GENETIC DEVELOPMENTS IN BREAST CANCER

Adolphus C Favors, Jr, MD

I. INTRODUCTION

It has been estimated that during 1995, 183,000 new cases of female breast cancer were diagnosed in the United States.\(^1\)
Six percent (10,960) will be found in women under age 40, 16 percent (30,000) between ages 40 to 49, but the vast majority (78 percent) will occur in women age 50 and older. While the incidence rate of age specific breast cancer has continued to increase over the last 20 years,\(^2\) mortality rates have remained flat because of early detection of small lesions and improved survival. The risk that a woman in the general population will develop breast cancer is one in 10 or 10 percent. This figure is a lifetime risk if a woman lives to be 80 years old.\(^3,4\)

The risk of breast cancer in the general population is low at the younger ages (0.5 percent at age 40 and two percent by age 50), but definitely increases with age, especially above 50.

Adenocarcinoma of the breast begins in the ductal epithelial cells. The pathway from normal function to invasion and metastasis requires multiple genetic alterations, perhaps between three and six changes that are rate-limiting in tumor progression.\(^1\) Cancer is genetic, ie., a normal cell is transformed to a malignant cell due to mutations of its DNA. These DNA mutations may occur after conception (somatic mutations) or be inherited from a parent (germline mutations). Most cancers are due to somatic mutations. So although most cancers are genetic, most are not inherited.

Most of the germline mutations which cause breast cancer appear to be inherited as autosomal dominants although the disease may not develop until the wild type allele (the normal allele) is lost or inactivated.

II. HEREDITARY BREAST CANCER

There are many inherited or germline mutations found in association with breast cancers (Table One) and these can be subdivided into two types, early onset and late onset; a division that is based on an inflection in the age specific incidence curve around age 50.\(^5\)

A. BRCA1

1. Early linkage studies. The Berkley Lab of Mary Claire King located this gene in a one to two million base pair region of chromosome 17q21.1, through linkage studies of 1500 families with three or more first degree relatives with breast and or ovarian cancer.\(^6\)

One in 200 women (600,000) have inherited susceptibility to breast cancer thought to be due to BRCA1 mutations. This represents five percent of breast cancers. Males may carry BRCA1, but rarely is it associated with breast cancer. A significant number of colon and prostate cancer have been reported in male BRCA1 carriers. The relative risk for both cancers was three to four fold greater than in those without the mutation.

2. Identification and cloning the BRCA1 gene. A team of 40 researchers from the Utah Medical Center, The National Institute of Environmental Health Sciences (NIEHS), Myriad Genetics, Inc., Eli Lilly, and McGill University identified and cloned the BRCA1 gene.\(^7\) They studied individuals from eight different kindreds that segregated 17q linked susceptibility to breast and ovarian cancer. All kindreds showed early onset breast cancer and four had at least one case of ovarian cancer. Methodology allowed construction of the composite full length BRCA1 complementary DNA. They found BRCA1 is:

SUMMARY

In this paper I have reviewed the current literature on the genetic mutations found in association with breast cancer. More emphasis has been given to BRCA1 because of the excitement generated with the cloning of this breast cancer susceptibility gene. A number of somatic mutations have been described (loss of heterozygosity, overexpression, and other mutations) in breast cancers. While strides have been made in unraveling the genetic basis of hereditary breast cancer (10 to 15 percent of all breast cancers), the genetic causes of most sporadic breast cancer (which comprise 85 to 90 percent) have yet to be discovered but they are likely the result of a step wise progression of cumulative genetic events, similar to those seen in colon cancer.
4. BRCA1 testing in sporadic cancers. The area involving BRCA1 shows loss of heterozygosity in 30 to 70 percent of sporadic breast and ovarian cancers, supporting the theory that BRCA1 is a tumor suppressor gene and responsible for both inherited and sporadic breast cancer.

The group responsible for cloning BRCA1 examined a number of sporadic breast and ovarian cancers hoping to find women with BRCA1 mutations. In 44 sporadic cases they found mutations in only one woman with ovarian cancer and three with breast cancer. One mutation was a stop codon while three were missense mutations but all four proved to be germline mutations. No somatic mutations were found. The mutations included six point mutations, one deletion, and one outside the normal coding sequence which resulted in complete loss of mRNA transcript. The surprise finding that BRCA1 does not appear to play a role in sporadic breast cancer was disturbing and a disappointment.

5. Mutations in BRCA1. A consortium of nine laboratories in North America and the United Kingdom screened 372 unrelated samples for BRCA1 mutations from high risk families. Three of these labs tested for two recurring mutations found in the familial samples using 714 additional breast and ovarian cancer cases of which 557 were unselected for family history.

