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PRINCIPAL INVESTIGATOR: Robert B. Dickson, Ph.D.

CONTRACTING ORGANIZATION: Georgetown University Medical Center
Washington, DC 20007

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

Robert B. Dickson, Ph.D.

Georgetown University Medical Center
Washington, DC 20007
E-Mail: dicksonr@georgetown.edu

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)

Because many of the familial breast cancer patients carry a mutation in BRCA1 on chromosome 17 or BRCA2 on chromosome 13, the first genetic event that may occur in their mammary glands to begin the progression toward cancer may be loss of heterozygosity (LOH) on one of these two chromosomes. It is unknown if these genetic changes correspond to a recognizable histopathological abnormality, nor what are the precise associated chromosomal changes leading to cancer. We hypothesize that such genomic changes may precede morphologic changes and thus we may detect evidence for such changes (eg LOH in morphologically normal breast tissues or benign lesions surrounding breast tumors in BRCA1/2 positive patients. We have recently developed a panel of markers to study LOH in morphologically normal breast tissues and carefully laser capture microdissected, breast tissues. We are evaluating a group of BRCA1/2 positive patients with breast cancer who are followed up by our Cancer Genetics Program at the Lombardi Cancer Center. Our studies so far support our hypothesis. Specifically, several of the markers studied show evidence of LOH in histologically normal looking tissues and in benign lesions surrounding breast cancer in both BRCA1 and 2 positive patients.
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Introduction:

No studies to date have systematically examined the early consequences of inheritance of a mutation in BRCA1 and BRCA2 genes (1, 2) for corresponding early changes in breast histopathology. In addition, no studies have addressed the correlation of such early abnormalities in the breasts of BRCA carriers with genomic gains, losses, loss of heterozygosity, or replication error repair instability. Our present study tests the hypothesis that genomic changes may be detected not only in histologically abnormal, premalignant, and malignant regions in the breasts of women with such an inherited predisposition to develop breast cancer but also in tissues adjacent to BRCA associated cancer and in contralateral prophylactic mastectomies in high risk women with breast cancer. These changes may also be present in “normal” specimens removed prophylactically from BRCA1 and BRCA2 carriers, as well. Such changes may represent the earliest detectable genomic aberrations that occur during the development and progression of breast cancer in these high-risk patients. These data will aid in improved early detection and diagnosis of hereditary breast cancer and provide more information when considering prevention strategies for such women at risk. There is evidence from previous studies (3, 4, 5) that genomic changes may precede morphologic changes in the process of cancer progression. To achieve our goal, we are studying a group of BRCA1/2 positive patients with breast cancer who are followed up at our Cancer Genetics Program at the Lombardi Cancer Center (LCC). We are evaluating carefully selected breast tissues, isolated using laser capture microdissection (LCM), with the help of an expert pathologist. We are looking for evidence of molecular genetic changes in these tissues. Specifically, we have developed a set of molecular markers to study loss of heterozygosity (LOH) on chromosome 17 (in patients with BRCA1 mutations), chromosome 13 (in patients with BRCA2 mutations) and 3p (in all patients). LOH is being evaluated in the tumors and the morphologically normal looking tissues or tissues with benign changes surrounding the tumors. Our studies conducted so far are very promising since we were able to detect LOH in morphologically normal breast tissues and in benign lesions in BRCA1/2 positive patients with breast cancer.

Body:

During the second year of this project, we have achieved major progress and completed important milestones.

A set of microsatellite markers on chromosomes 17 and 13 where the BRCA1 and BRCA2 genes map, respectively, has been established. We have also established the experimental conditions to study a set of markers which map to the 3p region. This is a region which is commonly lost in several tumors including early breast tumors and which harbors several tumor suppressor genes such the FHit gene (6, 7).

