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The promise of research into breast cancer genetics is that it will provide us with new insights into the etiology of breast cancer that can be translated to strategies for early diagnosis and treatment for the larger population of women who develop breast cancer without having a genetic predisposition.

An academic award represents an outstanding opportunity for me to critically appraise the emerging role of genetics in clinical breast cancer care and forge new avenues of research. Toward this goal, I plan to accomplish the following during the award period:

1) perform a thorough review of the cytogenetic and molecular genetics literature to identify potential chromosomal regions that may harbor genes whose abnormal function is critically involved in the development of breast cancer.

2) develop a robust panel of markers that can be used for clinical correlative studies of hereditary breast cancers.

3) develop a tissue repository composed of biological specimens from 500 patients with inherited breast cancer (e.g. fresh frozen tumor specimens, or paraffin embedded tumor specimens and normal blood lymphocytes, DNA and sera whenever possible).

These studies will lead to an improved understanding of the biology of breast cancer which will ultimately translate into more effective therapies.

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INTRODUCTION

As a physician-scientist, I have had extensive training in clinical oncology and in molecular biology and genetics; I am ideally positioned to bridge the gap between the two. The academic award has represented an outstanding opportunity for me to critically appraise the emerging role of genetics in clinical breast cancer care and forge new avenues of research. Toward this goal, I plan to accomplish the following during the period of my academic award.

1) perform a thorough review of the cytogenetic and molecular genetics literature to identify potential chromosomal regions that may harbor genes whose abnormal function is critically involved in the development of breast cancer.

2) develop a robust panel of markers that can be used for clinical correlative studies of hereditary breast cancers.

3) develop a tissue repository composed of biological specimens from 500 patients with inherited breast cancer (e.g. fresh frozen tumor specimens, or paraffin embedded tumor specimens and normal blood lymphocytes, DNA and sera whenever possible).

Using these unique resources, my future studies will characterize the molecular pathways which allow a normal breast cell to become cancerous in individuals who are genetically predisposed. I will also develop longitudinal follow up studies to correlate clinical outcomes with molecular characterization and epidemiologic risk factors. These studies will no doubt lead to an improved understanding of the biology of breast cancer which will ultimately translate into more effective therapies.

Task 1

perform a thorough review of the cytogenetic and molecular genetics literature to identify potential chromosomal regions that may harbor genes whose abnormal function is critically involved in the development of breast cancer.

Cytogenetic analysis of breast cancer has proven to be relatively difficult. Nonetheless, many of the tumor suppressor genes cloned to date that are important in solid tumors have been identified because their location was defined by recurring chromosomal aberrations in the particular tumors. I have begun a review of the cytogenetic and molecular genetic literature because another strategy to understanding the pathogenesis of breast cancer is the study of somatic mutations in breast cancer cells. For several reasons, this is an important complementary approach to the study of germline mutations. First, the large majority of breast cancers are sporadic and similar molecular pathways may be dysregulated in the tumor cells from sporadic and inherited cases. Secondly, genes that are identified as frequently altered in tumor cells are candidate genes for susceptibility loci e.g. TP53 and the Li-Fraumeni syndrome. Lastly, studies of the secondary genetic alterations may provide mechanistic clues for the development of breast cancer. As the Human Genome project has been completed, a number of genes with no known function are available in the database. A thorough review of the cytogenetic literature, especially Mitelman’s catalogue which is now available on the NCI website (Cancer Chromosome anatomy project) has provided me with a roadmap of chromosomal regions commonly deleted in breast cancer, as well as an overview of genes that are commonly mutated or dysregulated. We are also reviewing currently available technologies for assessing genetic alterations in breast cancer. For example, FISH may be good for simultaneously assessing multiple chromosomal and gene abnormalities in a single intact cell while comparative genomic hybridization can be used to survey the entire genome for unbalanced chromosomal changes in a single test, when a suspected genetic aberration is unknown. Several companies are developing a ready-to-use array of DNA or cDNA probes on a miniaturized chip so that simultaneous assessment of a large number of genes can be done. Thus, different technologies are now available to assess abnormalities of chromosomes, of individual genes within chromosomes and of specific DNA sequences within genes. No single technology is capable of assessing all of these different types of abnormalities. We are preparing two reviews on the genetics of breast cancer for publication.
Task II

develop a robust panel of markers that can be used for clinical correlative studies of hereditary breast cancers.

Little information exists on the secondary genetic changes which characterize hereditary. Previous studies have suggested that BRCA1-associated breast cancer has a distinct phenotype characterized by poor prognostic factors such as high tumor grade, increased proliferative rate, estrogen receptor negativity and p53 mutations. Paradoxically, the prognosis in women with BRCA1 hereditary breast cancer appears to be no worse if not better than controls. It may be that these high grade tumors with TP53 mutations respond better to chemotherapy and radiation therapy because of their inability to repair damaged DNA and genomic instability. Does BRCA1 modify the effects of other genes? To answer these questions, it will be necessary to characterize the secondary genetic changes in hereditary breast cancers. We have developed a robust panel of FISH probes and antibodies for immunocytochemistry that can be used on fresh or paraffin embedded tumors. Based on current data, the most common regions of LOH in invasive breast cancer are located on chromosome arms 17p, 17q, 16q, 13q, 11q, 1q and 18q. Analyses of DCIS have revealed frequent losses on 8p, 13q, 16q, 17p and 17q. Amplification of HER-2, MYC, Cyclin-D1 and the AB1 gene on 20q13.2 have also been described in breast cancer. LOH in the BRCA1 region has been described in more than 90% of BRCA1 associated tumors while LOH on 13q has been described for BRCA2 associated tumors. These data demonstrate that known breast cancer susceptibility genes are associated with LOH in the particular gene locus in tumors. It is my belief that other breast cancer susceptibility genes might be revealed by identifying regions of frequent chromosomal deletions in familial breast cancers. In addition, special interactions among different genes and molecular determinants of response to treatment can be observed in such studies.

We have developed FISH probes for BRCA1, BRCA2, CDKN2A, and HER-2 and we have optimized the immunohistochemical analysis for p53, BCL2, BCLX and RB. We have recently purchased a spectral karyotyping system comprised of a 24 color probe reagent set and a genetic workstation for image analysis of the multicolor karyotype provided by the reagent. Spectral karyotyping simplifies and improves the accuracy of karyotyping by providing a unique color for each chromosome. In collaboration with Dr. Janet Rowley, we are planning to perform spectral karyotyping on 20 of the best characterized breast cancer cell lines (e.g. MCF-7, T47Detc) to identify additional regions of subtle translocations or deletions. I will develop additional probes for specific genes or loci that are found to be commonly altered in breast tumors. As an example we are now collaborating with Dr. Charles Perou to examine the differentially expressed genes in BRCA1 tumors.

Task III

develop a tissue repository composed of biological specimens from 500 patients with familial or hereditary breast cancer (e.g. fresh frozen tumor specimens, or paraffin embedded tumor specimens and normal blood lymphocytes, DNA and sera whenever possible).

We are currently developing a clinical protocol for the tumor bank. The protocol has not yet been approved by the DOD Human Subjects Review Panel.

KEY RESEARCH ACCOMPLISHMENTS:
Too early to report

REPORTABLE OUTCOMES:
N/A

CONCLUSIONS:
N/A Too early
REFERENCES:
N/A

APPENDICES:
N/A