They found mutations in 63 individuals, 5.8 percent of 1,086 patients, had mutations found by screening the entire BRCA1 coding sequence. Of the 63 BRCA1 carriers, 38 had distinct mutations. The majority (54/63 or 86 percent), were frameshift (71 percent), nonsense, or splice/regulatory, resulting in a truncated protein product. The three most common mutations are shown in Table Two. Exon 1I, which contains 60 percent of the coding sequence of BRCA1, contains 22 of the 38 distinct mutations.

a. Factors affecting sensitivity: A number of families were reported in which the evidence of BRCA1 involvement by genetic linkage was strong, but no mutations were found. The possible explanations advanced for the failure to identify the mutation included:

- Mutations were missed by the single strand conformational polymorphism (SSCP) method. Direct sequencing could uncover these misses.
- Need to analyze for a single transcript on one chromosome because the copy of BRCA1 is not being properly transcribed from genomic DNA into messenger RNA.
- The frequency of presumed regulatory mutations outside of the region being analyzed (exon/intron junctions) will effect sequenced based test.

<table>
<thead>
<tr>
<th>Table One</th>
<th>Hereditary breast cancer mutations</th>
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<tr>
<td>AT (ataxic telangiectasia)</td>
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<tr>
<td>p53</td>
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<tr>
<td>ESR (estrogen receptor)</td>
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<td>BRCA1 (breast cancer -1)</td>
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<tr>
<td>BRCA2 (breast cancer -2)</td>
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<tr>
<td>Breast cancer susceptibility gene</td>
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<tr>
<td>Ovarian cancer susceptibility gene</td>
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<td>Androgen receptor</td>
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<tr>
<th>Table Two</th>
<th>BRCA1 mutations</th>
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<tr>
<td>Location of BRCA1 mutations</td>
<td>Frequency of mutations on cDNA</td>
</tr>
<tr>
<td>185 del AG</td>
<td>8</td>
</tr>
<tr>
<td>5382 ins C</td>
<td>7</td>
</tr>
<tr>
<td>4184 del 4</td>
<td>5</td>
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<tr>
<td>cDNA- complementary DNA transcript del- deletion del 4- deletion in Exon 4 ins- insertion</td>
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In addition, five common polymorphisms were identified.

3. Loss of heterozygosity. Loss of heterozygosity (LOH) is the loss of one copy of a gene, loss of a chromosomal segment, or the complete loss of one of the two chromosome pairs. LOH is an important concept because it implies that the affected allele may be a tumor suppressor gene, and LOH was found frequently prior to identification of many of the mutated breast cancer genes.

The proximal portion of 17q in which the BRCA1 gene locus lies is the frequent site of allelic loss or LOH. Futreal et al. found frequent deletions (25 to 79 percent) spanning a six-centimorgan interval between 17q11.2-17q21.1. Jacobs et al. found 64/130 (53 percent) of sporadic ovarian cancers had LOH at all loci analyzed on 17q, suggesting a tumor suppressor gene distal to BRCA1 which were frequently involved with sporadic ovarian cancer. Sporadic breast cancers are commonly affected by LOH in the general region of BRCA1. Alterations in BRCA1 may lead to breast cancer either by inheritance or by somatic alteration.
Factors affecting specificity: Usually the result of uncertain interpretation of missense mutations. All mutations do not have pathological significance and some may be polymorphisms which could possibly be distinguished from natural polymorphisms if a functional assay were available. The screening technique utilized has a great bearing on the ability to find mutations.

6. Ovarian cancer susceptibility. BRCA1 is also associated with an increased risk of hereditary ovarian cancer. DNA samples from 32 patients with sporadic and eight patients with familial ovarian cancer were studied to analyze chromosome 17 for other possible ovarian cancer causing genes, because BRCA1 mutations are associated with this disorder. Using genetic markers distal to BRCA1, they found loss of heterozygosity (LOH) in 73 percent (29/40) indicating a possible candidate locus involved in ovarian cancer distal to BRCA1. The results suggest a potential tumor suppressor ovarian cancer gene at locus 17q22-23 that may play a role in both sporadic and hereditary ovarian cancer.

B. BRCA2

In 1987, Lundberg, et al. studying breast cancer in 12 pre-menopausal females and two males, found loss of heterozygosity at three distinctive loci on chromosome 13q12 to 13q22 in primary tumor tissue of three females and one male. This suggested the site of a predisposing recessive mutation that might be hereditary and involved in the pathogenesis of human breast cancer. Other groups had demonstrated allelic loss of as many as seven markers on the long arm of chromosome 13.

