GRANT NUMBER DAMD17-94-J-4177

TITLE: Breast Cancer Resource for Research and Banking, with Emphasis on Early Tumors and Precursor Lesions

PRINCIPAL INVESTIGATOR: Helen Feiner, M.D.

CONTRACTING ORGANIZATION: New York University Medical Center
New York, New York 10016

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Fort Detrick, Frederick, Maryland 21702-5012

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Breast Cancer Resource for Research and Banking, with Emphasis on Early Tumors and Precursor Lesions

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13. ABSTRACT (Maximum 200)
The Breast Cancer Resource for Research and Banking has accrued breast cells or tissues from 1,467 patients during the grant period (3 year grant with 9 month extension without additional funding). The emphasis of this project has been on the collection of microscopic at risk and precursor lesions as imprints/scrapes. Additionally, throughout the grant period, all invasive carcinomas with available tissue have been accrued, since most investigators who have requested samples have requested frozen pieces of tumor tissue paired with normal tissue from the same patient, i.e. their interest has been in established carcinomas and not in precursor lesions. During the last year we also filled requests for specimens for microdissection. Over the entire grant period 26 investigator requests for tissue have been filled. At termination of the grant the Breast Cancer Resource was transferred to the auspices of the Resource for Tumor Tissue and Data of the NYU Kaplan Comprehensive Cancer Center.

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For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

[Signature]

PI - Signature  Date
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INTRODUCTION

Basic, clinical, and translational research on breast cancer in the United States has been stimulated in recent years by increased government and private funding. Translational research, in particular, requires the availability of human breast cancer tissue, as well as breast tissue with "precursor" and "at risk" lesions. At risk lesions are the proliferative and atypical proliferative components of fibrocystic change, and the precursor lesion is carcinoma in situ. These lesions have been defined histologically and their roles in breast carcinogenesis have been validated epidemiologically. This grant was funded in the category of "Infrastructure Enhancement" specifically to make breast cancer tissue, precursor, and at risk lesions available to investigators in the field of human breast carcinogenesis.

METHODS

Clinical cancers, that is invasive carcinomas that have resulted in a palpable mass lesion, were banked in standard fashion as snap-frozen pieces of tissue, together with pieces of non-neoplastic breast tissue from the same patient, and a portion of lymph node when available.

Collecting at risk and precursor lesions of breast cancer, which are almost invariably microscopic, is difficult, firstly because the lesions are so small, and secondly because good medical practice requires that all the tissue excised be subjected to routine histopathologic examination in order to properly classify the lesion.

Accordingly, in years 1 to 3 of the grant we collected at risk and precursor lesions of breast cancer as slide imprints/scrapes prepared from excised breast tissue prior to histopathologic examination. By the end of year two 782 imprint samples had been collected from mammographically detected (non-palpable) lesions. These covered the spectrum of fibrocystic change (non-proliferative, proliferative, and proliferative with atypia), as well as ductal carcinoma in situ and lobular carcinoma in situ. It was disappointing that despite considerable effort to publicize this collection of material, investigators did not request it. The reasons were twofold. Firstly, most basic scientists are unfamiliar with the histopathologically defined at risk and precursor lesions of breast cancer; they are more interested in established cancers (mass lesions). Secondly, the samples are small and comprised of mixtures of cells (of necessity stromal cells and lymphohistiocytic cells are admixed with lesional cells in imprint/aspirate specimens). At about the same time the technique of microdissection was evolving, and provided an alternate method for acquiring such lesions for research purposes. Accordingly in year 3 and in the extension period we have been supplying investigators with material prepared for microdissection. This technique allows one to obtain pure specimens of microscopic precursor and at risk lesions, from either fixed paraffin embedded tissue (for DNA - PCR studies) or from frozen sections (for RNA based studies). Specimens obtained by microdissection are superior to imprints and aspirates inasmuch as the histologic context from which samples are obtained can be documented, and the samples are pure. Because of the aforesaid, in year 3 we turned our efforts away from imprints and toward providing samples for microdissection.
Use of the Resource has been stimulated by the award of 16 pilot projects from developmental funds from the Kaplan Comprehensive Cancer Center's NCI Breast Cancer Program Grant during the 1995-1998 period.

Outside NYU, the Resource has been included in the Breast Cancer Specimen and Data Information System, a collaborative project sponsored by the National Action Plan for Breast Cancer Biologic Resources Banks Working Group and the NCI. The DOD Breast Cancer Research Program "Era of Hope" in Washington D.C. in October/November 1997 provided another forum for publicizing the Resource.

