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The expression of genes involved in signal transduction (e.g. protein kinases) is often altered in tumors. The aberrant expression of several of these genes typically parallels the progression toward a more malignant phenotype. We developed a cDNA micro-array - based screening system to measure the level of expression of tyrosine kinase (tk) genes. The hardware for preparation of cDNA micro-arrays and basic protocols for hybridization were developed in the first year. In the second year, we finished isolating RNA and cDNA synthesis from 16 breast cancer cell lines and 10 frozen tissues. We optimized protocols for tk-specific PCR amplification and cloning. We continued our DNA sequencing effort and added additional targets to our micro-arrays. Using well-characterized breast cancer cell lines, the system delivered reproducible results about tk gene expression during cell transformation and progression toward a more malignant phenotype. Comparing the absolute expression levels from cDNA micro-arrays with data from Northern blot analyses suggested that our initial approach using mixed-based oligonucleotide primers led to lowered representation of highly abundant transcripts. This problem has been addressed with a new oligonucleotide primer design based on known gene sequences and a two step DNA amplification protocol that now allows to investigate the tk gene expression in small tissue samples.

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## Table of Contents


Cover........................................................................................ 1

SF 298........................................................................................ 2

Introduction.............................................................................. 4

Body.......................................................................................... 4

Key Research Accomplishments............................................ 12

Reportable Outcomes.............................................................. 12

Conclusions.............................................................................. 14

References................................................................................ 14

Appendices................................................................................14

## INTRODUCTION:

Aberrant expression of receptor or cytosolic tyrosine kinase genes and, in particular, their hyper-expression are common phenomena in breast cancer, which are believed to alter cell growth and response to external signals such as growth factors, hormones etc. Knowledge about the relative levels of expression of many tyrosine kinase genes, all at the same time, might contribute significantly to a better understanding of the processes of tumor development and progression. We developed a rapid assay using innovative cDNA micro-arrays carrying small amounts of individual tyrosine kinase gene-specific targets to simultaneously determine the expression levels of up to 100 tyrosine kinase genes using a small number of cells. Our research and development lead to the definition of a set of gene-specific markers associated with breast cancer progression and a simple device capable of performing inexpensive expression profiling of these markers. The research and development efforts in the first two years of this project focused on the design and testing of robotic instrumentation to prepare DNA micro-arrays and the preparation of prototype arrays carrying sets of more than 50 gene-specific tyrosine kinase fragments. Tests with cDNA samples prepared from cell lines suggested the in vitro DNA amplification lead to unequal representation of gene transcripts, so-called 'biased' quantification. The third and fourth year efforts were therefore directed towards the optimization of in vitro DNA amplification protocols and validation of results from cDNA micro-array experiments.

During the third reporting period (1 Aug 01 –31 Jul 02), Dr. Weier's effort averaged 15%. This reduction in effort was due to Dr. Weier being on leave under the federal Family Medical Leave Act of 1993 (FMLA) since November 2001 due to his wife's disability and the birth of their twin daughters. A postdoctoral fellow, Lisa Chu, Ph.D., was hired in January 2002 to assume some of the duties. In February of 2002, LBNL notified the Commander, U.S. Army Medical Research and Material Command, that the Laboratory is making a one-time extension through June 30,. 2003 in accordance with Article 5, Approvals and Other Authorizations. This no-cost extension was approved by the Grants Officer, U.S. Army Medical Research Acquisition Activity on March 19, 2002 to complete the research as proposed.

## BODY:

Here, we report our progress as it relates to the approved 'Statement of Work'.

### Task 1. Identify tyrosine kinase (tk) genes expressed in normal and neoplastic breast tissues (months 1-24)

Completed as proposed and described in previous progress reports.

Briefly, we isolated total RNA from 16 different breast cell lines provided by our collaborators Paul Yaswen, Ph.D., Martha Stampfer, Ph.D., Daniel Callahan, Ph.D., and Ruth Lupo, Ph.D., from LBNL, and Chris Benz, M.D., from the Buck Institute for Aging Research, Novato, CA. (Table I). Normal breast tissue and breast cancer tissue specimens were obtained from the University of California Comprehensive Cancer Center (Joe W. Gray, Ph.D., B.M. Ljung, M.D., and K. Chew). We received a total of 10 frozen tissue specimens representing 3 normal tissues and 7 cancer tissues.

