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We have successfully established a breast tissue repository which contains over 250 individual breast specimens. In addition, we have cryopreserved and cultured cells from human breast tumor tissues and have established 21 new human breast tumor cell lines. The repository also includes breast epithelial and stromal cell strains derived from non cancerous breast tissue as well as peripheral blood mononuclear cells.

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JW Shaw 7/1/97

PI Signature Date

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A Comprehensive Repository of Normal and Tumor Human Breast Tissues and Cells
Jerry W. Shay, Ph.D., Program Director
(Total grant period: July 1, 1994 - July 31, 1998)
(Progress report period: July 1, 1996 - June 30, 1997)

Introduction

Statement of background/purpose:

Progress in understanding the development of human breast cancer has been made by studying tumor tissue obtained from patients during surgery and by establishing breast tumor cell lines. However, in such cases, the researcher may be analyzing one or more late events in the progression of the disease. The development of breast cancer is likely to be a multi-step, progressive process with several heritable alterations accumulated during the evolution to malignancy. Since one of the major objectives of breast cancer research is to provide a means for early intervention, it is important to define the specific molecular alterations at each stage in this process. The study of such alterations is greatly aided by having not only tumor cells but also corresponding non-malignant breast cells of stromal and epithelial origin. Because non-malignant breast cells may have undergone genetic alterations, a self-replicating source of constitutional DNA is also of great importance. The purpose of our project is to establish a unique repository of materials for the biologic and genetic study of breast cancer. The repository will contain cryopreserved and cultured cells from tumor tissues, non-malignant epithelial and stromal cells as well as peripheral blood mononuclear cells. Patient demographic and clinical data, and family history will be collected and entered onto a relational database.

Statement of the principal objectives of the program are to:

1) obtain and cryopreserve from breast cancer patients, peripheral blood mononuclear cells, tumor tissue, and non-malignant adjacent breast tissue; include samples from women with familial breast cancer and with non-invasive breast cancers; collect patient demographic, family, clinical and pathological data;
2) prepare and cryopreserve breast tissue organoids from which both epithelial and stromal cells can be cultured;
3) characterize breast epithelial and stromal cells
4) establish and characterize breast tumor cell lines from patients with breast carcinoma;
5) establish and cryopreserve Epstein Barr Virus-transformed B-lymphoblastoid cells as a source of constitutional DNA;
6) maintain computerized records of all data, materials accessioned, and cell characterization;
7) make samples available and publicize information about the repository and to make its resources readily available to the scientific community with minimal restrictions.
8) maintenance of cell repository and backup
9) obtain future stable monetary support for repository

Body

Task 1. **Obtain and Cryopreserve Normal and Tumor Surgical Specimens**

In July 1994 we initiated a repository for multiple areas of breast cancer research. It was an ambitious project, and during the first year of the parent grant we obtained and cryopreserved 84 tissue samples. Of these samples we established and cryopreserved a total of five human breast tumor cells lines. During the second year of the parent grant we obtained and cryopreserved 55 additional tissue samples. Efforts were undertaken to obtain early and premalignant breast tissue samples and during the second year of the parent grant we obtained one ductal carcinoma *in situ*, three lobular carcinoma *in situ*, 20 fibroadenomas, and 17 other benign conditions.

Prior to the initiation of our breast tumor and cell repository 85 breast cancer specimens were accessioned. These consisted of 62 primary breast cancers and 23 metastatic lesions. When available, primary tumor tissue, adjacent non-malignant tissue, and cryopreserved peripheral blood mononuclear cells were obtained. In summary, during the first three years of the parent breast tumor and cell repository grant, we have obtained and cryopreserved approximately 165 samples so that at the present time we have accessioned a total of 250 individual breast specimens. Thus most of our effort during the first three years has been to accession samples and establish the cell lines. During the third year we have also initiated and nearly completed the characterization of all our primary tumors and tumor derived cells lines and several manuscripts are presently being prepared. One of our tumor derived cell lines obtained during the third year has a BRCA-1 (inherited breast cancer susceptibility locus) mutation. This line will continued to be characterized and additional interesting specimens will be obtained as they become available. All patient demographic, family, clinical and pathological data are maintain on the computerized database (see appendix, pages 12-32).

The samples have been characterized (see appendix, pages 22-24) for DNA ploidy, karyotype, progesterone/estrogen receptors, Ber-EP4 (breast specific antigen), BRST-1 (breast specfic antigen), BRST-2 (breast specific antigen), cytokeratins, Her2-neu (breast amplified oncogene), p53 mutations and telomerase activity (references 2,4,6,7). Detailed experimental methods are described in the published manuscripts.

Task 2. **Culture and Cryopreserve Organoids from "Normal" Breast Tissue Samples and Separate Epithelial from Stromal Cells**

We have continued to be successful in culturing and cryopreserving breast epithelial and stromal cell cultures. During the first three years a total of 23 human breast epithelial and 25 stromal cell strains have been cryopreserved. In addition, we have 50 additional organoid cultures frozen which have not been established into epithelial and stromal strains. Due to limited manpower, we have elected to only characterize those epithelial and stromal cells in which tumor cell lines are established. Since it requires at least 4-6 months of culture to be confident that a primary tumor is successfully established, we generally make breast tissue organoids and in some instances primary

cultures and then cryopreserve them until such time as the tumor cell data are obtained. We now have matched tumor derived cell lines and normal epithelial and stromal cells from five of our accessioned specimens (see Table 1, page 22 appendix). We are finalizing the characterization of these strains, scaling them up and freezing back early passages for distribution from the repository.