To obtain feedback on the satisfaction of investigators with the material sent to them, two contacts are made with each recipient. The first is to determine the state of material given or shipped and occurs within a day or two of shipping. The second contact is made 6 - 18 months later to determine the level of satisfaction in terms of results obtained. User files are maintained for each recipient of samples. User records are initiated with "Investigator Request" forms (Appendix 1).

To obtain information on the quality and durability of banked tissues and cells, specimens obtained in 1995, 1996 and 1997 have been subjected to a variety of analyses. These analyses were immunohistochemistry, immunofluorescence microscopy, fluorescence in situ hybridization, and RT-PCR. Analyses were done in various laboratories at NYU that have expertise in these assays. Records are maintained on "Evaluation of Banked Material" forms (Appendix 2).

The Resource technician has also culled the NYU departmental records retrospectively so that all patients with mammographically detected lesions from 1991-1994 have been entered into the database. Even though no fresh samples (imprints/aspirates) are available in these cases, the ability to microdissect the archival samples has made them a valuable addition to the Resource.

RESULTS

The numbers of the various types of breast tissue samples that have been banked and entered into our database during each grant year, as well as the cumulative numbers of samples for the entire collection period are shown in Tables 1 to 4. Tables 1 and 2 use the format of previous annual reports. Tables 3 and 4 show data for all four years.

In Table 1 the breakdown is by type of samples available. In Table 2 the breakdown is by type of lesion as defined histopathologically. Total number of samples in Table 1 exceeds total number of cases in Table 2 because some cases (patients) generated more than one sample type.
TABLE 1
BANKED CASES OF BREAST CANCER AND PRECURSOR LESIONS
AT NYU MEDICAL CENTER BY SAMPLE TYPES

<table>
<thead>
<tr>
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<th>Grant Year #4 12/97 - 8/98</th>
<th>4 Yr. Cumulative 12/94 - 8/98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imprints/scrapes</td>
<td>0</td>
<td>633</td>
</tr>
<tr>
<td>Aspirated cells</td>
<td>115</td>
<td>642</td>
</tr>
<tr>
<td>Snap frozen tissue fragments*</td>
<td>69</td>
<td>757</td>
</tr>
<tr>
<td>TOTAL</td>
<td>184</td>
<td>2,032</td>
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</tbody>
</table>

*includes 308 paired samples of breast cancers with normal tissue.

TABLE 2
BANKED CASES OF BREAST CANCER AND PRECURSOR LESIONS
AT NYU MEDICAL CENTER BY HISTOPATHOLOGIC DIAGNOSIS

<table>
<thead>
<tr>
<th></th>
<th>Grant Year #4 12/97 - 8/98</th>
<th>4 Yr. Cumulative 12/94 - 8/98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive ductal carcinoma</td>
<td>67</td>
<td>383</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>12</td>
<td>59</td>
</tr>
<tr>
<td>Ductal carcinoma in situ*</td>
<td>7</td>
<td>151</td>
</tr>
<tr>
<td>Lobular carcinoma in situ*</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>Secondary carcinoma, lymph node</td>
<td>27</td>
<td>110</td>
</tr>
<tr>
<td>Lymph node without tumor</td>
<td>0</td>
<td>81</td>
</tr>
<tr>
<td>Fibrocystic change, non-proliferative</td>
<td>1</td>
<td>190</td>
</tr>
<tr>
<td>Fibrocystic change, proliferative**</td>
<td>1</td>
<td>215</td>
</tr>
<tr>
<td>Fibrocystic change, proliferative with atypia**</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>Other (mostly fibroadenoma)</td>
<td>13</td>
<td>153</td>
</tr>
<tr>
<td>TOTAL</td>
<td>128</td>
<td>1,468</td>
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</table>

*precursor lesion **at risk lesion

Table 5 indicates the number of patients from whom samples were obtained during years 1 and 3 and the extension period, and during the entire grant period. Table 6 indicates the numbers of requests for specimens that have been filled over similar time periods.
### TABLE 3