**Table I. Breast cancer cell lines used in our experiments.**

| | |
|---|---|
| ZR75 | SKBR3 |
| BT474 | BT468 |
| BT549 | MCF-7 |
| 184A1 | 184A1TH-6 |
| 184B5 | 184B5-ME |
| T47D | MDA-MB-453 |
| MDA-MB-436 plus Vit. A | MDA-MB-436 plus pCMr3.1 |
| 600MPE | HMEC |

RNA was extracted using a commercial kit (Qiagen) and transcribed into cDNA immediately after isolation. Remaining RNA was stored at –80 degrees. We prepared cDNAs from the RNA by random priming and reverse transcription. Commercial kits (Qiagen, Roche, Ambion) were used for all steps. Typically, 1 µg of total RNA produced sufficient quantities of cDNA for cloning and/or repeated micro-array analyses.

***1.2 Perform RT-PCR reactions and clone PCR products in plasmids (months 3-18)***

***1.3 Perform pre-screening with known tk fragments, cDNA sequencing and database searches (months 5-22)***

***1.4 Add novel clones to the panel of expressed tk gene fragments (months 6-24)***

Tk gene-specific DNA fragments were obtained by in vitro DNA amplification and clones in plasmid vectors for further characterization (Weier et al., 2001). The DNA from plasmid clones with inserts of about 125-190 bp was isolated, fingerprinted or screened against known tk genes and 'novel' clones were sequenced at the UC Berkeley, Biochemical Core Facility. The list of genes cloned and identified contained several sequences reported to have transforming activity, such as brk, trk, axl/ufo or to be overexpressed in various types of cancer.

We cloned and partially characterized about 240 tk fragment containing plasmid clones derived from breast cancer tissues and cell lines. These clones join more than 500 tk fragments containing clones that were previously isolated from thyroid and prostate tumors. From these clones, DNA was isolated, bound to nylon filters and prescreened with probes prepared from known tk clones. Following this pre-screening step, we performed cDNA sequencing and database searches. In about 100 tk containing plasmids that were sequenced, we found two potentially novel tk genes. The other sequenced clones contained two known kinases that we added to our panel. The remaining clones contained sequences that were already part of the panel suggesting insufficient stringency during the pre-screening process. At this point we decided to focus our efforts on the optimization of the PCR amplification (see 2.3, below). DNA isolated from this panel of tk gene fragments is then arrayed and printed onto glass slides to measure tk gene expression in cell lines, normal tissues, and tumor tissues. Our present panel of kinase gene tags used to prepare cDNA micro-arrays contains 60 genes among them four novel sequences and HLA-A, which happens to be amplified by our PCR primers.

To search for full length cDNA clones for some of the novel sequences found expressed in breast cancer, we employed the services of a local company (Pangene of Fremont, CA). Pangene used two of our sequences to screen their proprietary cDNA libraries for full length clones. In first attempts, the expressed sequence tags (EST's) were too short to isolate a cDNA clone. However, one EST

could be mapped to chromosome 1 by database searches, and expanded by using flanking genomic sequences. This provided sufficient sequence information for screening and we obtained one cDNA clone with a 5.5kb insert sequences in the 6.5 kb of vector plasmid pEAK8. Exactly 1,316bp of the clone were analyzed by sequencing. 1,248 bp of 1,316 bp were identical (~100%) to the exons 72-74 of heparan sulfate proteoglycan 2 (HSPG2).

Heparan sulfate proteoglycan 2 (HSPG2) is the major heparan sulfate proteoglycan of basement membranes and the gene located on chromosome 1p36.1. It possesses angiogenic and growth-promoting attributes primarily by acting as a co-receptor for basic fibroblast growth factor (bFGF). BFGFs have diverse functions in cell growth and differentiation. In addition, multiple roles have been proposed for bFGFs in cancer origin and progression. BFGFs signal through transmembrane receptor tyrosine kinases. This signalling is regulated by a balance of HSPGs that either stimulate or inhibit bFGF binding to its bFGF receptor. Even though HSPG2 is not a tyrosine kinase, the involvement of HSPG in breast cancer progression as reported in the literature, makes this gene a worthwhile addition to our expression panel.

However, Pangene Corp. was not able to provide a cDNA clone for our second sequence of interest. We therefore initiated a collaborative effort with Dr. Melvin Simon at the California Institute of Technology, Pasadena, CA, to screen BAC libraries for genomic clones containing our genes of interest. Seven screenings using DNA sequence information from six of our clone tk fragment resulted in 16 BAC clones.

**Task 2. Measure tk gene expression in cell lines, normal and tumor tissues (months 3-24)**

Completed as proposed and described in previous progress reports.

