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Characterization of Early Genomic Changes in Mammary Glands of High Risk Women

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Because many of the familial breast cancer patients carry a mutation in brca-1 on chromosome 17 or brca-2 on chromosome 13, the first genetic event that may occur in their mammary glands to begin the progression toward cancer is on one of these two chromosomes. This genetic event is termed loss of heterozygosity (LOH). It is unknown if these genetic changes correspond to a recognizable histopathological abnormality, nor what are the precise associated chromosomal changes leading to cancer. At the Lombardi Cancer Center, we have a large ongoing study to test high-risk women for brca mutations and to counsel them on their prevention options. One of the options is prophylactic mastectomy, and our Histopathology and Tissue Shared Resource is maintaining a repository and database, with associated blood specimens of such material. We now propose to utilize these "normal glands" removed for preventive purposes, as well as brca- positive breast cancer and associated mastectomy tissue to learn more about the precise earliest histopathologic changes and associated chromosomal changes in brca- carriers. From these studies we hope to learn more about the natural history of brca positive breast lesions and to improve their molecular diagnosis and the decision making for the patients.

Breast Cancer

Unclassified

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

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

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

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

A major advance in breast cancer research over the past few years has been the identification of genes which are responsible for hereditary breast cancer, namely BRCA1 and BRCA2 genes (1, 2). However the actual roles of these two genes in breast tumorigenesis are not clear. For example, the basis of the vast variation in penetrance of different mutations in these two genes, variations of the same mutation in different individuals of a similar family and variations among families, are not clear. We would like to begin to understand the earliest steps in histopathologic appearance and potentially associated genomic alterations as breast cancer begin to arise in high risk, BRCA-carrying individuals. No studies to date have systematically examined the early consequences of inheritance of a mutation in the BRCA-1 or BRCA-2 genes for corresponding early changes in breast histopathology. In addition, no studies have addressed the correlation of such early abnormalities in the breasts of BRCA mutation carriers with genomic gains, losses, loss of heterozygosity (LOH), or replication error repair instability. Studies of morphologically normal lobules adjacent to sporadic breast cancer have shown the presence of LOH in these morphologically "normal" tissues suggesting the presence of a "field effect" of preexisting genomic damage in the gland, which gives rise to the tumor (3). Two recent studies have shown cytogenetic abnormalities in prophylactic mastectomy specimens characterized by hyperplasia without atypia, from patients with a positive family history of breast cancer (unknown BRCA status) (4, 5). Taken together, these studies suggest that there may be detectable early genomic changes in the breasts of high risk patients with inherited predisposition to breast cancer. Characterization of such changes may provide further insight into the onset and progression of breast tumors in these patients.

In this project we are evaluating the early genomic changes that occur in the mammary glands of patients with increased predisposition to breast cancer because of germline mutations in the BRCA-1 or BRCA-2 genes. To address this question we are analyzing mammary tissues from a group of patients with such mutations. Our analysis will include tumor tissues from patients with breast cancer, normal surrounding tissues to breast tumors, contralateral prophylactic mastectomy samples from patients with breast tumors and prophylactic mastectomy samples from patients with no breast tumors. Our evaluation of these samples is done using a combination of molecular tests and expert pathology review. Following careful evaluation of each sample by pathologist, we will study loss of heterozygosity (LOH) at loci on chromosome 17 in patients with BRCA-1 mutations and on chromosome 13 in patients with BRCA-2 mutations. In addition, a genome wide search for chromosomal gains and losses will be conducted on all samples using comparative genomic hybridization (CGH). The overall purpose of this work is to learn more about the natural history of BRCA-positive breast lesions and to improve the molecular diagnosis of BRCA malignancy-associated changes. These studies should aid to improve early detection and diagnosis of hereditary breast cancer and provide more information when considering prevention strategies for such women at risk.