**SPECIMENS BY SAMPLE TYPE**

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<tr>
<td>Imprints/TP</td>
<td>367</td>
<td>415</td>
<td>102</td>
<td>0</td>
<td>633</td>
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<tr>
<td>Aspirates</td>
<td>149</td>
<td>219</td>
<td>159</td>
<td>115</td>
<td>642</td>
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<tr>
<td>Tissue</td>
<td>233</td>
<td>199</td>
<td>256</td>
<td>69</td>
<td>757</td>
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<td><strong>TOTAL</strong></td>
<td>749</td>
<td>833</td>
<td>517</td>
<td>184</td>
<td>2,032</td>
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</table>

### TABLE 4

**SPECIMENS BY HISTOPATHOLOGIC DIAGNOSIS**

<table>
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<th></th>
<th></th>
<th></th>
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<tr>
<td>Invasive ductal carcinoma</td>
<td>118</td>
<td>112</td>
<td>86</td>
<td>67</td>
<td>383</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>16</td>
<td>20</td>
<td>11</td>
<td>12</td>
<td>59</td>
</tr>
<tr>
<td>In situ ductal</td>
<td>55</td>
<td>50</td>
<td>39</td>
<td>7</td>
<td>151</td>
</tr>
<tr>
<td>In situ lobular</td>
<td>11</td>
<td>25</td>
<td>10</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>Secondary carcinoma</td>
<td>25</td>
<td>23</td>
<td>35</td>
<td>27</td>
<td>110</td>
</tr>
<tr>
<td>FCD - proliferative</td>
<td>86</td>
<td>92</td>
<td>90</td>
<td>1</td>
<td>269</td>
</tr>
<tr>
<td>FCD - non-proliferative</td>
<td>71</td>
<td>107</td>
<td>11</td>
<td>1</td>
<td>190</td>
</tr>
<tr>
<td>Other</td>
<td>48</td>
<td>104</td>
<td>97</td>
<td>13</td>
<td>262</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>430</td>
<td>533</td>
<td>379</td>
<td>128</td>
<td>1,470</td>
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### TABLE 5

**NUMBER OF PATIENTS WITH BANKED SAMPLES**

<table>
<thead>
<tr>
<th>Grant Year #1</th>
<th>Grant Year #2</th>
<th>Grant Year #3</th>
<th>Grant Year #4</th>
<th>4 Yr. Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>430</td>
<td>537</td>
<td>365</td>
<td>135</td>
<td>1,467</td>
</tr>
</tbody>
</table>
### TABLE 6

**REQUESTS FOR SPECIMENS FILLED**

<table>
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<tr>
<th></th>
<th>Grant Yr. #1 12/94 - 11/95</th>
<th>Grant Yr. #2 12/95 - 11/96</th>
<th>Grant Yr. #3 12/96 - 11/97</th>
<th>Grant Yr. #4 12/97 - 8/98</th>
<th>Cumulative 12/94 - 8/98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imprints/scrapes</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Frozen tissue</td>
<td>2</td>
<td>4</td>
<td>9</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Tissue for</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>microdissection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>7</td>
<td>26</td>
</tr>
</tbody>
</table>

As shown in Table 3, we reduced the numbers of imprint/scrape samples collected in years 3 and 4 of the grant. These represent samples of microscopic lesions, mainly in situ carcinoma and proliferative fibrocystic changes. The reason for this reduced collection is twofold. Firstly, we now have a large collection of these lesions and requests for such samples have been very low. Secondly, the technique of microdissection has been gaining increasing favor as an alternative method for obtaining samples of microscopic lesions. Current amplification techniques allow the analysis of cells from a single microdissected duct or lobule of breast tissue. Microdissection can be done on frozen or on fixed, paraffin embedded tissue. Furthermore, the purity of specimens can be monitored by examination of sections before and after the microdissection is done. The success of this technique may be the reason for the underutilization of our imprint/scrape samples. Prior to the use of microdissection, scrapes/imprints represented the only means for obtaining precancerous and microscopic breast lesions for research purposes. The disadvantages of imprint/scrapes as compared to microdissection relates to the fact that imprint/scrape samples represent mixtures of cells, albeit the lesional cells predominate. In both instances the samples are small, but investigators prefer to use samples of known and verifiable purity.

There have been several opportunities for publicizing the Resource at NYU. It has been written up three times in the Kaplan Comprehensive Cancer Center newsletter, "LAB NOTES". The principal investigator has lectured on the Resource to the Kaplan Comprehensive Cancer Center Core Grant Working Group and at the NYU Breast Cancer Research Program (BCRP). She is also a major participant at the NYU monthly clinical multidisciplinary breast cancer conferences and a member of the Executive Committee of the NYU Breast Center, both of which provide forums for continually updating colleagues on the size of the Resource and the spectrum of available material. Additionally, the Kaplan Comprehensive Cancer Center Breast Cancer Research Program Grant has funded pilot projects for translational research from 1995-1998 generating intramural users (Appendix 3).