***2.1 Prepare DNA microarrays carrying about 100 different sequences (months 3-24)***

***2.2 Optimize hybridization conditions to provide quantitative information (months 6-24)***

CDNA micro-arrays were prepared in-house on poly-L-lysine coated slides and hybridized with CY3- and CY5-labeled probes (Hsieh et al., 2001). CDNA probes prepared from other tissues or cell lines such as thyroid cells lines known to express a particular tk gene at high levels were used as controls. The second year effort was directed towards the optimization of cDNA preparation, labeling, hybridization and detection protocols.

***2.3 Optimize PCR parameters for quantitative amplification of target genes (months 12-18)***

We have worked out an effective method to generate probe for our microarray experiments. In our original method, we isolate total RNA from frozen tissues cell lines and prepared cDNAs from the RNA by random priming and reverse transcription, before we amplify tk-specific cDNA fragments with our mixed-base F-/R-TYRK primers.

In the course of our experiments, we have discovered that this probe synthesis method can generate quality microarray data. This method is particularly good at assaying the expression of tk genes that are poorly or moderately expressed. We subjected our results to independent validations with colleagues expert in the expression of tk genes in the breast cells. We have the learned that our original method seems to recapitulate the results of other investigators (using Northern blots) for moderate and low expressing tk genes. However, we have also learned that highly expressed tk gene expression is less accurately represented in out experiments than we would prefer. The rank

ordering of the high expressing genes is maintained (i.e. for tk genes that are highly expressed, a lower-expressed tk gene indeed appears to be expressed less than a higher-expressed tk genes). However, the apparent difference two highly expressed genes are less than one might expect. In essence, our original probe synthesis scheme is not completely representative of total tk expression.

### *2.4 Develop algorithms for array readout and comparisons between measurements (months 9-24)*

A Axon GenePix 4000 (Axon Inc.) array scanner used to acquire all images has a preview resolution of 40 μm and a scanning resolution of 10 μm. The photomultiplier sensitivity can be adjusted by the user during preview to optimize the signal intensity. GenePix 3.0 (Axon Inc.), an image acquisition and analysis software, was used to analyze the images acquired from our tk arrays and provided numerical data that was imported into spreadsheets (Microsoft Excel) for further analysis. For display purposes, the images were saved in standard formats and imported into common graphics programs such as Adobe Photoshop. Given the relatively small numbers of arrays that have been hybridized so far, it was sufficient to normalize the data and compare numeric values in spreadsheets.

## Task 3. To validate assays for multigene expression profiling in small amounts of tissue (months 18-36)

In accordance with the approved milestones our efforts focused on improving system parameters to allow tk gene fragment amplification from small tissue samples and validation of micro-array results. The analysis of small samples required a modified DNA amplification scheme as described below (Task 3b). CDNA micro-array results were validated using cDNA in situ hybridization (Lersch et al., 2001). Briefly, selected cDNA clones were purchased from Research Genetics and used to prepare probes for RNA FISH. We established a FISH protocol that allowed us to hybridize five to seven uniquely labeled cDNA probes simultaneously. We use a Spectral Imaging approach to identify and quantitate the amount of bound probe, which reflects the intracellular amount of the target RNA. This assay seems to work well on cultured cells and tissue sections.

### *Task 3.1. Develop software for databasing, automated analysis of expression profile datasets and their annotation (months 18-24)*

Given the rather small number of cDNA samples analyzed to date, we decided to handle all results in form of Microsoft Excel spreadsheets.

### *Task 3.2. Test the system with serial dilutions of cells (months 24-30)*

The RT-PCR approach using mixed-base primers defined by the conserved amino acids may be the method of choice to clone and identify novel tk genes expressed in these tumors, but it has two major disadvantages. First, it leads to non-linear amplification of individual cDNA species. Expression levels measured with the cDNA microarray method differed from those estimated by

Southern blot analyses, and generally showed a reduced dynamic range. We attributed this to the fact that the initial set of mixed-base oligonucleotide primers was comprised of 1024 and 6144 different sequences for the forward and reverse primers, respectively. This rather high number of different sequences had two consequences: 1. The concentration of any one particular primer was very low, and 2. forward and reverse primers were used in different concentrations. We therefore redesigned our PCR primers using the published cDNA sequences and optimized the PCR amplification scheme. Although the new primer set is similar to the previous set, the complexity of the new primers was decreased 1.8-fold for the new forward primer and 3-fold for the reverse primer. This lead to increased specificity and amplification efficiency.

