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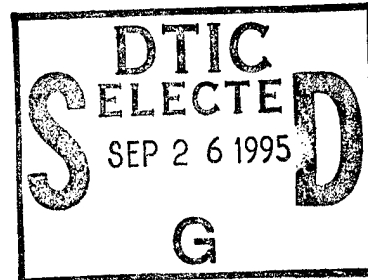
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Human Breast Tissues and Cells

PRINCIPAL INVESTIGATOR: Jerry W. Shay, Ph.D.

CONTRACTING ORGANIZATION: University of Texas Southwestern Medical Center
Dallas, Texas 75235-9016

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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. We have established a unique repository of materials for the biologic and genetic study of breast cancer. The repository contains cryopreserved and cultured cells from tumor tissues, non-malignant epithelial and stromal cells and when available peripheral blood mononuclear cells. Patient demographic and clinical data, and family history are collected and entered onto a relational database.

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enclosure

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JWS Jerry W. Shay 12 July 1995
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 1994 - July 1995)

Introduction

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;
- 2) prepare and cryopreserve breast tissue organoids from which both epithelial and stromal cells can be cultured;
- 3) establish breast tumor cell lines from patients with primary breast carcinoma;
- 4) establish and cryopreserve EBV-transformed B-lymphoblastoid cells as a source of constitutional DNA;
- 5) include samples from women with familial breast cancer and with non-invasive breast cancers;
- 6) collect patient demographic, family, clinical and pathological data;
- 7) maintain computerized records of all data, materials accessioned, and cell characterization;
- 8) publicize information about the repository and to make its resources readily available to the scientific community with minimal restrictions.

The samples will be characterized for DNA ploidy, karyotype, progesterone/estrogen receptors, BRST-1, BRST-2, CEA, cytokeratins, MFGMA, p53 mutations and telomerase activity. The repository will provide researchers with a comprehensive and unique source of frozen tissue and self-replicating tumor cells and corresponding epithelial and stromal breast cells as well as constitutional DNA, along with relevant clinical and demographic information.

Body

Task 1. Obtain Normal and Tumor Surgical Specimens

In July 1994 we initiated a repository for multiple areas of breast cancer research. It is an ambitious project, and during the first year of the parent grant we have obtained and cryopreserved 84 tumor and adjacent "normal" tissue samples. Of these samples we have established and cryopreserved a total of five human breast tumor cells lines as well as 30 "normal" breast tissue organoids and breast epithelial and stromal cells primary cultures. Almost all our repository samples have been obtained from Parkland Memorial Hospital in Dallas County. The patient population at Parkland has a strong ethnic mix of minority groups with black and hispanic patients accounting for the vast majority of patients. During the first year very few early stage breast tumors were

obtained due in large part to lack of availability of sufficient material. While approximately 50% of all breast cancer patients in the U.S. are diagnosed with early stage breast cancer, it is unfortunate that 80-90% of the patients seen at Parkland Memorial Hospital present with advanced breast cancer. Therefore, a goal for the next year of this program is to obtain more early stage and premalignant breast tissue samples for our repository.

Prior to the initiation of our breast tumor and cell repository approximately 85 breast cancer specimens were accessioned. These consisted of 62 primary breast cancers and 23 metastatic lesions. Primary tumor tissue, adjacent non-malignant tissue, and cryopreserved peripheral blood mononuclear cells were preserved. During the first year of the parent breast tumor and cell repository grant, we have obtained and cryopreserved approximately 84 additional patient samples so that at the present time we have accessioned approximately 169 breast tumor specimens. Thus most of our effort during the first year has been to accession samples and establish the cell lines. We have characterized all our primary tumors for the presence of telomerase activity. The development of a novel telomerase activity assay has been successful and widely acknowledged as a major breakthrough in cancer research (see references).

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

We have been successful in culturing and cryopreserving approximately 30 breast epithelial and stromal cell cultures. One of the epithelial cell cultures obtained from a patient with Li-Fraumeni syndrome spontaneously immortalized (see references).

Task 3. Characterize Breast Epithelial and Stromal Cells

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 have initiated the cultures and then cryopreserved them until such time as the tumor cell data are obtained.

Task 4. Establish Breast Tumor Cell Lines from Primary Breast Carcinoma

We recognized at the onset that this would be the rate limiting component to the success of the repository. We have only clearly established one additional breast tumor cell line during the first year (for a total of 5 new breast tumor cell lines). We will be characterizing these and making them available for distribution during the next review period.

Task 5. Establish EBV-transformed B-lymphoblastoid Cell Lines

We have cryopreserved peripheral blood mononuclear cells but will only transform samples with EBV when we have evidence that the tumor lines and normal epithelial and stromal cells are successfully established and cryopreserved.

Task 6. Maintain a Computerized Database

All entries are currently 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 Wheelless, Computer Specialist, an employee of the Simmons Cancer Center 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). 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.

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

Even though we have not advertized our repository during the first year, approximately 12 individuals have obtained tissues and cells from our repository.

Task 8. Maintenance of Cell Repository and Backup

At present all samples are maintained in both Dr. Gazdar's and Dr. Shay's laboratories. During the next review period, we propose to survey existing breast tissue banks and initiate efforts to coordinate data base interconnections. At the NCI there is a Cooperative Breast Cancer Tissue Repository directed by Roger L. Aamodt, Ph.D. This registry provides tissue sections from formalin-fixed, paraffin-embedded primary breast tumors. This registry can provide clinical and outcome data, including demographic data, diagnosis, extent of disease, treatment, follow-up, recurrence, survival and vital statistic. These formalin-fixed materials should complement our frozen repository materials, and we will initiate discussions with Dr. Aamodt about interconnecting our efforts. In addition, Martha Stampfer, Ph.D. (Lawrence Berkeley Laboratory, California) has cooperated with us in the past with her breast tumor and cell repositories, and we intend to have discussions with her about coordinating our resources. We have already contacted Dr. Stampfer about the possibility of interconnecting our repository with hers and establishing an electronic bulletin board that could include some of the following: availability of cells and tissues; posted information from others who have resources to share (cells, antibodies, cDNAs etc); posted questions and requests from others on the network; indexed information about latest experimental results/abstracts they would like to share.

Task 9. Future Stable Monetary Support for Repository

Since we have only completed one year of the grant we have decided to delay until the third year pursuing additional long term support for the repository.

Conclusions

Overall we have had a very successful first year effort. Initially we had to recruit and train a new research assistant and establish lines of communication for successfully obtaining and distributing samples. We were somewhat disappointed that we have not clearly established more tumor cells lines but are optimistic that this will improve in the following years. Our biggest success was the development of an improved telomerase activity assay (see references). 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).

References

The following three manuscripts acknowledge support from the parent grant DAMD17-94-J-4077:

1. 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 Shay J. W., Specific association of human telomerase activity with immortal cells and cancer, *Science*, 266:2011-2015, 1994.
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3. Piatyszek, M. A., Kim N. W., Weinrich S. L., Hiyama, K., Hiyama, E., Wright W. E., Shay, J. W. Detection of telomerase activity in human cells and tumors by a telomeric repeat amplification protocol (TRAP). *Methods Cell Sci.*, 17:1-15, 1995.