Our Internet listing through the National Action Plan has generated 6 outside users of the Resource, one in 1996, three in 1997, and two in 1998.

Based on investigator feedback, our efforts in filling requests for specimens and determining investigator satisfaction with specimens has produced results ranging from good to
excellent. All investigators have been very satisfied with the state in which they have received specimens shipped or delivered to them. Feedback from 1995, 1996, and early 1997 recipients indicates that the material was suitable for the research techniques that they used. An example of such feedback and publications referring to the Resource and its funding source are shown in Appendix 1.

Several slide-based techniques performed in the principal investigator's department and elsewhere in the Medical Center produced good results of immunohistochemistry (Appendix 2) and immunofluorescence microscopy on archived samples. Fluorescent in situ hybridization (Appendix 2) results have been excellent on 1997 samples, and good on 1995 and 1996 samples.

At termination of the grant the Breast Tissue Resource is being transferred to the auspices of the Resource for Tumor Tissue and Data of the Kaplan Comprehensive Cancer Center. Thus, the Resource technician, materials, and database will remain available. Collection of samples will continue and the materials collected will remain available to investigators.

CONCLUSIONS

The Resource has acquired 2,032 specimens from 1,467 patients.

Requests for snap frozen samples of established breast cancers, matched with normal tissue from the same patient are the most frequent requests received.

Sample preservation is good.

We have met investigator's needs in all instances.

The Resource has provided the principal investigator with outstanding opportunities for ongoing collaboration in various aspects of breast cancer research (1-13).

REFERENCES


12. Cui, X., Li, H., Liu, J., and Feiner, H. Genetic analysis of tubular carcinoma using microdissected samples. (Submitted)

13. Cui, X., Feiner, H., and Li, H. Development of tubular carcinoma of the breast may involve a distinct gene set. (Submitted)

LIST OF PERSONNEL RECEIVING PAY FROM THIS EFFORT

Helen Feiner, M.D.
Jaishree Jagirdar, M.D.
Ms. Yara Delgado
<table>
<thead>
<tr>
<th><strong>Name:</strong></th>
<th>Dr. CHARLES CARMECI/ D. THOMPSON Ph.D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title:</strong></td>
<td>MD/Ph.D</td>
</tr>
<tr>
<td><strong>Address:</strong></td>
<td>STANFORD UNIVERSITY. DEPTO OF SURGERY MSLP 229. 1201 WELCH RD STANFORD, CA 94305</td>
</tr>
<tr>
<td><strong>Phone:</strong></td>
<td>(415) 725-1671 (415) 498-5510</td>
</tr>
<tr>
<td><strong>Fax:</strong></td>
<td>(415) 725-8762</td>
</tr>
<tr>
<td><strong>e-mail:</strong></td>
<td>--</td>
</tr>
<tr>
<td><strong>Grant Support:</strong></td>
<td>YES</td>
</tr>
<tr>
<td><strong>Material Requested:</strong></td>
<td>BREAST TISSUE IN VIAL CONFIRMED ER+ AND ER-</td>
</tr>
<tr>
<td><strong>Date Shipped:</strong></td>
<td>8-5-96  4-21-97</td>
</tr>
<tr>
<td><strong>Date Received:</strong></td>
<td>NEXT DAY</td>
</tr>
<tr>
<td><strong>State of Specimen on receipt:</strong></td>
<td>GOOD</td>
</tr>
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</table>

**Brief Summary of intended use:**
(Use additional page if necessary)
TO IDENTIFY AND CHARACTERIZE GENES THAT ARE COORDINATELY EXPRESSED WITH ER AND DETERMINE THEIR INFLUENCE ON BREAST CANCER PROTOTYPE.
Dear Dr. Feiner,

Thank you for the primary breast cancer specimens. They arrived in excellent condition.

We have recently isolated and partially characterized several genes from breast cancer cell lines which are coordinately expressed with the gene for estrogen receptor. We feel that this set of genes plays a critical role in determining the differing phenotypes between ER positive and ER negative carcinomas. Using Northern blots from the samples which you have provided, we aim to determine the expression of these genes in primary tumors. The NIH has provided funding for this project (Grant #: NIH/NRSA#1F32CA69715-01A1 PI: Ronald Weigel, MD, PhD).