Task 3. **Characterize Breast Epithelial and Stromal Cells**

One of the epithelial cell cultures obtained from a patient with Li-Fraumeni syndrome spontaneously immortalized in cell culture (see reference 2). The 5 stromal and epithelial samples with matching tumor derived lines are being characterized for DNA ploidy, karyotype, progesterone/estrogen receptors, Ber-EP4 (breast specific antigen), BRST-1 (breast specfic antigen), BRST-2 (breast specific antigens), cytokeratins, Her2-neu (breast amplified oncogene), p53 mutations, telomere length, and telomerase activity. During the fourth year we will complete the characterization of the 5 strains in which we have matched tumor derived cell lines.

Task 4. **Establish and Characterize Breast Tumor Cell Lines from Primary Breast Carcinoma**

We recognized at the onset that establishing breast tumor cell lines would be the rate limiting component to the success of the repository. At the end of the first year of the parent grant we had clearly established one additional breast tumor cell line (for a total of 5 new breast tumor cell lines). During the second and third years we made a special effort to initiate and obtain additional human breast tumor cell lines. We were successful in establishing 16 additional lines for a total of 21 lines that are currently in the repository (see Tables 1-3, pages 22-24 in the appendix). These new human breast tumor cells lines were almost all derived from primary invasive ductal breast carcinomas and have been characterized for DNA ploidy, karyotype, progesterone/estrogen receptors, Ber-EP4, BRST-1, BRST-2, cytokeratins, Her2-neu, p53 mutations and telomerase activity. In addition, a manuscript describing the FRA3b and FHIT characterization of the cells has been submitted for publication (reference 9). In the near future two additional manuscripts will be submitted for publication (reference 10 and 11). Within the next six months, tumor derived breast cell lines will be provided to the American Type Culture Collection for distribution to the scientific community. Our final report will include copies of these manuscripts.

Task 5. **Establish and Cryopreserve EBV-transformed B-lymphoblastoid Cell Lines**

We have cryopreserved peripheral blood mononuclear cells from patients from whom we obtained permission, but decided that we would transform only those samples with EBV when we had preliminary evidence that the tumor lines were successfully established and cryopreserved. Of the 21 breast tumor cell lines that we have established, we have 16 EBV-transformed peripheral blood mononuclear cultures established as lines for a source of constitutional DNA. In addition, two of these EBV-transformed B-lymphoblastoid cell lines have accompanying normal breast and stromal cell strains as well as a tumor derived cell line. This is a unique combination of materials

from these two individuals and will be a valuable asset for breast cancer research.

Task 6. **Maintain a Computerized Database**

All entries are currently made and will continue to be made on a MacIntosh computer in the co-investigator's laboratory (Dr. Gazdar). Patient demographic information, and relevant clinical and family data are collected and entered onto a computerized relational database written in the Fourth Dimension software program with access by password. A database has been appropriately modified by Mr. David Wheeless, Computer Specialist, at the University of Texas Southwestern Medical Center. Only Drs. Shay, Gazdar, and personnel with a need to know have access to patient identification. Informed consents and other hard copies of patient data are stored in locked, limited access cabinets. Responsibility for computer entries are given to a single person (with the confirmation of correct entry given to a second person). Backup of the data base is made weekly onto a tape drive (automatic via network).

Task 7. **Making Samples Available to Breast Cancer and Other Researchers**

Although our homepage announcing the availability of our tissue/cell repository will be on line this year (see pages 12-32 in the appendix for a draft of our homepage), during the first three years over 75 individuals have obtained tissues and cells from our repository. We have contacted existing breast tissue banks to coordinate data base interconnections. We have contacted Dr. Steve Ethier at the University of Michigan Cancer Center who has just established a web site for their breast tumor repository (http://www.cancer.med.umich.edu/). In addition, Dr. Martha Stampfer (Lawrence Berkeley Laboratory, California) has also established a home page on human mammary epithelial cells (http://www.lbl.gov/~mrgs) and we have contacted her. We have now establish our own web site and will link our site with the Michigan and California site as well as the Cell Line Data Base and the Breast Cancer Information Core. In addition, many of our reagents (especially the tumor derived cell lines) developed will be submitted to the American Type Culture Collection for broad distribution to the scientific community (The ATCC will also be linked to our homepage). Those reagents such as primary biopsies, which are limited in quantity, will be maintained in our repository for distribution. We are attaching with this report in the appendix (pages 12-32) an overview of our web page. Our goal was to have this web page on line by July 1, 1997 but we will slightly delay this until we finalize the characterization of the cell lines and send them to the American Type Culture Collection for distribution. Thus, while we are slightly behind schedule on this subtask, we expect this to only be a minor delay and we should be fully functional and on line by the end of October, 1997. In addition, it is our intention to widely distribute information about our repository at the DOD Breast Cancer Research Program Meeting "Era of Hope" October 31-November 4th in Washington, D.C.