The second disadvantage of the use of mixed-based primers is that each primer is present in a rather low concentration in the RT-PCR reaction. Different amounts of individual primer sequences are needed, since genes are expressed at vastly different levels. Thus, during DNA amplification some primers are used up faster than others leading to reduced amplification efficiencies, so-called 'amplification bias'. We addressed this problem by designing a two-step amplification protocol using tk-specific primers during the first few cycles, followed by high efficiency PCR using a 5'-adaptor primer in subsequent amplification cycles.

***Task 3.3. Test the assay with microdissected tissue from breast cancer sections (months 30-36)***

Tests with serial dilutions of cells from several of the cell lines used in the initial stages of this project suggested that the assay was sensitive enough to measure the levels of tk gene expression in as little as 100-200 cells. We selected frozen tissues blocks from normal ('NL') breast and tumor tissues and cut sections of about 8-10 μm. The tissue sections were mounted on positively charged microscope slides (Fisher 'Probe on Plus') and stained with 0.5% Methyl Green for 30 sec. Microdissection was performed manually using a dissecting microscope and scalpels to collect between 100 and 100,000 cells from normal tissues and 3 tumor specimens (Table II, Fig.1). The RNA was isolated from duplicate samples using a Qiagen RNeasy kit and transcribed into cDNA using Promega's Reverse Transcription kit. Aliquots of the cDNA representing an amount of cDNA prepared from the equivalent of 10, 100 or 1000 cells were used in PCR reactions using our tk-specific primer set TYRK4.

Following the initial PCR amplification, one half of each PCR reaction was subjected to a second in vitro DNA amplification reaction using the 5-adaptor primer (H3T7). This template DNA was equivalent to TYRK4 PCR products generated from 5, 50 or 500 cells (Fig. 2). The PCR products from the H3T7 reaction were concentrated 7.5-fold by alcohol precipitation and resuspension in 10 μl. One microliter of each of the concentrated H3T7 PCR products was then labeled in 25 μl random priming reactions using Cy3- or Cy5-dUTP. Probes were concentrated 2-fold using Microcon-30 columns and 1 μl of each probe was hybridized for 16 hours to the tk arrays (Table III). Hybridization results (Fig.3) demonstrated consistent tk gene overexpression in all three tumors. Most notably, we found high levels of expression of abl, ufo and MKK3 (Fig.3), which were expressed at much lower levels in normal breast tissue (Fig. 4). The results for sample sizes equivalent to the amount of cDNA prepared from 10 cell equivalents are shown in Fig. 3 and 4. In Fig. 3A the tumor-derived cDNA sample was labeled with Cy5 (shown in red) and was hybridized against the tk gene PCR product from normal tissue (Cy5-labeled, shown in red). We confirmed the overexpression of abl, ufo and MKK3 by repeating the experiments with the tumor-derived cDNA labeled with CY3 and the normal control tissue-derived cDNA labeled in Cy3 ('dye swapping' experiment, Fig. 3B).

| Sample | Approximate # Cells Dissected | ul RNeasy Buffer RLT | # Eppendorf Tubes |
|---|---|---|---|
| **Normal Breast #1—Slide #3** | **100 (1x10$^{2}$)** | **300** | **2** |
| **Normal Breast #1—Slide #3** | **1,000 (1x10$^{3}$)** | **300** | **2** |
| **Normal Breast #1—Slide #4** | **100,000 (1x10$^{5}$)** | **300** | **2** |
| **B859 Tumor** | **100 (1x10$^{2}$)** | **300** | **2** |
| **B859 Tumor** | **1,000 (1x10$^{3}$)** | **300** | **2** |
| **B859 Tumor** | **100,000 (1x10$^{5}$)** | **300** | **2** |
| **B865 Tumor** | **100 (1x10$^{2}$)** | **300** | **2** |
| **B865 Tumor** | **1,000 (1x10$^{3}$)** | **300** | **2** |
| **B865 Tumor** | **100,000 (1x10$^{5}$)** | **300** | **2** |
| **B778 Tumor** | **100 (1x10$^{2}$)** | **300** | **2** |
| **B778 Tumor** | **1,000 (1x10$^{3}$)** | **300** | **2** |
| **B778 Tumor** | **100,000 (1x10$^{5}$)** | **300** | **2** |
| **B767 Tumor** | **100 (1x10$^{2}$)** | **300** | **2** |
| **B767 Tumor** | **1,000 (1x10$^{3}$)** | **300** | **2** |
| **B767 Tumor** | **100,000 (1x10$^{5}$)** | **300** | **2** |