Thank you for providing such a valuable resource.

Charles Carmeci, MD
As discussed, information to add to your publication(s):

Acknowledgment. Breast cancer tissue was obtained from the Breast Cancer Resource of the Department of Pathology, N.Y.U. Medical Center, Dr. Helen Feiner, Director. The resource is funded by The Department of the Army, as Grant DAMD 17-94-J-4177.
Dear Dr. Caremeci:

This is to confirm that on August 5, 1996 we shipped you 18 frozens breast tissue specimens, as follows: 8 estrogen receptor positive carcinomas
8 estrogen receptor negative carcinomas
2 non tumor breast tissue

Please let us know the state in which the specimens were received, a brief statement of the intended use, and how well the material served your purposes.

Many thanks in advance for this important feedback.

Yours sincerely,

Helen Feiner, M.D.
Director, Anatomic Pathology
Director, Breast Cancer Resource
PH (212) 263-8826 FAX (212) 263-7916

cc: Rita Demopoulos, M.D.
TO: Helen Feiner

PH. NO: (212) 263 - 5470

FAX NO: (212) 263 - 7916

NO. OF PGS: 3 (Including this page)

FROM: Devon Thompson

PH. NO: (415) 498 - 5510

COMMENTS:

If you have an email address I could forward you the list of tumours so you would have it on your computer.
July 9th 1997

Dear Dr. Feiner,

I have been working in collaboration with Charles Carmeci, M.D., with whom you have had previous discussions. We have received 31 breast tumour and 4 normal breast samples from your Breast Cancer Tissue Bank. This source has been invaluable to us. We have used these samples to extract RNA and then perform RT-PCR to detect several different genes. At this juncture it would be extremely useful if we could obtain any information you have in your files pertaining to the specific tumours that you have provided to us. Information such as, histological grade, the method(s) used to establish the estrogen receptor phenotype and quantitative values for the ER levels determined. Following I have listed our ID number and your ID number for each of the tumours that we have received.

I will be away on vacation from July 12th until July 26th. You can contact me by e-mail devont@leland.stanford.edu, by phone (415) 498-5510, or fax (415) 725-8762 after this date. Thank you for your help with this matter.

Sincerely,

Devon A. Thompson, Ph.D.
devont@leland.stanford.edu
<table>
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<th>NYU Tumour ID</th>
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<td>s95 10787</td>
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<tr>
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<td>s97 1792</td>
<td>normal breast</td>
</tr>
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Subject: NYU breast cancer samples.
From: Helen Feiner 7/24/97 12:50 PM
To: devont@leland.stanford.edu at PMDF

Dear Dr. Thompson,

I have sent you, by mail, two reports from each of the patients listed in your communication of July 9th. One is the surgical pathology report from which you can derive a histologic grade. The most common grading system utilizes architectural grade + nuclear grade + mitotic rate. The second report is from our molecular pathology lab from which you can obtain the estrogen receptor quantitative values. Let me know if you need help with any of these data.

Method used to obtain ER phenotype: Indirect immunoperoxidase technique. Estrogen receptor antibody is obtained from AMAC (clone ER1D5, Westbrook, ME) and Novo Castra (clone 6F11, distributed by Vector, Burlingame, CA). Secondary antibody is horse anti mouse IgG. A standard avidin-biotin-peroxidase technique is used on formalin fixed, paraffin embedded tissue sections. Antibody expression is evaluated in 10 40x fields in a CAS Image Analyzer. Result is expressed as percent positive nuclear area.

Helen Feiner M.D.
Dear Dr. Feiner,

August 25 1998

Thank you for the information pertaining to the breakdown of race status, with regard to patients from whom the breast tumour specimens are obtained. Enclosed are reprints from some papers in which we have used the tumour specimens that you provided. These frozen tumours have been invaluable to us in extrapolating our findings in breast cancer cell lines to breast tumour biology. We hope to continue using the Breast Cancer Resource of the Department of Pathology, New York University Medical Center, to obtain breast cancer samples.