During the first year of the grant, we have established and validated the experimental conditions to evaluate each of the markers. Major progress has been reached during the second year, as we were able to successfully study each of these markers, in a reliable and reproducible way, using DNA prepared from laser capture microdissected (LCM)
specimens. This step is critical for our study, as it allows us to select specific epithelial cells for molecular analysis following accurate histologic characterization by the pathologist.

At present our panel includes the following markers:

- Chromosome 17 markers: TP53 (17p13), D17S849 (17p13), D17S250 (17q11.2-q12), D17S786 (17p12), D17S579 (17q21.3), D17S806 (17q21), D17S855 (17q21.2, BRCA1 gene intragenic marker), D17S785 (17q24), D17S784 (17q25).

The Cancer Genetics Program at the Lombardi Cancer Center (LCC) continues to actively accrue patients with familial breast cancer (both with and without BRCA1/2 mutations) and their unaffected 1st degree relatives. Dr. Luciane Cavalli, a post doctoral fellow in Dr. B. Haddad's lab, is analyzing the presently available tumor samples from both BRCA1 and BRCA2 positive patients. For each patient studied, the markers on chromosome 17 (for the BRCA1 positive patients) and on chromosome 13 (for the BRCA2 positive patients), in addition to the markers on chromosome 3p (for all patients), are 1st evaluated using DNA prepared from the patient's blood to identify the markers which are informative. For each patient studied, tissue sections from the breast with a tumor and from the contralateral normal breast removed prophylactically, are carefully evaluated by Dr. B. Singh, an expert breast pathologist. Dr. Singh marks each section to clearly indicate the malignant and premalignant tissues, and the morphologically normal looking tissues surrounding the tumor, and the areas with benign changes such as sclerosing adenosis (SA), usual ductal atypia, or atypical ductal hyperplasia. Over the past two years, Dr. Cavalli has acquired an extensive experience in the technique of laser capture microdissection (LCM). She has attended two conferences on the topic and continues to interact with colleagues at other labs where this method is performed. Following the evaluation of each tissue section by the pathologist, she uses LCM to isolate cells from the different areas described above. LOH analysis is then performed on DNA isolated from each area.

Using our panel of markers described above, we have evaluated 14 areas showing normal morphology or sclerosing adenosis surrounding breast tumors from three different patients (2 patients with BRCA1 mutations and 1 patient with BRCA2 mutation) and from an area of sclerosing adenosis from a prophylactically removed breast from a BRCA1 positive patient with breast cancer in the contralateral breast. In addition, tumor tissues microdissected from each of the cases using LCM, were also studied for LOH for comparison.

Our studies revealed that markers showing LOH in the tumors, did also show LOH in morphologically normal areas and areas with benign changes (i.e. sclerosing adenosis) surrounding the tumor. An interesting finding was the detection of LOH of the D17S855 marker (intragenic to BRCA1 gene), in areas showing sclerosing adenosis in the contralateral breast removed prophylactically from the patient with a BRCA1 mutation.
Figures 1-6 show examples of LOH analysis for different markers at areas with malignant changes, normal morphology or benign changes (sclerosing adenosis).

Revised and approved 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. [Completed].

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:

-Development of a panel of microsatellite markers to study LOH on chromosomes 13, 17 and 3p in laser capture microdissected (LCM) specimens.
-LOH analysis of morphologically normal breast tissues and breast tissues with benign changes, carefully microdissected from BRCA1/2 positive patients with breast cancer.

Reportable Outcomes:

Cavalli, LR., Singh, B., Issacs C., Dickson RB., and Haddad, BR. Evidence of genomic instability in morphologically normal breast tissues and in benign breast lesions in BRCA1/2 positive patients with breast cancer. Manuscript in preparation.