**Table II:** The microdissection experiments using normal breast and tumor tissue specimens. Using a dissecting microscope and a scalpel, we picked up between 100 and 100,000 (estimated) cells from Methyl Green stained sections.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | GAPDH | HPRT | RPL13A | K-SAM | c-mer | CLK3 | flg | HSIGFIRR | blk | c-src | EphA2 | EphA4 |
| 2 | HSLK/KIAA0204 | PTK6 | c-syn/FYN | EGFR | HLA-A | HSUO7349 | c-abl | c-yes-1 | blk-like | EphA1 | EphB4 | c-fms |
| 3 | pkC-delta | fer | HSEGF01 | HUMP4K | HUMPKSCD | JAK3 | MKK3 | RON | tyk2 | EphA4 | lck | MLK-3 |
| 4 | TEK | TYRO3 | ZAP-70 | LYN | EphB1 | UFO | IRR | MEK3 | PDGF | tie | arg | JAK1 |
| 5 | met | PYK2/FAK2 | DRR1 | MKK3 | pra src pp60 | clk1 | MAPK1 | no match | serium inducible | v-abl | Ste20-like | cosmid B1E7 |
| 6 | G-protein-coupled | GAPDH | HPRT | RPL13A | TRK | ret (PTC3) | EphA2 | ret (PTC3) | ret (HHCC55 | TRK | erbB2 | EphB2 |
| 7 | GAPDH | HPRT | RPL13A | K-SAM | c-mer | CLK3 | flg | HSIGFIRR | blk | c-src | EphA2 | EphA4 |
| 8 | HSLK/KIAA0204 | PTK6 | c-syn/FYN | EGFR | HLA-A | HSUO7349 | c-abl | c-yes-1 | blk-like | EphA1 | EphB4 | c-fms |
| 9 | pkC-delta | fer | HSEGF01 | HUMP4K | HUMPKSCD | JAK3 | MKK3 | RON | tyk2 | EphA4 | lck | MLK-3 |
| 10 | TEK | TYRO3 | ZAP-70 | LYN | EphB1 | UFO | IRR | MEK3 | PDGF | tie | arg | JAK1 |
| 11 | met | PYK2/FAK2 | DRR1 | MKK3 | pra src pp60 | clk1 | MAPK1 | no match | serium inducible | v-abl | Ste20-like | cosmid B1E7 |
| 12 | G-protein-coupled | GAPDH | HPRT | RPL13A | TRK | ret (PTC3) | EphA2 | ret (PTC3) | ret (HHCC55 | TRK | erbB2 | EphB2 |

**Table III:** The organization of our tk DNA micro-arrays used to study gene expression in microdissected breast cancer tissue specimens.