Task 8. **Maintenance of Cell Repository and Backup**

At present, all samples are split and maintained in both Dr. Gazdar's and Dr. Shay's laboratories. All samples are coded, divided and maintained in both liquid nitrogen and -150°C freezers (with automatic alarms). The freezers are located in

separate buildings. Only designated personnel are able to access the repository. During the fourth year of this project many of the reagent will be provided to the American Type Culture Collection as a permanent source for the distribution of the tumor derived cell lines.

Task 9. **Future Stable Monetary Support for Repository**

Since we have now completed three of the four years of the grant, we will submit several applications during the next several months for the long term support for the repository. We are currently deciding if we wish to only maintain versus expand the repository after the final year of the grant. We believe for the final year of the work plan, that expansion of the repository will only be in areas that are exceptionally interesting or important (e.g. additional LiFraumeni Syndrome and BRCA-1 mutations). Thus, we will attempt to conserve funds during the final year so that we may be able to carryover funds should our grant applications for maintenance of the repository not be immediately funded. We are planning to apply for funds to expand as well as sustain the repository from the NIH, Mary Kay Ash Foundation, Susan G. Komen Foundation, and if rules permit from the USAMRC. Finally, we have letters of basic maintenance financial "guarentees" from Drs. John Minna and Perrie Adams from our University if other financial resources are not available. Thus, while an important area for this year, this is not considered a crucial item.

Conclusions

There are no subtasks that are behind far behind schedule and we expect to have completed all of the original tasks the end of the fourth year. We have had a very successful effort since the initiation of the repository. Initially we had to recruit and train new research assistants and establish lines of communication for successfully obtaining and distributing samples. We were somewhat disappointed in the first year that we had not clearly established more tumor cells lines, but during the second and third years we have had considerably more success. One of our biggest successes was the development of an improved telomerase activity assay which we used to characterize almost all the 250 human breast tumors, 55 adjacent noncancerous breast tissue specimens, and other noncancerous lesions including 20 fibroadenomas and 17 fibrocystic disease specimens (see references 1,2,3,4,7,8,10). In addition, we successfully established a breast epithelial cell line from a patient with Li-Fraumeni syndrome (one of the first spontaneously immortalized human breast epithelial lines reported, reference 2) and have another breast cell line with a BRCA-1 mutation. Finally, and perhaps most importantly for future breast cancer research, we have successfully established 21 new human breast tumor cell lines and from 16 of these we have corresponding non-malignant blood lymphocytes. In addition, we also have non cancerous human breast epithelial and stromal cell strains from 5 of these patients. Overall, we have tumor derived cell lines, lymphocytes, epithelial and stromal cells from two of individuals. These new reagents should facilitate progress in breast cancer research in the future.

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HAMON CENTER FOR THERAPEUTIC ONCOLOGY RESEARCH

BREAST TISSUE REPOSITORY

Dr Adi F Gazdar MD
Dr Jerry W Shay PhD

- **Overview** Breast tumor repository (BTR) was initiated in the early 1990s to address the apparent lack of resources to obtain tumor and non-involved adjacent breast tissue from the same patient. Our objective is to make a comprehensive breast tissue/cell repository as a research resource for breast cancer researchers worldwide. The repository maintains novel cell lines generated from the breast tumor tissues with detailed information on the cellular, biochemical and molecular analysis of these cell lines along with a comprehensive record of clinical and pathological data. The repository also includes cryopreserved epithelial and stromal cell strains established from normal mamary tissues. These strains demonstrate a finite lifespan in culture. Five of these strains correspond to tumor derived cell lines which provides an opportunity to compare differences between tumor-derived cell lines and their "normal" counterparts. We believe that this repository represents a unique resource that should prove critical to our understanding of breast cancer development possibly resulting in new therapies.

  Human breast tissue is composed of a series of ducts that terminate in ductules embedded in basement membrane-contained stroma. Ducts and ductules consist of epithelial cells, which, like all human cells, are extraordinarily difficult to immortalize. Immortalization indicates an escape from cellular senescence and may represent one of the major events in the progression normal cells to malignancy. Immortalization is accompanied by numerous cellular and molecular abnormalities. Studies on cell lines will be useful in the elucidation of molecular mechanisms underlying this phenominon.

- **Breast Tumor Cell Lines** The breast tumor repository hosts a total of 21 cell lines of which 18 are derived from primary breast tissues and 3 are cultured from metastatic breast tissues. Of the 21 tumor cell lines, 13 are paired cell lines for which we have established corresponding blood lymphocyte (BL) cell lines. These cell lines have been analyzed extensively for cellular, molecular and genetic abnormalities, and a comprehensive database is now available.

GAZDAR GROUP (Arvind Virmani PhD, Venkatesh Kurvari PhD, Duli Kodagoda, Max Westerfield, ADI F. GAZDAR MD)

- Primary Breast Tumor Cell Lines
- Metastatic Breast Tumor Cell lines
- Paired Cell Lines
- Brief Summary of All Cell Lines
- Data Tables

- **Want to see a "sticker" (adhering) phenotype? Click HERE**
- **How about a "floater" (non-adhering) phenotype? Click HERE**
- **Or a tumor cell line that forms duct-like and gland-like structures? Click HERE**

- **Human Mammary Epithelial / Stromal Cells** We have established cultures of normal epithelial and/or stromal mammary cells from approximately 25 primary cultures, five of which correspond to tumor derived cell lines. These primary tissues are mechanically and enzymatically dispersed and plated in medium specific, for epithelial or stromal cell growth. During growth and expansion, the cells are immunocytostained for lineage specific markers, characterised for morphological changes and population doubling levels in culture.