Conclusion:

Taken together, our data so far, is consistent with our hypothesis, namely that genetic changes may indeed occur in morphologically normal breast tissues, or breast tissues showing benign changes in BRCA1/2 positive patients with breast cancer. As we continue to evaluate more tumors in the coming year, we will be able to test this hypothesis further. Such findings might lead to the identification of genetic markers that could be used to assist in the early detection of breast cancer in women at risk.
References:


Appendices:

-Figures 1-6

-Abstract Presented at the United States and Canadian Academy of Pathology Annual Meeting March 3-9, 2001, Atlanta, GA.
marker D17S855

Figure 1: Marker D17S855 (17q21.2, BRCA1 gene intragenic) showing LOH in 4 morphologically normal areas at different distances, surrounding a breast tumor in a BRCA1 positive patient.
Top panel, shows the marker to be heterozygote (informative) in the patient's blood. The marker shows also LOH in the tumor.
marker D17S855

Blood

LCM - tumor

LCM - 7mm away from the tumor

LCM - 7.5 mm away from the tumor

LCM - 8 mm away from the tumor

LCM - 8.5 mm away from the tumor

Figure 2: The same marker in Figure 1 (D17S855), showing LOH in 4 areas with benign changes (sclerosing adenosis) surrounding a breast tumor in a \textit{BRCA1} positive patient.
Figure 3: Marker D17S855 showing LOH in an area with sclerosing adenosis, in the contralateral breast removed prophylactically from a BRCA1 positive patient with breast cancer. Morphologically normal areas of the same breast do not show LOH for this marker.
marker D17S785

Figure 4: Marker D17S785 (17q24) showing LOH in both the tumor and a surrounding benign (sclerosing adenosis) area in a BRCA1 positive patient.
marker D3S1300

Figure 5: Marker D3S1300 (3p14.2, FHIT gene intragenic) showing LOH in both the tumor and a surrounding benign (sclerosing adenosis) area in a BRCA1 positive patient.
marker D13S153

![Marker D13S153 showing LOH in tumor cells compared to blood and LCM-tumor samples.](image)

**Figure 6:** Marker D13S153 (13q14.1-q14.3) showing LOH in the tumor cells obtained using LCM in a *BRCA2* positive patient.
Abstract Presented at the United States and Canadian Academy of Pathology Annual Meeting March 3-9, 2001, Atlanta, GA.

Spectrum of Benign Breast Disease in Familial Breast Cancer Patients With and Without BRCA Mutations. B. Singh, L. Seltzer, M. Ossandon, S. Constable, M. Joyner, B. Haddad, J. Stead, C. Lerman, C. Isaacs Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC

**Background:** The pathology of familial breast carcinoma with and without BRCA mutations has been reported. However, a systematic review of benign breast disease has hitherto not been performed in this cohort of patients.

**Design:** Hematoxylin and Eosin stained slides from formalin fixed, paraffin embedded archival tissue from 20 cases with BRCA1/2 mutations and 12 cases of familial breast carcinoma without BRCA mutations were examined. One representative slide from of benign breast disease from seventeen patients with BRCA mutations was immunostained for estrogen, progesterone receptors and Ki67 to categorize the proliferative status of the terminal duct lobular units and classify them as Lobule type I, II or III.

**Results:** BRCA mutants had sclerosing adenosis in 76% (16/21), usual ductal hyperplasia (UDH) in 38%, small duct papilloma in 10% and radial scar in 5% cases. Cases of familial breast carcinoma without BRCA mutations had sclerosing adenosis in 50% (12/24), UDH in 8%, atypical ductal hyperplasia in 4%, radial scar in 12.5% cases. 12% (2/17) of cases with BRCA mutants predominantly had lobules type I and 88% cases contained lobules, type II.

**Conclusion:** Proliferative fibrocystic changes were observed in 76% of cases with BRCA mutations and in 50% of cases with familial breast carcinoma without BRCA mutations. The frequency of proliferative changes in BRCA mutants is similar to that reported in sporadic breast carcinoma patients and is less frequent in non-mutants. The lobules in BRCA mutants, all of which were parous women, revealed low proliferative activity as assessed by Ki67 staining.