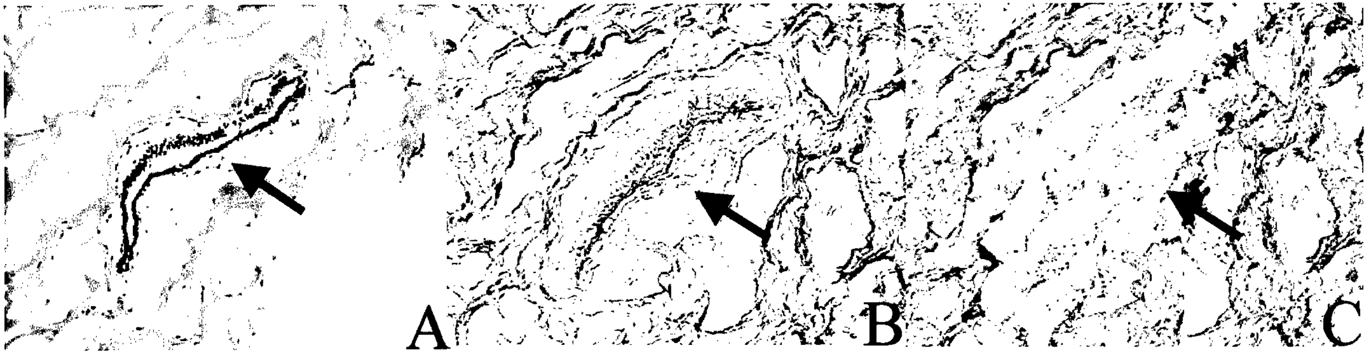


**Figure 1:** A breast tissue section before (A: H&E stain, B: Methyl Green) and after (C) dissection.

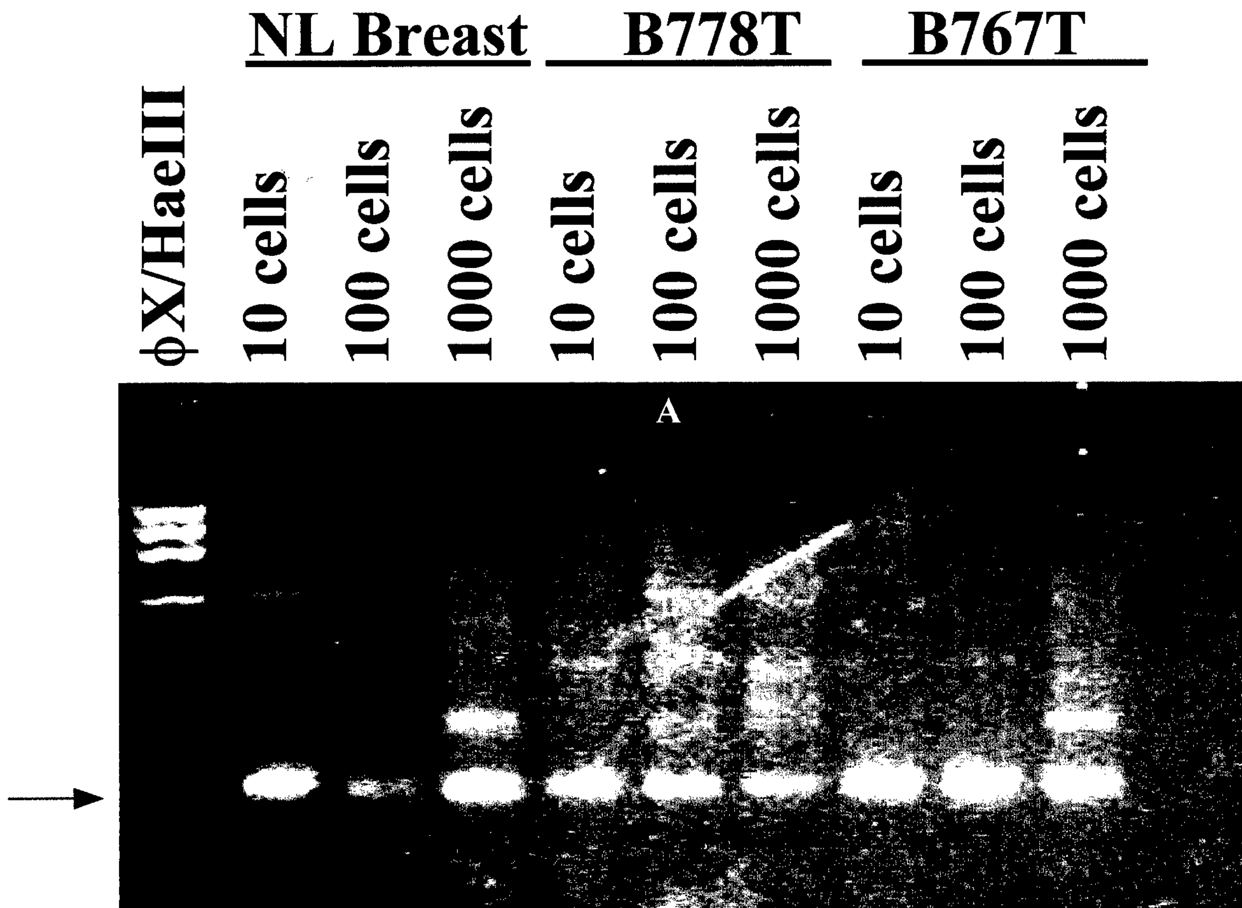


**Figure 2:** The amplification products using the tk gene specific primers. The arrow points at the tk domain-specific products of about 200 bp.