  SHAY GROUP (Lauren GollahonPhD and JERRY SHAY PhD)

  - **Normal Epithelial Cell Lines**
  - **Stromal Cell Lines**

- **Search Breast Tumor Repository**
- **Request HME Cells/Tissue**
- **Request a Tumor Cell Line**
- **Links to Other Breast Cancer Databases**
- **UT Southwestern Links**

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Page maintained by Dr. Venkatesh Kurvari. Last modified on June 23, 1997

Breast Tumor Repository

Cell line Information

Each cell line in the Repository is given an identification number that is prefixed with HCC for Hamon Cancer Center (eg. HCC1569). The following is a brief description of cell lines established at the Hamon Center for Therapeutic Oncology at UT Southwestern.

HCC38

- **Growth in Culture:** Grows as epithelial stickers; some floaters
- **Initiation Period:** 32 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIB, Grade 3, Invasive ductal carcinoma with 3/28 lymph node metastasis)
- **Culture media:** ACL4 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** ER-, PR- and Her2- negative; p53 positive

HCC70

- **Growth in Culture:** Grows as medium-sized epithelial stickers; no floaters
- **Initiation Period:** 44 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIIA, Grade 3, Invasive ductal carcinoma with 4/17 lymph node metastasis)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** none available
- **Molecular analysis:** ER-positive, PR- and Her2- negative; overexpresses p53

HCC202

- **Growth in Culture:** Grows as medium-sized epithelial stickers; partial floaters
- **Initiation Period:** 41 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIIA, Grade 3, Invasive ductal carcinoma with 4/19 lymph node metastasis)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum

- **Non-Malignant Cell Line/Strain:** none available
- **Molecular analysis:** ER-, PR- and p53- negative; positive for Her2-neu

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HCC712

- **Growth in Culture:** Grows as large epithelial monolayers; some ducts and tubules
- **Initiation Period:** 13 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIB, Grade 2, Invasive intraductal and ductal carcinoma with 44/46 lymph node metastasis)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** ER- and PR- positive; low expression of p53; negative for Her2-neu

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HCC1007

- **Growth in Culture:** Grows as epithelial stickers; some floaters; vacuolated
- **Initiation Period:** 9 months
- **Relevant clinical & pathological information:** Derived from metastatic lymph node; ductal carcinoma (Stage IIA, Grade 3, 12/12 lymph node metastasis)
- **Culture media:** ACL4 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** ER- and PR- negative; p53- and Her2-neu positive

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HCC1008

- **Growth in Culture:** A floater; forms structures that are strikingly similar in appearance to ducts and glands
- **Initiation Period:** 12.5 months
- **Relevant clinical & pathological information:** Derived from primary breast cells; ductal carcinoma (Stage IIA, Grade 3, 12/12 lymph node metastasis)
- **Culture media:** ACL4 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** ER- and PR- negative; p53- and Her2-neu positive

HCC1143

- **Growth in Culture:** Medium-sized epithelial stickers
- **Initiation Period:** 29 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Invasive ductal carcinoma, Stage IIA, Grade 3, with 0/15 lymph node metastasis)
- **Culture media:** ACL4 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** In preparation
- **Molecular analysis:** ER-, PR- and Her2-neu negative; p53-positive

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HCC1187

- **Growth in Culture:** Small poorly-adherent epithelial cells
- **Initiation Period:** 4.5 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Invasive ductal carcinoma, Stage IIA, Grade 3)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** ER expression undetectable by immunohistochemistry; however, cytosolic protein detectable (57 fmol/mg); p53 positive; PR- and Her2-neu negative

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HCC1395

- **Growth in Culture:** Large epithelial cells; vacuolated
- **Initiation Period:** 14 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage I, Grade 3 Invasive ductal carcinomaprimary breast cells with 0/34 lymph node metastasis). Familial breast cancer history (Patient's mother)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line, stromal and epithelial strains are available
- **Molecular analysis:** ER-, PR- and Her2-neu negative; p53-positive

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HCC1419

- **Growth in Culture:** Large epithelial cells; exhibit sticker phenotype in island-like formations
- **Initiation Period:** 9 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIIA, Grade 2, Invasive ductal carcinoma and CIS primary breast cells with 5/5 lymph node metastasis). No history of familial breast cancer history
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** Stromal and epithelial strains are available
- **Molecular analysis:** ER- and p53 negative; PR-negative by immunohistochemistry, but positive in cytosolic protein assay (133 fmol/mg); Her2-neu overexpression detectable by immunohistochemistry and by ELISA

HCC1428

- **Growth in Culture:** Large epithelial cells; finely vacuolated
- **Initiation Period:** 15 months
- **Relevant clinical & pathological information:** Derived from metastatic adenocarcinoma and pleural effusion cells (Stage IV, Grade 3). Familial history of breast cancer (Patient's maternal grand mother)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** ER- and PR- positive both by immunohistochemistry and cytosolic protein assays (ER, 55 fmol/mg; PR, 984 fmol/mg). Negative for p53 expression