Sincerely,

Devon A. Thompson, Ph.D.
Characterization of a gene that is inversely correlated with estrogen receptor expression (ICERE-1) in breast carcinomas

Devon A. THOMPSON and Ronald J. WEIGEL
Department of Surgery, Stanford University, Stanford CA, USA
(Received 22 September/10 December 1997) - EJB 97 1350/1

Differential screening and suppression subtractive hybridization identified genes differentially expressed in an estrogen receptor-positive breast carcinoma cell line

Wayne W. Kuang, Devon A. Thompson, Renee V. Hoch and Ronald J. Weigel*
Department of Surgery, Stanford University, Stanford, CA 94305, USA
Received June 10, 1997; Revised and Accepted December 18, 1997
DDBJ/EMBL/GenBank accession no. AF007170

Identification of a Gene (GPR30) with Homology to the G-Protein-Coupled Receptor Superfamily Associated with Estrogen Receptor Expression in Breast Cancer

Charles Carmeci,* Devon A. Thompson,* Huijun Z. Ring,† Uta Francke,† † and Ronald J. Weigel*†
*Department of Surgery, †Department of Genetics, and †Howard Hughes Medical Institute, Stanford University, Stanford, California 94305
Received Apr 2, 1997; revised Jun 11, 1997
<table>
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<th>Name: Dr. KEN TAKASHITA</th>
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<tr>
<td>Title: ASSISTANT PROFESSOR</td>
</tr>
<tr>
<td>Address: NYU MEDICAL CENTER</td>
</tr>
<tr>
<td>DEPT. OF HEMATOLOGY</td>
</tr>
<tr>
<td>Phone: (212) 263-5465</td>
</tr>
<tr>
<td>Fax: (212) 263-8444</td>
</tr>
<tr>
<td>e-mail: --</td>
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<tr>
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**Material Requested:** FROZEN SECTIONS OF METASTATIC BREAST CANCER.

- Date Shipped: 8-15-97
- Date Received: SAME DAY
- State of Specimen on receipt: GOOD

**Brief Summary of intended use:**

(Use additional page if necessary)

TO PERFORM IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY, IN ORDER TO DETERMINE WHETHER THE DECREASED EXPRESSION OF RAR-ALPHA RETINOIC ACID RECEPTOR EXPRESSION SEEN IN METASTATIC BREAST CA. IS DUE TO A TRANSCRIPTIONAL DEFECT OR A TRANSLATIONAL DEFECT.
August 15, 1997

Dr. Helen Feiner
Department of Pathology
Breast Cancer Archives

Dear Dr. Feiner:

I am writing to notify you that we have requested and received from Yara Delgado of the breast tumor registry frozen sections of lymph nodes containing known breast cancer metastasis from 7 different patients. We received 6 slides for each patient.

These sections will be used to perform in situ hybridization and immunohistochemistry. The objective of this experiment is to determine whether the decreased expression of RAR-alpha retinoic acid receptor expression seen in metastatic breast cancer is due to a transcriptional defect or a translational defect.

We are grateful for your assistance in our studies. Please contact me if you have any questions.

Sincerely yours,

Ken Takeshita, M.D.
Assistant Professor of Medicine
Feiner, Helen, D.
DAMDM17-94-J-4177

APPENDIX 2
RECORD OF EVALUATION OF BANKED MATERIAL:

Type of Specimen: Imprint / Frozen Tissue

Date of evaluation: 9/4/97

Duration in freezer: 2 years

Type of evaluation: IMMUNOHISTOCHEMISTRY

Results: Excellent

Entered by: HELEN FEINER MD

Signature and date: 9/13/97
IMMUNOHISTOCHEMISTRY

RESIDENT/ ATTENDING

PATIENT  Anonymous  SURG PATH#:  95-95-16437 Block *
(*only single block/case will be stained - except by special request)

DATE  5/12/98 SITE  Biopsy  SPECIMEN:  biopsy  major  Imprint

DIAGNOSTIC ISSUE  None  QC material

ANTIBODIES:  CIRCLE (if limited tissue, number antibodies according
to priority, and request "numbered PLL" slides under special requests)

LEU- M1  Calretinin  CK19  GFAP  B72.3  Adenovirus
Muscle Specific  CAM 5.2 (CK)
ACTIN  AE1/AE3  CHRO  *HCG  PSA  *HBsAg
DESMIN  EMA  NSE  *SYN  *AFP  FVIII  CMV
SMA  EMA  NSE  *SYN
VIMENTIN  34BE12  *CALCITON  pCEA  CD34  *HSV
*  S-100  THYRO  MCEA  CD 68  ER/PR
HMB45  CK20  *MYOglobin (LCA)  BerEP4  Brst-2