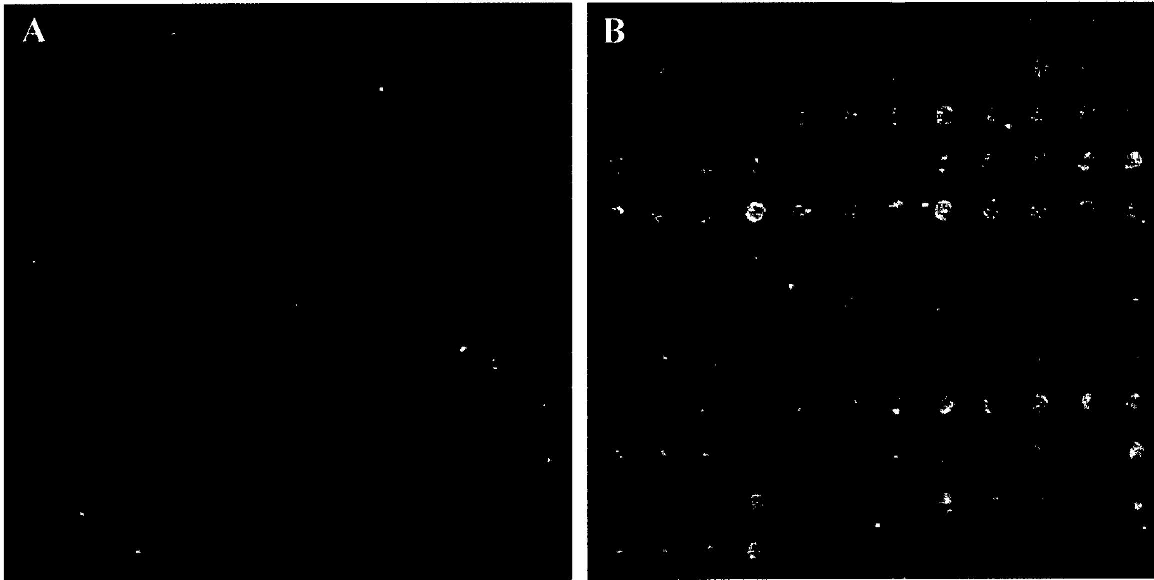


**Figure 3:** The amplification products using the tk gene specific primers hybridized to the tk array. This picture illustrates the 'dye swapping' experiment. Figure A show the results using Cy5-labeled products from tumor sample 767T (10 cell equivalents, shown in red) hybridized against the PCR products from normal breast tissue (NL, 10 cell equivalents) labeled with Cy3 (shown in green). The hybridization result in B shows the opposite labeling scheme: cDNA from tumor sample 767T (10 cell equivalents) was labeled with Cy3 (green) and hybridized against Cy5-labeled PCR products from normal breast tissue (shown in red).

**Figure 4:** The amplification products prepared from normal breast tissue (10 cell equivalents) labeled with Cy3 hybridized against Cy5-labeled PCR products from the same normal tissue.

## KEY RESEARCH ACCOMPLISHMENTS:

- Finished the isolation of RNA and preparation of cDNA from 16 breast cancer cell lines and 10 frozen tissue specimens
- Completed the PCR-amplification of tk-specific DNA fragments and cloned the products into plasmids
- Pre-screening more than 400 breast cancer cell line-derived clones and sequenced an additional 100 clones, database searches identified two clones containing potentially novel tyrosine kinase genes
- Expanded the panel of tyrosine kinase genes used for expression profiling and printed second generation cDNA micro-arrays
- Generated artificial mixtures of tk DNA fragments to be used as reference DNA
- Reconfirmed tk gene expression changes as breast epithelial cells become tumorigenic and grow anchorage-independent using second generation tk micro-arrays
- Isolated BAC clones for novel tk genes
- Developed a Spectral Imaging-based system to quantitate expression levels of 5 different tk genes on a cell by cell base
- Developed a 2-step in vitro DNA amplification protocol that provides improved efficiency while minimizing amplification bias
- Demonstrated the feasibility of tk gene expression profiling using cDNA prepared by tissue microdissection from as little as 5-10 cell equivalents

## REPORTABLE OUTCOMES:

- **manuscripts**


1. Hsieh H-B, Lersch RA, Callahan DE, Hayward S, Wong M, Clark OH, Weier H-UG (2001) Monitoring signal transduction in cancer: cDNA microarray for semi-quantitative analysis. J Histochem Cytochem 49: 1057-1058
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11. Lersch RA, Fung J, Hsieh H-B, Smida J, Weier H-UG(2001) Monitoring signal transduction in cancer: from chips to FISH. 2001 Annual Meeting of the Histochemical Society, February 3-6, 2001, Santa Fe, NM.
12. Weier H-UG, Zitzelsberger HF, Hsieh H-B, Sun MV, Wong M, Lersch RA, Yaswen P, Smida J, Kuschnick C, Clark OH (2001) Monitoring signal transduction in cancer: tyrosine kinase gene expression profiling. 2001 Annual Meeting of the Histochemical Society, February 3-6, 2001, Santa Fe, NM.
13. Lersch RA, Chu LW, Ito Y, Weier H-UG (2001) Toward Tyrosine Kinase Expression Profiling at the Single Cell Level. The Sixth Joint Meeting of the of the Japan Society of Histochemistry and Cytochemistry and the Histochemical, July 18-21, 2001, Seattle, WA.
14. Weier H-UG, Ito Y, Fung J, Lehmann L, Lersch RA, Chu LW, Zitzelsberger HF (2001) Chromosome rearrangements in a cell line derived from a case of childhood papillary thyroid cancer (chPTC) with radiation history. The Sixth Joint Meeting of the of the Japan Society of Histochemistry and Cytochemistry and the Histochemical, July 18-21, 2001, Seattle, WA.
15. Ito Y, Fung J, Hsu J, Katzir N, Lersch RA, Weier H-UG (2001) Phenotype analysis of tumor cells with eight color FISH. The Sixth Joint Meeting of the of the Japan Society of Histochemistry and Cytochemistry and the Histochemical, July 18-21, 2001, Seattle, WA.