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HCC1500

- **Growth in Culture:** Small, tightly adherent epithelial cells
- **Initiation Period:** 14 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIB, Grade 2, Invasive ductal carcinoma with 4/24 lymph node metastasis).
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** Stromal and Epithelial strains available
- **Molecular analysis:** ER- and PR- positive by immunohistochemistry, but undetectable by cytosolic protein assays. p53-positive, Her2-neu negative

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HCC1569

- **Growth in Culture:** Large epithelial stickers
- **Initiation Period:** 19 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IV, Grade 3, Invasive metaplastic carcinoma with 4/18 lymph node metastasis).
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** Stromal and Epithelial strains available
- **Molecular analysis:** ER- and PR- negative by immunohistochemistry, but PR expression was detectable by cytosolic protein assay. p53-negative; Her2-neu overexpression detectable by immunohistochemistry and ELISA

HCC1599

- **Growth in Culture:** Epithelial floaters; appear as irregular masses
- **Initiation Period:** 10 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIIA, Grade 3, Invasive ductal carcinoma).
- **Culture media:** ACL4 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** ER-, PR-, p53 and Her2-neu negative

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HCC1739

- **Growth in Culture:** Large epithelial cells; float when heavy
- **Initiation Period:** 15.5 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage I, Grade 3, Invasive ductal carcinoma and CIS with no lymph node metastasis). No history of familial breast cancer
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line, stromal and epithelial strains available
- **Molecular analysis:** ER-, PR-, and Her2-neu negative; p53 positive

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HCC1806

- **Growth in Culture:** Medium-sized epithelial cells; tendency to form monolayers

- **Initiation Period:** 10 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIB, Grade 2, Acantholytic squamous carcinoma with no lymph node metastasis). No history of familial breast cancer
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** In preparation
- **Molecular analysis:** ER-, PR-, p53 and Her2-neu negative

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HCC1937

- **Growth in Culture:** Large epithelial cells; tendency to float when heavy
- **Initiation Period:** 11.5 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIB, Grade 3, Invasive ductal carcinoma). Familial history of breast cancer reported (Patient has an identical sister who also developed breast cancer)
- **Culture media:** ACL4 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** ER-, PR-, p53 and Her2-neu negative

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HCC1954

- **Growth in Culture:** NA
- **Initiation Period:** 4 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIA, Grade 3, Invasive ductal carcinoma with no lymph node metastasis)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** BL-line available
- **Molecular analysis:** Overexpresses Her2-neu in ELISA assay

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HCC2157

- **Growth in Culture:** Granular epithelial cells; floaters
- **Initiation Period:** 8 months
- **Relevant clinical & pathological information:** Derived from primary breast cells (Stage IIIA, Grade 2, Ductal carcinoma)

- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** In preparation
- **Molecular analysis:** ER-negative; PR-, p53 and Her2-neu positive

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HCC2185

- **Growth in Culture:** Large epithelial cells; single cell floaters
- **Initiation Period:** 7.5 months
- **Relevant clinical & pathological information:** Derived from metastatic lobular carcinoma (Stage IV, Grade 2 with 39/39 lymph node metastasis)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** In preparation
- **Molecular analysis:** ER- and PR-negative; p53 and Her2-neu positive

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HCC2218

- **Growth in Culture:** Loosely adherent epithelial floaters
- **Initiation Period:** 6 months
- **Relevant clinical & pathological information:** Derived from primary breast tumor (Stage IIIA, Grade 3 with 42/43 lymph node metastasis)
- **Culture media:** RPMI-1640 with 5% fetal bovine serum
- **Non-Malignant Cell Line/Strain:** In preparation
- **Molecular analysis:** ER-negative; PR and p53-positive; highly positive for Her2-neu

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Breast Tumor Cell Lines
Data Tables

Table 1. Clinical and pathological features of breast tumors used for initiation of cell lines

Where available, Table 1 describes patient's race, age and familial breast cancer history; describes tumor source, grade and LN metastasis; also indicates tumor cell line's initiation period and optimal media.

Table 2. Characterization of breast tumor cell lines

Table 2 describes characterization of the tumor cell lines by FACS, RT-PCR and DNA finger printing.

Table 3. Molecular analysis of breast tumor cell lines

Table 3 describes molecular characterization of breast tumor cell lines for ploidy index, ER, PR, p53 and Her2-neu expression. Also describes morphological features of growth in culture.