Wooster et al. (1994) began looking for other genes that might predispose to breast cancer susceptibility in those with male and female breast cancer. They conducted a linkage analysis of 15 families with multiple cases of early breast cancer not linked to BRCA1, which included male and female breast cancers along with ovarian cancer. Examination of chromosome 13q yielded the second hereditary gene—BRCA2.

BRCA2 is located in a six-centimorgan region delimited by 13q12-13. The risk of breast cancer with BRCA2 is 87 percent by age 80. BRCA2 carriers are thought to represent another five percent of hereditary breast cancer cases.

BRCA1 and BRCA2 mutations are generally considered to be dominant mutations, i.e., only one of the pair of genes needing to be mutated to initiate disease (breast cancer). Table Three summarizes and compares the information presently known about BRCA1 and BRCA2.

C. Li-Fraumeni syndrome (LFS)

This is an extremely rare genetic syndrome due to homozygous germline p53 mutations. It has been found in nearly 100 families who develop several different cancers but commonly breast cancer and soft tissue sarcomas. Other tumors include pancreas, lung, brain, skin, acute leukemias, and other malignancies. In this autosomal recessive disorder the probability of developing breast cancer in homozygous affected individuals is greater than 30 percent before age 30 and nearly 100 percent by age 50. Homozygous germline p53 mutations account for only one percent of hereditary breast cancers.

D. Ataxic teleangiectasia (AT)

Ataxic Teleangiectasia (AT) has received a lot of attention because of some new data linking this gene with increased cancer rates. It is a rare autosomal recessive disorder affecting one in 40,000 people. There are only 500 homozygotes in the USA, but the heterozygote frequency is nearly one percent with an estimated 2.5 million carriers. Epidemiological data suggest that a single copy of the defective gene gives women a five-fold increased risk of breast cancer, and may account for up to eight percent of all breast cancers.

E. ESR (estrogen receptor)

The ESR (Estrogen receptor) gene locus may be a candidate gene for inherited susceptibility to breast cancer, especially in women with late onset (over 50) disease, and also those who have tumors that are positive for the estrogen receptor.
F. Androgen receptor mutations

Androgen receptor mutations were described by Wooster as a rare syndrome causing breast cancer in two brothers. A germline mutation of the androgen receptor on the X chromosome was found with evidence of androgen resistance, known as the Reifenstein's syndrome.

G. Third breast cancer susceptibility gene

In the collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene published in early 1995, the authors state that in addition to BRCA1 and BRCA2 there was evidence of a third locus.

When all these genes are isolated and characterized, a diagnostic test for inherited breast cancer susceptibility may be offered.

III. SPORADIC BREAST CANCER

Hereditary breast cancer, resulting from germline mutations, probably accounts for only 10 to 15 percent of all breast cancers. The vast majority of these tumors occur as a result of somatic mutations involving DNA found only in breast tissue. We have already noted disappointment with the early screening of sporadic breast cancers for BRCA1 mutations which has not been very fruitful. Sporadic breast cancer is associated with a number of somatic gene mutations (Table Four) indeed multiple mutations can be found simultaneously in the same breast tumor. To date, no mutation is known to account for the majority of sporadic breast cancers. The mutations to be discussed are those that are found with some frequency in sporadic breast cancer.

A. p53

While known to cause the rare autosomal recessive Li-Fraumeni syndrome of multiple cancers, other studies have documented heterozygous p53 mutations in up to 50 percent of all forms of cancer.

Deng et al. found that the overall prevalence of mutations and nuclear immunopositivity was 59 percent in 133 specimens. Studies done at the Mass General Hospital confirmed that p53 mutant protein detected by immunohistochemical methods proved to be an independent marker for shortened survival, especially in those with positive nodes.

B. Cyclin D1

It is reported that upwards of 45 percent of human breast cancers have significant elevations of Cyclin D1 RNA. Other studies have shown 11q13 amplification in 10 to 15 percent of sporadic breast cancers. In addition to gene amplification, loss of heterozygosity has also been detected at 11q13 in both in situ and invasive breast cancers. This suggested four different sets of breast cancer cases: a small set showing amplification (oncogene) and a larger set demonstrating LOH (tumor suppressor gene).

Gillet et al. used a Cyclin D1 specific antibody to stain breast cancer sections known to have amplification of the PRAD1 (Cyclin D1) locus on chromosome 11q13. Nearly all tumors amplified at the region showed strong staining for Cyclin D1 antibody indicating an increased copy number. The intensity of staining and the degree of DNA amplification were in close agreement.

Eight tumors not amplified showed strong staining for Cyclin D1 antibody demonstrating elevated expression without a measurable change in copy number. The variability in overexpression of Cyclin D1, suggests that it may play a role in tumorigenesis.