- **funding obtained**

California Cancer Research Program, 'Tyrosine Kinase Gene Expression Profiling in Prostate Cancer', Pilot and Feasibility Study Award, H.-U. Weier (P.I.), 7/01/00-6/30/03

National Institute of Health, 'Spectral Karyotyping for Phenotype Analysis of Cancer Cells', R21/R33 grant, H.-U. Weier (P.I.), 9/01/00-8/31/03

Department of Defense Prostate Cancer Research Program, Tyrosine Kinase Gene Expression Profiling in Prostate Cancer, H.-U. Weier (P.I.), 03/28/00-03/31/04

Deartment of Defense Prostate Cancer Research Program, Dietary determinants of prostate cancer progression, Chu, Lisa W. (Post Doctoral Fellow), 01/01/03-12/31/04

## CONCLUSIONS:

This IDEA project demonstrated the feasibility of performing gene expression profiling in breast cancer specimens using short gene specific DNA fragments, and delivered information about changes in tk gene expression as cell and cell lines progress towards a more malignant phenotype. A modified 2-step in vitro DNA amplification procedure lead to increased sensitivity and minimized amplification bias. While the measurements in the present study were performed with cDNA microarrays on glass, other approaches for DNA quantitation such as electrochemical sensors might lower the cost per assay without sacrificing assay sensitivity.

## REFERENCES:


Hsieh H-B, Lersch RA, Callahan DE, Hayward S, Wong M, Clark OH, Weier H-UG (2001) Monitoring signal transduction in cancer: cDNA microarray for semi-quantitative analysis. J Histochem Cytochem 49: 1057-1058

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Weier H-UG, Zitzelsberger HF, Hsieh H-B, Sun MV, Wong M, Lersch RA, Yaswen P, Smida J, Kuschnick C, Clark OH (2001) Monitoring signal transduction in cancer: tyrosine kinase gene expression profiling. J Histochem Cytochem 49:673-674.


## APPENDICES:

1. The approved Statement of Work
2. Personnel Supported

## III-A.4.f. Statement of Work

**Title: Expression Profiling of Tyrosine Kinase Genes**

**P.I.: Heinz-Ulrich G. Weier**

*Task 1.* To identify tyrosine kinase (tk) genes expressed in normal and neoplastic breast tissues (months 1-24)

- prepare cDNAs from ten cell lines and ten frozen tissue specimens (months 1-18)
- perform RT-PCR reactions and clone PCR products in plasmids (months 3-18)
- perform pre-screening with known tk fragments, cDNA sequencing and database searches (months 5-22)
- add novel clones to the panel of expressed tk gene fragments (months 6-24)

*Task 2.* To measure tk gene expression in cell lines, normal and tumor tissues (months 3-24)

- prepare DNA microarrays carrying about 100 different sequences (months 3-24)
- optimize hybridization conditions to provide quantitative information (months 6-24)
- optimize PCR parameters for quantitative amplification of target genes (months 12-18)
- develop algorithms for array readout and comparisions between measurements (months 9-24)

*Task 3.* To validate assays for multigene expression profiling in small amounts of tissue (months 18-36)

- develop software for databasing, automated analysis of expression profile datasets and their annotation (months 18-24)
- test the system with serial dilutions of cells (months 24-30)
- test the assay with microdissected tissue from breast cancer sections (months 30-36)

## Appendix 2

## Personnel

1. **Fiscal Year 1999**

**Weier, H.-U.G.**

2. **Fiscal Year 2000**

**Weier, H.-U.G.**

3. **Fiscal Year 2001**

**Guan, J.**

**Weier, H.-U.G.**

4. **Fiscal Year 2002**

**Guan, J.**

**Ito, Y.**

**Weier, H.-U.G.**

5. **Fiscal Year 2003**

**Guan, J.**

**Li, Z.**

**Weier, H.-U.G.**

**Zhang, J.**