Body:

During the first year of this project we have actively proceeded to achieve the groundwork for the success of this study. Specifically, we have recruited Dr. Luciane Cavalli, a postdoctoral fellow with an excellent experience in cytogentics and molecular biology. Dr. Cavalli started her fellowship in October 1999. Her CV is attached. Progress relative to our statement of work (revised and previously approved according to reviewers' suggestions) is as follows.

Patient accrual:
Over the past year, we have continued to collect tissues from high risk patients with known BRCA status. At present we have accrued 15 patients with BRCA mutations: 10 patients with BRCA-1 mutations and 5 patients with BRCA-2 mutations. (This number represents the subset of the high risk patients who have BRCA-1/2 mutations).

Pathology review of the BRCA 1/2 positive patients:

Tissue is available from 14 of 15 cases:

Sclerosing adenosis and cystic change are present in all fourteen cases. Usual ductal hyperplasia is seen in seven and atypical ductal hyperplasia in one case. Atypical lobular proliferation is seen in one case. Fibroadenomatoid hyperplasia is present in three cases. A small duct papilloma is seen in one case.

Laser capture microdissection (LCM):

Dr. Cavalli, with the assistance of our pathologist, Dr. Baljit Singh, was trained to use the LCM system which is available in our tumor bank. She has spent several hours of training using sporadic breast tumor samples available from our tumor bank. In addition Dr Cavalli has established a contact with the NIH LCM core facility, directed by Drs. Robert Bonner and Lance Liotta. She has visited the facility and had the chance to interact with the core staff and discuss the protocol they follow. This interaction is very helpful to our group because it gives us access to the lab that discovered and developed the LCM technique. In June 2000, Drs. Haddad, Singh and Cavalli attended the LCM symposium held at NIH where several presentations and discussions took place from different groups using this approach. The meeting allowed very helpful interactions to take place among users and experts in the field. At present, Dr. Cavalli is well trained to use LCM on our BRCA-1/2 samples and will be starting this in the second year of the grant.

LOH studies:

The PCR conditions to study several of the LOH loci on chromosomes 13 and 17 which are needed for this project have been established and validated using DNA extracted from blood and from tumor tissues. We are presently in the process of optimizing the conditions for DNA obtained from laser capture microdissected (LCM) specimens. To achieve that optimization, we are using sporadic breast samples which are available from our tumor bank. Once this is completed, we will test our 15 BRCA-1/2 mutation positive samples (plus all newly accrued patients).

Figure 1 shows LOH analysis performed in our lab on samples from one of our patients with a BRCA-1 mutation and breast cancer for the locus D17S855 (a BRCA-1 intragenic locus). Analysis of DNA prepared from the patient's lymphocytes (Fig 1, "Blood") and the normal breast tissues (prophylactic mastectomy) (Fig 1, "Normal Breast") show 2 peaks representing 2 alleles while the DNA from the breast tumor (Fig 1, "Breast tumor") show loss of heterozygozity for that locus.
CGH evaluation:

CGH analysis using fresh tissues and/or archival formalin-fixed, paraffin-embedded tumors is routinely performed in our lab. Dr. Cavalli was trained to use this technique and will apply it to evaluate our samples. Figure 2 shows the analysis from the breast tumor described in the previous paragraph from the same patient with BRCA-1 mutation. The profile shows both gains and losses of chromosomal material. The five vertical lines on the right side of the chromosome ideograms reflect different values of the fluorescence ratio between the test DNA prepared from tumor cells and the control DNA. The values are 0.5, 0.75, 1, 1.25 and 1.5 from left to right. The ratio profile (curve) was computed as a mean value of at least 8 metaphase spreads (n is the number of chromosomes used to generate each ratio profile). Gains are noticed on chromosomes 6q, 9, 10p, and 11q, amplification on chromosome 8q and losses on chromosomes 4, 8p and 18. We plan to perform this assay on all our samples during the second year of the project.