# PLL SLIDES REQUESTED  (circle # if ordered on gross sheet:
SPECIAL_REQUESTS: (CIRCLE): RUSH / USE H&E SECTION /

RCVD STAINED SIGNED OUT TURNAROUND

SPECIAL PROCESSING

IHC INTERPRETATION:

Abbreviations and Clone #:  *= POLYCLONAL ANTIBODY
SMA= SMOOTH MUSCLE ACTIN (1A4), ACTIN SPECIFIC ACTIN (HHF-35), AE1/AE3 & CAM 5.2= LOW MOL WT
KERATINS, 34BE12= HIGH MOL WT KERATIN, CALC= CALCITONIN, THYRO= THYROGLOBULIN (Tg6), PSA= PRO
ACID PHOS (PASE/4LT), PSA= PROSTATE SPECIFIC ANTIGEN (EA-PR8), FVIII=FACTOR VIII (F8/86),  * SYN= SYN
CHRO= CHROMOGRANIN (1A2/H10), CMV= CYTOMEGALOVIRUS (DDG9, CCH2), DESMIN (D35), VIMENTIN (V9), LCA= LYMPHOCYTE COMMON ANTIGEN
28/11), CEA= (A5B7), EMA= (E29), AFP= ALPHA FETOPROTEIN (M1 A/301), HSV= HERPES.  PLAP= PLACENTAL A
PHOSPHATASE (886)

Histology:  Date/time submitted date/time cut
**RECORD OF EVALUATION OF BANKED MATERIAL:**

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<tr>
<th><strong>Entered by:</strong></th>
<th>Dr. H. FEINER</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Signature and date:</strong></th>
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IHC# 1
IMMUNOHISTOCHEMISTRY  * Place completed form in Tisch-379 (ext 8922)
RESIDENT/ATTENDING  95-11102
PATIENT _______ SURG PATH#: 98-_______ Block ___
(*only single block/case will be stained - except by special request)
DATE 9/5/97 SITE Breast SPECIMEN: biopsy/ major
DIAGNOSTIC ISSUE 2 material

ANTIBODIES: CIRCLE (if limited tissue, number antibodies according
to priority, and request "numbered PLL" slides under special requests)

LEU- M1 Calretinin CK19 GFAP B72.3 Adenovirus
Muscle Specific CAM 5.2 (CK) NSE *HCG PSA *HBsAg
ACTIN AE1/AE3 CHRO PLAP PAP *HBcAg
DESMIN EMA *SYN *AFP FVIII CMV
VIMENTIN 34BE12 *CALCITON pCEA CD34 *HSV
*S-100 CK7 THYRO mCEA CD 68 ER/PR
HMB45 CK20 *MYOglobin LCA BerEP4 Brst-2

# PLL SLIDES REQUESTED _____ (circle # if ordered on gross sheet)
SPECIAL REQUESTS: (CIRCLE): RUSH / USE H&E SECTION /

RCVD ______ STAINED ______ SIGNED OUT ______ TURNAROUND _____

SPECIAL PROCESSING ___________________________________________________________

IHC INTERPRETATION: NEGATIVE CONTROL- 0 POSITIVE CONTROLS -(*)

remedial step(s): result- Conclusion-

ABBREVIATIONS AND CLONE #  * = POLYCLONAL ANTIBODY
SMA= SMOOTH MUSCLE ACTIN (1A4), MUSCLE SPECIFIC ACTIN (HHF-35), AE1/AE3 & CAM 5.2= LOW MOL WT
KERATINS, 34BE12= HIGH MOL WT KERATIN, CALC= CALCITONIN, THYRO= THYROGLOBULIN (Tg6), PSAP= PRO
ACID PHOS (PASE/ALT), PSA= PROSTATE SPECIFIC ANTIGEN (EA-PR3), FVIII=FACTOR VIII (F8/85), SYN= SYNAP
CHROMOGRANIN (12H10), CMV= CYTOMEGALOVIRUS (DDG9, CCH2), DESMIN (D33), VIMENTIN (V9), LCA= LYMPHOCYTE COMMON ANTIGEN
281), CEA= (A5B7), EMA= (E29), AFP= ALPHAFETOPROTEIN (M1A1301), HSV= HERPES. PLAP= PLACENTAL A
PHOSPHATASE (J84)

Histology: Date/time submitted date/time cut
RECORD OF EVALUATION OF BANKED MATERIAL:

Type of Specimen: Imprint __________ Frozen Tissue _________

Date of evaluation: 9/9/98

Duration in freezer: 2 yrs

Type of evaluation: FISH

Results: Attached

Entered by: J. Teiner

Signature and date: 9/18/98
MOLECULAR CYTOGENETIC ANALYSIS

SPECIMEN TYPE: Tissue Imprints
QUALITY OF PREPARATION: adequate
NO. OF CELLS EXAMINED: 50+

INTERPRETATION:

Slides were received from air-dried material described as imprints/scrapes from breast tissue.