Cyclin dependent kinase four (cdk4), is one of several cdk enzymes whose activity propels cells through the cell cycle and into cell division. Cdk's must be activated by proteins called Cyclins. Cyclin D1 is known to behave like an oncogene. The normal control of cell growth requires a balance between Cyclin D1 activation of the cdk's and inhibitory proteins such as p16. Production of excess Cyclin D1 or loss of inhibition of p16 or p21 proteins could tip cells towards cancer growth. Cyclin D1 and p16 are in competition for cdk4.

C. c-erbB2 (HER-1/ neu)

An oncogene that is homologous to epidermal growth factor receptor (EGFR) c-erbB1, encodes a transmembrane glycoprotein receptor (structure similar to EGFR with tyrosine kinase activity) c-erbB2, which is also associated with breast cancer. Amplification of c-erbB2 oncogene or overexpression of its mRNA transcript is found in 21 percent of breast cancers. There is a correlation between relapse and survival in patients with amplification of c-erbB2.

In a CALGB randomized adjuvant chemotherapy trial comparing high, medium, and low doses in node positive breast cancer, a series of factors were also examined to determine their usefulness as markers of survival. There was an inverse correlation seen with c-erbB2 ie., the lower the value the greater the survival.

Doxorubicin plus CMF in full doses was effective against tumors over expressing c-erbB2. When there was little or no c-erbB2, the response to chemotherapy was not dose related. CMF was effective.
against tumors with no or low c-erbB2. The addition of prednisone was felt to affect the response observed.6

D. nm23

A novel gene, nm23 located on chromosome 17q that is associated with tumor metastasis, was discovered due to its decreased expression in murine melanoma cell lines of high metastatic potential.7 Using a nm23 value of 2.4 as the cut-off point, an English study of breast cancer patients revealed that 69 percent of node negative patients expressed high levels of nm23 versus only 37 percent from node positive patients.8 High levels of nm23 were associated with absence of lymph node involvement, well differentiated tumor grade, and a better prognosis.

There are two highly homologous human nm23 alleles, H1 and H2 which cross react with both DNA probes and most antibodies.9 These two genes encode the two sub-units of a nucleoside diphosphate (NDP) kinase. This kinase activity has no connection with the metastatic suppression activity. Nm23-H2 protein also functions as a transcription factor that transactivates the c-myc promoter.10 Nm23 is a tumor suppressor which correlates best with the phosphoprotein level (NDP).

Howlett and others11 have conducted experiments in which the nm23-H1 gene is a prerequisite for certain steps of differentiation in normal breast tissue. When a metastatic human breast cancer cell line was transfected with nm23 and cultured with reconstituted basement membranes, the cells expressed basement membrane components (type IV collagen and laminin) and displayed other characteristics of breast cell differentiation (reduced growth rate).

E. c-myc

This oncogene, located on chromosome eight is noted to be amplified, that is to have increased DNA copy number, in about 12 percent of breast cancers.12

F. RB1

Structural rearrangement of the retinoblastoma gene (RB-1) is found in seven percent of primary breast cancers. These include homozygous partial and total deletions.13 The retinoblastoma gene is located in the same region where BRCA2 is found and could account for the finding of loss of heterozygosity. The presence of many recombinant markers between the retinoblastoma gene and the BRCA2 locus in linkage studies indicates that the retinoblastoma gene was not BRCA2.

G. Cathepsin D

This major protease is found and secreted by breast cancer cells and is responsible for degradations of the extracellular matrix. The gene is overexpressed in breast cancer cells.14

H. DCC

Chromosome 18 contains the DCC gene (deleted in colon cancer)15 which is fundamental in the progression of colon cancer. The frequency of the loss of heterozygosity at the DCC locus ranges upwards to nearly 40 percent in breast cancer.

I. The ras genes

Protein tyrosine kinases formed the internal domain of many membrane growth factor receptors and others which act within the cytoplasm. Three ras genes code for highly related proteins, known as p21 and mutations have been found in 10-15 percent of human solid tumors including breast cancer.16 There is a crucial connection between protein tyrosine kinases and the ras proteins. Signal transduction involves protein tyrosine kinases and ras genes to transmit signals from outside the cell to inside by a chain of protein-protein interactions.17 The ras gene which is bound to GTP shifts to an active state and is affiliated with cell growth and division. When GTP is converted to GDP, ras becomes active. Mutations in ras result in a cell that will become permanently active and dividing.

Although many studies of the molecular biology of breast cancer have been completed in the last decade, there remains much work to do. We have learned that breast cancer is not a single disease, but many, resulting from many differing etiologies. It is likely that this knowledge will lead to improved methods of therapy and a far better understanding of the mortality and morbidity risks associated with the different types of the disease.

References


15. Friedman L. Personal communication. 3-30-95