Revised statement of work:

Year 1: In the first year, we will obtain hereditary breast tumors with associated mastectomy tissue as well as prophylactic mastectomies from BRCA carriers. [Completed]. We will also fully establish and validate all necessary LOH assays, following pathologic review of all specimens, for comparison of their genomic changes relative to nearby pathologically reviewed and microdissected non-tumor tissue (Aims 1 and 2). [Completed].

Year 2: In the second year, specimen collection will continue, Aims 1 and 2 will continue, and Aims 3 and 4 (study of pathologically reviewed contralateral prophylactic mastectomy tissues and pathologically reviewed bilateral prophylactic mastectomy tissues) will begin.

Year 3: In the third year, all 4 aims will be completed and data analyzed. Specifically, pathologic diagnosis will be correlated with genomic and chromosomal changes for each aim.

Key Research Accomplishments:

- Accrued 15 patients with BRCA 1/2 mutations who underwent mastectomy and/or prophylactic mastectomy.
- Reviewed tissues by an expert pathologist.
- Optimized conditions for LOH studies using DNA from both lymphocytes of patients with BRCA 1/2 mutations and LCM samples from archival tumors.
- Optimized conditions for CGH studies of archival breast tumor tissues from BRCA 1/2 mutation carriers.

Reportable outcomes: N/A

Conclusions:

In this first year we have established the groundwork for the success of this project. As described in our "revised statement of work", we have obtained hereditary breast tumors with associated mastectomy and prophylactic mastectomy tissues from BRCA carriers. We established and validated the necessary LOH assays. A pathologist evaluated the tissues and helped in establishing the LCM technique in our lab. We will now address our primary hypothesis: Genomic changes (genomic gains, losses, LOH, or replication error repair instability) may be
detected in the histologically abnormal, premalignant and malignant regions in the breasts of BRCA carriers. These changes may also be present in tissues adjacent to BRCA-associated cancer and in prophylactic mastectomy specimens, thus representing the earliest detectable changes. In the upcoming two years we will look for these changes in our collection of specimens. These data should aid in improved early detection and diagnosis of hereditary breast cancer and provide more information when considering prevention strategies for such women at risk.

References:


Appendices:

CV of Dr. Cavalli
Figure 1. LOH
Figure 2. CGH
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2. Photocopy this page or follow this format for each person.

NAME
Luciane Regina Cavalli

POSITION TITLE
Postdoctoral Research Fellow

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

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<td>BS</td>
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<td>Masters</td>
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<td>Federal University of Parana, Brazil</td>
<td>PhD *</td>
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<td>post doctoral</td>
<td>oct 1st / 99-present</td>
<td>Molecular Cytogenetics</td>
</tr>
<tr>
<td>Georgetown University Medical Center (Institute for Molecular and Human Genetics)</td>
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RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE

1990-91: English teacher at the Open English House - Curitiba, Parana, Brazil
1993-95 / 1997-99: Biologist at the Laboratory of Human Cytogenetics at the Clinical Hospital of the Federal University of Parana, Brazil

PUBLICATIONS


LECTURES AND COURSES (last three years)

1. Genetic and Cytogenetic of Breast Tumors. At the Oncology Surgery Service of the Hospital Nossa Senhora das Graças, Curitiba, Paraná, Brazil, December, 1997.

2. Cytogenetic and Molecular Genetics of Hematologic Diseases and Solid Tumors. At the IV Genetic Meeting of the state of Paraná, Londrina, Paraná, Brazil, June, 1998.

4. Genetic and Cytogenetic of Breast Tumors. At the Oncology Surgery Service of the Hospital Nossa Senhora das Graças, Curitiba, Paraná, Brazil, September 1st, 1998.


RELEVANT ABSTRACTS (last 3 years)


5. Schmid-Braz A, Cornélrio DA, Cavalli LR, Lima RS, Cavalli IJ. Cytogenetic study in ovarian tumors. 44th N National Congress of Genetics, Brazil, September, 1998 (Genetics and Molecular Biology, 21 (3), 1998)


Figure 1