TABLE 1. Clinical and pathological features of breast tumors used for initiation of cell lines

Cell line	Patient Age	Race	Tumor Source	Diagnosis	TNM Stage	Grade	LN metastasis	Breast CA Family history	Cell line Initiation period (months)	Optimal media	Paired non-malignant strain/line
HCC38	50	*NA*	Pr.Breast	Invasive Ductal CA	IIB	3	3 / 28	*NA*	32	ACL4-5	BL
HCC70	49	B	Pr.Breast	Invasive Ductal CA	IIIA	3	4 / 17	*NA*	44	RPMI-5	*NA*
HCC202	82	*NA*	Pr.Breast	Invasive Ductal CA	IIIA	3	4 / 19	*NA*	41	RPMI-5	*NA*
HCC712	41	A	Pr.Breast	Inv Intraduct & Duct CA	IIB	2	44 / 46	*NA*	13	RPMI-5	BL
HCC1007	67	B	LN/Met.	Ductal CA	IIA	3	12 / 12	*NA*	9	ACL4-5	BL
HCC1008	67	B	Pr.Breast	Ductal CA	IIA	3	12 / 12	*NA*	12.5	ACL4-5	BL
HCC1143	52	W	Pr.Breast	Invasive Ductal CA	IIA	3	0 / 15	*NA*	29	ACL4-5	*IP*
HCC1187	41	W	Pr.Breast	Invasive Ductal CA (IV)	IIA	3	*NA*	*NA*	4.5	RPMI-5	BL
HCC1395	43	W	Pr.Breast	Invasive Ductal CA	I	3	0 / 34	Yes (Mother)	14	RPMI-5	BL, St, Ep
HCC1419	42	H	Pr.Breast	Inv Duct CA & CIS (IV)	IIIA	2	5 / 5	No	9	RPMI-5	*NA*, St, Ep
HCC1428	49	W	Pl.Eff/Met	Metastatic AdenoCA (IIIB)	IV	3	*NA*	Yes(Mat.gr.mother)	15	RPMI-5	BL
HCC1500	32	B	Pr.Breast	Invasive Ductal CA	IIB	2	4 / 24	*NA*	14	RPMI-5	*NA*, St, Ep
HCC1569	70	B	Pr.Breast	Invasive Metaplastic CA (IV)	IV	3	4 / 18	*NA*	19	RPMI-5	*NA*, St, Ep
HCC1599	44	*NA*	Pr.Breast	Invasive Ductal CA	IIIA	3	*NA*	*NA*	10	ACL4-5	BL
HCC1739	51	W	Pr.Breast	Inv Duct CA & CIS (II)	I	3	0 / 33	No	15.5	RPMI-5	BL, St, Ep
HCC1806	60	B	Pr.Breast	Acantholytic Sq CA	IIB	2	0 / 18	*NA*	10	RPMI-5	*IP*
HCC1937	24	W	Pr.Breast	Invasive Ductal CA (I)	IIB	3	*NA*	Yes (Identical sister)	11.5	ACL4-5	BL
HCC1954	61	EI	Pr.Breast	Invasive Ductal CA (III)	IIA	3	0 / 27	*NA*	4	RPMI-5	BL
HCC2157	48	B	Pr.Breast	Ductal CA	IIIA	2	1 / 9	*NA*	8	RPMI-5	*IP*
HCC2185	49	*NA*	Pl.Eff/Met	Metastatic Lobular CA	IV	2	39/39	*NA*	7.5	RPMI-5	*IP*
HCC2218	38	*NA*	Pr.Breast	Ductal CA	IIIA	3	42 / 43	*NA*	6	RPMI-5	*IP*

TABLE 2. Characterization of breast tumor cell lines

Cell line	Ber-EP4 FACS	RT-PCR	BRST1 FACS	BRST3 FACS	DNA Finger printing	LDL	GYPA	HBGG	D7S8	GC
HCC38	+ (58)		-	-	MATCH					
HCC70	+ (120)	+	+ (34)	-						
HCC202	+ (85)	+	-	-						
HCC712					MATCH	AB/AB	AB/AB	B/B	AB/AB	AB/AB
HCC1007	+ (181)		+ (140)	+ (58)						
HCC1008	+ (50)	+	-	-	MATCH	AB/AB	B/B	C/C	**AB/B**	**AB/B**
HCC1143										
HCC1187	+ (54)		-	-	MATCH	A/A	A/A	B/B	B/B	C/C
HCC1395	+ (30)	+	-	-	MATCH	A/A	A/A	A/A	A/A	C/C
HCC1419	+ (119)	+	-	-						
HCC1428	+ (154)	+	+ (47)	-	MATCH	AB/A	AB/AB	**AB/B**	B/B	AC/AC
HCC1500	+ (30)		-	-						
HCC1569	+ (127)		+ (100)	+ (120)						
HCC1599	+ (165)		+ (157)	+ (98)		AB/AB	AB/A	B/B	AB/AB	AC/AC
HCC1739	+ (290)	+	+ (49)	-	MATCH	AB/AB	AB/AB	A/A	AB/AB	**AC/C**
HCC1806										
HCC1937		+			MATCH	B/B	AB/AB	B/B	AB/AB	C/C
HCC1954	+ (324)		-	-	MATCH	B/B	A/A	A/A	A/A	AC/AC
HCC2157		+			MATCH	B/B	AB/AB	A/A	B/B	B/B
HCC2185										
HCC2218										