Interphase molecular cytogenetic analysis was performed using fluorescent in situ hybridization (FISH) with investigational DNA probes specific for the centromeric region of the X chromosome (Vysis CEP X-alpha probe set). Random sections of the slide were examined by two independent readers. Adequate signal for analysis was seen over the majority of the hybridization area. Results indicated over 85% of cells contained two signals for the X chromosome consistent with two copies of the X. No evidence was seen of X chromosome aneuploidy.

MOLECULAR CYTOGENETIC DIAGNOSIS: nuc ish Xcen(DXZ1x2)

Note: Since this is an in vitro test, accuracy may be limited by technical or cultural artefacts.
APPENDIX 3
BREAST CANCER PILOT PROJECTS AWARDED
1995 - 1998

1995 GRANT YEAR
Pamela Cowin, Ph.D.
Assistant Professor
Cell Biology

"The Role of Plakoglobin in Breast Cancer"
($30,000)

Xiao-Hong Sun, Ph.D.
Assistant Professor
Cell Biology

"The Role of ID Proteins in Breast Cancer"
($28,450)

Mary Ann Perle, Ph.D.
Assistant Professor
Pathology

"Chromosomes 7, 18, 20 and X in Mammogram Detected Atypical Ductal Hyperplasia and Ductal Carcinoma in situ"
($8,950)

1996 GRANT YEAR
Sandra Reynolds, Ph.D.
Res. Assistant Professor
Dermatology

"Peptide Epitopes Recognized by CD8+ T Cells in Patients with Breast Cancer"
($10,000)

Herbert Samuels, M.D.
Professor
Medicine

"Retinoid-Regulated Genes and Breast Cancer"
($25,000)

Jan Sap, Ph.D.
Assistant Professor
Pharmacology

"Receptor Protein Tyrosine Phosphatases and Breast Cancer"
($20,000)

Kenichi Takeshita, M.D.
Assistant Professor
Medicine

"9-cis Retinoic Acid and Retinoid X Receptor RXR in Breast Cancer"
($20,000)

Stephen Tomlinson, Ph.D.
Assistant Professor
Pathology

"The Role of Complement Inhibitors in Tumorigenicity"
($10,000)

Stanislav Vukmanovic, MD,PhD
Assistant Professor
Pathology

"Effector Function of Vaccine Induced CD8+ Cells"
($10,000)

1997 GRANT YEAR
Harry Ostrer, M.D. (P.I.)
Professor
Pediatrics

"Genetic Susceptibility to Breast Cancer"
($15,000)

Ruth Oratz, M.D. (Co-P.I.)
Assistant Professor
Medicine
W. Fraser Symmans, M.D. (P.I.)
Assistant Professor
Pathology
Matthew Volm, M.D. (Co-P.I.)
Instructor
Medicine

Carolyn Wasserheit, M.D. (P.I.)
Assistant Professor
Medicine
Kenichi Takeshita, M.D. (Co-P.I.)
Assistant Professor
Medicine

1998 GRANT YEAR
Ruben Abagyan, Ph.D.
Associate Professor
Biochemistry

Alan Frey, Ph.D.
Assistant Professor
Cell Biology

Giorgio Inghirami, M.D.
Associate Professor
Pathology

Carole Oddoux, Ph.D.
Assistant Professor
Pediatrics

"A Response Biomarker for Paclitaxel Chemotherapy in Patients with Breast Cancer"
($29,875)

"Biological Correlates of 9-Cis Retinoic Acid and Tamoxifen"
($15,000)

"Toward A New Chemotherapy for Breast Cancer: Rational Design of A Retinoid X Receptor-Selective Agonist"
($29,968)

"Translational Arrest of IL-2 mRNA in Human Breast Cancer Tumor Infiltrating Lymphocytes"
($30,000)

"Molecular Characterization of BRCA1"
($30,000)

"Heritable Susceptibility to Invasive and Non-Invasive Breast Cancer"
($15,000)