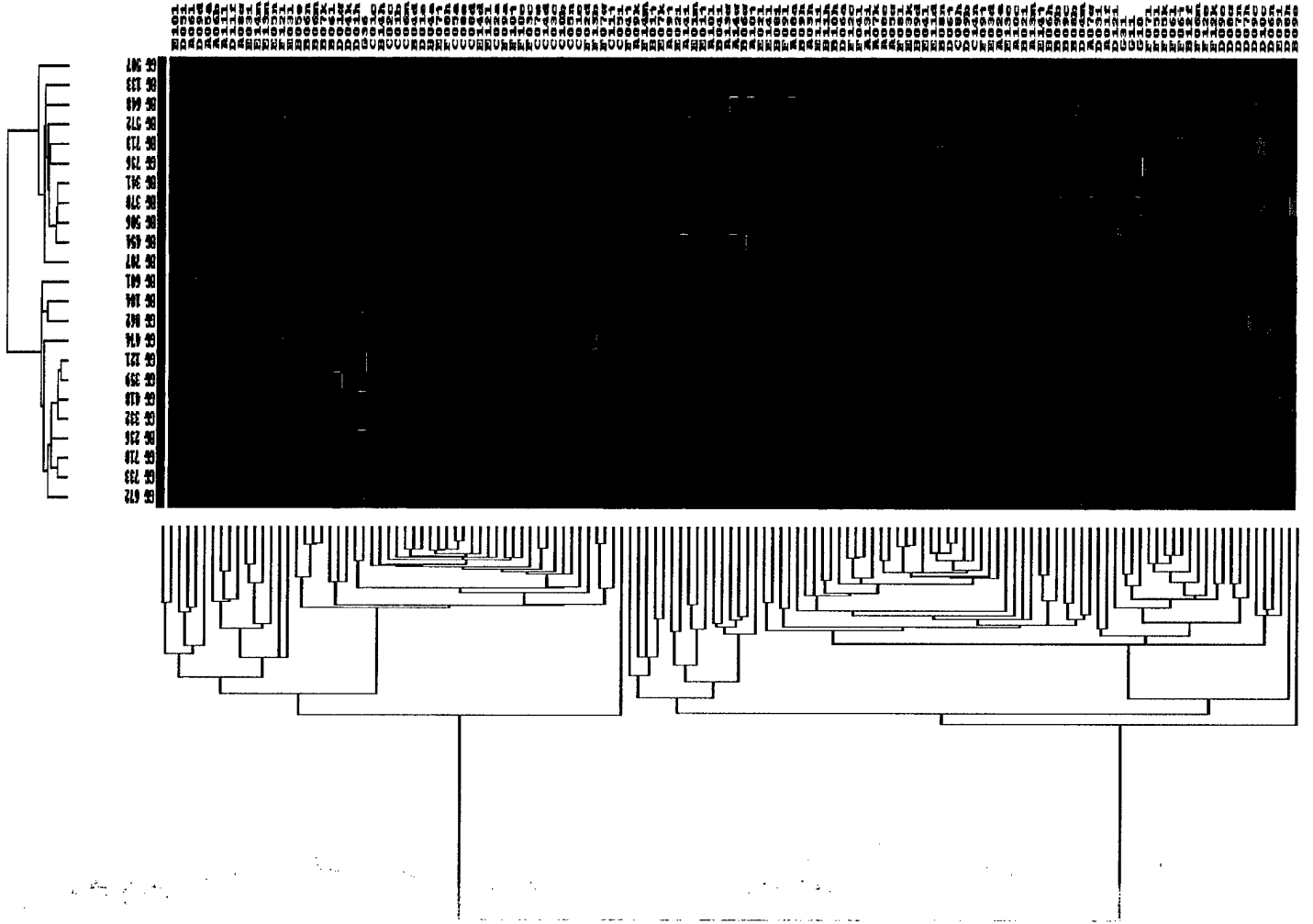
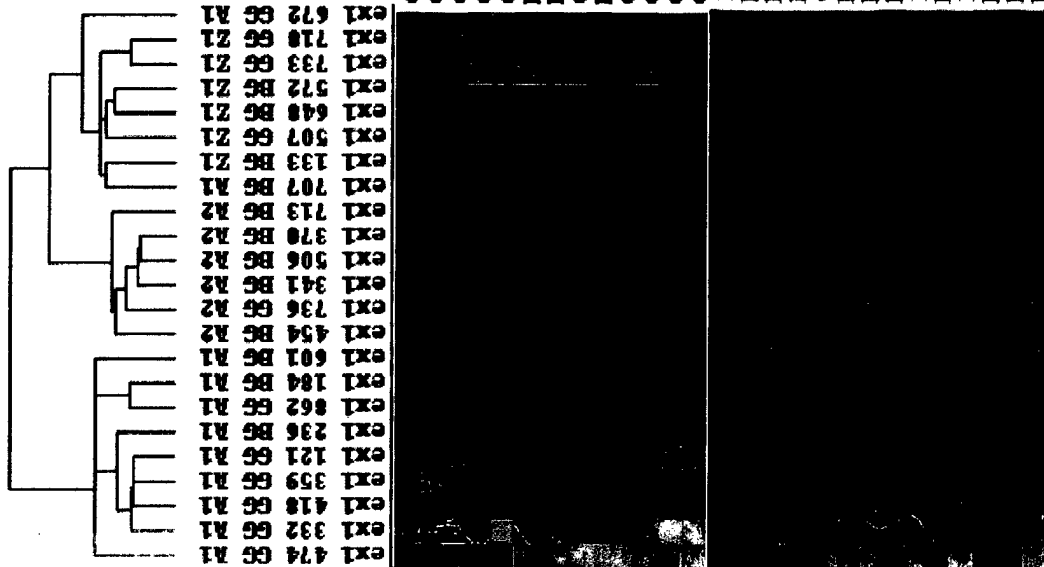


Figure 2: Cluster analysis generated dendrograms classifies the two types of tumors into outcome-related clusters. (scale: Dark Red-highest expression; Dark green lowest expression)



- C02b-CD27BP (Siva)
- C01c-BHD proteina; bcl-2 binding
- C06a-decoy receptor 2
- C02g-DNA ligase III (LIG3):
- C01f-activator 1 40-kDa subunit
- F101-KIAA0265
- F03e-fatty acid synthase
- C05e-P53-BINDING PROTEIN
- F10e-mvosin-DXB
- C08d-inhibitor of apoptosis proteina 3
- C07f-DNA primase small subunit:
- C02a-SL cytokine precursor:
- C01a-HIK serine/threonine proteina
- A02b-FB1 proteina
- E11f-laterleukia-3 precursor (IL-3):
- B09a-tyrosine-protein kinase HCK; P59-HCK
- D06k-tyrosine kinase receptor
- C08i-telomerase reverse
- F07d-CTP synthase; UTP-aseonia ligase:
- E10f-tissue inhibitor of metalloproteinase
- D09c-autoimmunotoxic cancer
- A12a-protein-tyrosine phosphatase PTEA
- E09k-ubiquitin C-terminal
- A12l-CDK25C; K-ase inducer phosphatase 3
- F06m-laterferos-induced 56-kDa
- D01a-uracine nucleotide-binding
- F12g-BCL7B proteina