TABLE 3. Comparison of properties of tumors with cell lines

HCC ID	Morphology	Ploidy index	ER (IHC)	ER (cytosol) (fmol/mg)	PR (IHC)	PR (cytosol) (fmol/mg)	p53 (IHC)	Her2-neu (IHC)	Her2-neu (ELISA) fold/HME
HCC38	Ep.stickers; some floaters	1.9	-	- (<15)	-	- (<15)	+	-	6.5
HCC70	Medium-sized ep. stickers; no floaters	2.09	+	- (<15)	-	- (<15)	++	-	2
HCC202	Medium-sized ep. stickers; partial floaters	2	-	- (<15)	-	- (<15)	-	+	30
HCC712	Ep. monolayers; large; some ducts and tubules	1.2	+	repeat	+	repeat	±	-	4
HCC1007	Ep. stickers; some floaters; vacuolated	1.76	-	- (<15)	-	- (<15)	++	++	11
HCC1008	Duct-like; gland-like structures	>2	-	- (<15)	-	- (<15)	++	++	12
HCC1143	Medium-sized ep. stickers	>2	-	- (<15)	-	- (<15)	++	-	4
HCC1187	Small, poorly adherent ep. cells	>2	-	+ (57)	-	- (<15)	++	-	12
HCC1395	Large ep. cells; vacuolated; multinucleated	>2	-	- (<15)	-	- (<15)	+	-	3
HCC1419	Large ep. cells; stickers in islands	1.89	-	- (<15)	-	+ (133)	-	++	29
HCC1428	Large ep. finely vacuolated	1.95	+	+ (55)	+	+ (984)	-	*NA*	2.5
HCC1500	Small, tightly adherent ep. cells	0.9	+	- (<15)	++	- (<15)	+	-	3
HCC1569	Large ep. stickers	2.26	-	- (<15)	-	+ (43)	-	++	30
HCC1599	Ep. floaters; irregular masses	>2	-		-		-	-	4
HCC1739	Large ep. cells; float when heavy	2.68	-	- (<15)	-	- (<15)	+	-	1.5
HCC1806	Medium-sized ep. cells; monolayered	1.43	-	- (<15)	-	- (<15)	-	-	4
HCC1937	Large ep. cells; float when heavy	>2	-	- (<15)	-	- (<15)	-	-	4
HCC1954									28.5
HCC2157	Granular ep. cells; amorphous; floaters	1.67	-		+		+	+	4
HCC2185	Large ep. cells; single cells; floaters	1.38	-	- (<15)	-	- (<15)	++	++	10
HCC2218	Loosely adherent ep. floaters	2.05	-		-		+	++	28

Breast Tissue/Tumor Databases

The University of Michigan Human Breast Cell/Tissue Bank and Database

A comprehensive resource that includes 9 tumor cell lines and a large number of human breast tissues in a variety of forms suitable for diverse experimental conditions. The resource provides molecular data (eg. ER expression), and the relevant demographic and clinical information.

Human Mammary Epithelial Cell (HMEC)

Provides information on human mammary cell resources at Lawrence Berkeley National Laboratory at the University of California, Berkeley. The resource includes a variety of normal, benign, tumor-derived, and *in vitro* transformed cultures of human mammary epithelial cells (HMEC).

Cell Line Data Base (CLDB)

Contains information regarding 3200 human and animal cell lines that include 2226 human cell lines and 861 tumor cell lines.

Breast Cancer Information Core (BIC)

Acts as a repository of information regarding breast cancer susceptibility genes (eg. BRCA1), and other frequently observed mutations in the development of breast cancer. Accessible by a password provided to BIC members who have agreed to abide by a set of guidelines.

American Type Culture Collection (ATCC)

GO Back to UT Southwestern Breast Tissue Repository

Request for Tumor Line

Please complete all fields before sending the request.

Principal Investigator: .....

Phone #: .....

Contact Person (if different): .....

Academic Title: .....

e-mail address: .....

Mailing Address:

.....

.....

Fax #: .....

Project Title: .....

Funding: .....

Requested Strain (s)

Tumor Cell line (HCC) Number: .....

Number of samples requests:.....

Clinical Data Required ? .....

Pathologic Data Required ?.....

Briefly describe project, including needs for and uses of tissue and database. Please justify use of requested number of samples.

Request for HME Strain

Please complete all fields before sending the request.

Principal Investigator: .....

Phone #: .....

Contact Person (if different): .....

Academic Title: .....

e-mail address: .....

Mailing Address:

.....

.....

Fax #: .....

Project Title: .....

Funding: .....

Requested Strain (s)

HME strain Number: .....

Number of samples requests:.....

Clinical Data Required ? .....

Pathologic Data Required ?.....

Briefly describe project, including needs for and uses of tissue and database. Please justify use of requested number of samples.

Dr Adi F Gazdar MD

W Ray Wallace Distinguished Chair in Molecular Pathology

Research Interests & Publications

Awards, Honors & Major Accomplishments

Biographical Sketch

Laboratory Home Page

Selected Publications

Research Interests

Long term goals of Dr. Adi Gazdar's research efforts are to determine molecular and genetic basis of human cancers, and to develop molecular insights to provide prognostic and diagnostic therapies in the treatment of human cancers. Towards these goals, research efforts in his laboratory are aimed at in-depth molecular and genetic analysis of primary tumors derived from human cancers, and the in vitro established tumor cell lines. As the Head of Tumor Cell Biology Section at the National Cancer Institute, Dr. Gazdar collected, catalogued and analyzed over 2,200 human cancer specimens with an emphasis on lung cancer and lymphomas. As Professor of Pathology at the Hamon center for Therapeutic Oncology at UT Southwestern, his efforts resulted in the collection and analysis of over 2,500 human tumor specimens, and establishment of over 21 breast tumor cell lines. Dr. Gazdar's current efforts are directed at elucidating the molecular events preceding the onset of invasive cancers and the development of molecular technology for early detection of cancers. He has been an author of over 350 research publications and three medical text books. His greatest thrust has been in the cancers of lung, breast and cervix.

Awards, Honors & Major Accomplishments

1. .....
2. .....
3. .....
4. .....
5. .....

Biographical Sketch

Dr. Adi Gazdar earned his medical degree from the University of London, and came to the United States in 1964 for Residency in Pathology at Harvard Medical School and its affiliated Hospitals. Originally from Mumbai, India, Dr. Gazdar led the Viral Pathology Section and Medical Oncology Branch at the National Cancer Institute, Bethesda, MD. In 1991, he moved to the University of Texas Southwestern Medical Center at Dallas, TX where he holds W. Ray Wallace Distinguished Chair in Molecular Pathology at The Hamon Center for Therapeutic Oncology. He likes reading historical and biographical books and playing racquet ball.

Selected Publications

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Dr Jerry W Shay PhD

Professor of Cell Biology and Neuroscience

Research Interests

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Biographical Sketch

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Selected Publications

Research Interests

In contrast to cancer cells which have unlimited growth potential, normal cells display a limited capacity for growth and then undergo a process called cellular senescence or aging. Immortal cells, those with an unlimited lifespan, have escaped from normal aging controls. Such an escape mechanism may also be one of the major changes leading normal human cells to become malignant tumors. Consequently, an understanding of the genes and mechanisms involved in aging and immortality would provide information about important steps in the development of cancer. We have shown that several genes originally discovered as tumor suppressors are involved in regulating cellular senescence. Our objective is to define the molecular basis of cellular senescence in human cells, with the long term goal of applying this knowledge to the biology of cancer.

Human telomeres (the ends of chromosomes) are composed of multiple repeats of the sequence TTAGGG. The enzyme telomerase adds these sequences to the end of DNA molecules in germline cells as well as

immortal and tumor cells. Because telomerase is absent in normal cells, telomeres shorten 50-200 b.p. every time a cell divides. We have proposed that the regulatory loci controlling cellular senescence are located adjacent to telomeres and that their expression is modified by telomere length as normal cells divide. We have shown that induction of the M1 (Mortality Stage 1) mechanism constitutively activates the tumor suppressor proteins pRB and p53 into their antiproliferative states.2 Blocking M1 in fibroblasts, with agents that bind pRB and p53, permits cells to divide until most telomeric repeats are lost, thereby causing M2 (Mortality Stage 2). In mammary epithelial cells, agents that bind only p53 are sufficient to block M1. Escape from M2 and consequent immortalization is due to inactivation of factor(s) that normally repress telomerase. Derepression of telomerase then results in an immortal cell line.

The specific goals of the laboratory are directed towards establishing molecular proofs for these hypotheses by: 1) cloning and characterizing expressed genes in the subtelomeric DNA, showing that their expression is regulated by telomere length, and demonstrating their functional roles in the regulation of M1; 2) using insertional mutagenesis to clone M2 genes, and investigating their functional role in M2; 3) identifying and cloning genes that specifically inhibit telomerase activity, resulting in the progressive shortening of telomere length and the restoration of the cellular senescence program.

Awards, Honors & Major Accomplishments

Editorial Board (1997-2000), J Clinical Pathology
Associate Editor (1997) Cancer Research
Associate Editor (1993-) In Vitro; Cellular and Developmental Biology
Editorial Board (1994-) Methods in Cell Science
Editorial Academy (1992-) International Journal of Oncology
Executive Editor (1992-) Cellular and Molecular Differentiation
Scientific Advisory Board (1992-) Geron Corporation
Scientific Advisory Board (1992-) BioWhitaker (1994-1996)
Scientific Review Committee Chairman (1996-) Mary Kay Ash Charitable Foundation
Research Grant Review Committee (1995-) Susan Komen Breast Cancer Foundation
Member, UT Southwestern Cancer Center Review Committee (1977-1994)
Elected Member, Tissue Culture Association Education Committee (1978-1982; 1988-1992)
AlliedSignal Award for Research on Aging (1995-1997)
NIH Research Career Development Award (1978-1983)

Biographical Sketch

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Laboratory Home Page

Check out Jerry's extensive and colorful laboratory page! You get a glimpse of their party pictures, his collaborative efforts with Dr. Woody Wright , and their new **nature medicine** cover picture.

Link to Lauren Gollahon's Home Page

Selected Publications

Kim, N-W., Piatyszek, M.A., Prowse, K.R., Harley, C.B., West, M.D., Ho, P.L.C., Coviello, G.M., Wright, W.E., Weinrich, S.L., and J.W. Shay. Specific association of human telomerase activity with immortal cells and cancer. Science, 266:2011-2015, 1994.

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Shay, J.W., Werbin, H., and W.E. Wright. You haven't heard the end of it: Telomere loss may link human aging and cancer. Canadian J. Aging 14:511-524, 1995.

Shay, J.W. Aging and cancer: Are telomeres and telomerase the connection? Mol. Med. Today 1:378-384, 1995.

Shay, J.W. and W.E. Wright. Telomerase activity in human cancer. Curr. Opin. Oncol. 8:66-71, 1996.

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