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Molecular cloning of BRCA1: a gene for early onset familial breast and ovarian cancer

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Summary

Molecular analyses allow one to determine genetic lesions occurring early in the development of tumors. With positional cloning approaches we are searching for a gene involved in the development of early onset familial breast and ovarian cancer that maps to human chromosome 17q21 and is termed BRCA1. This involves localizing the region genetically within families with multiply affected members, capturing the region identified by genetic analyses in YACs (yeast artificial chromosomes), converting those YACs to smaller manipulable pieces (such as cosmids), and searching for genes via a variety of approaches such as direct screening of cDNA libraries with genomic clones, direct selection by hybridization, "exon trapping", and CpG island rescue. Once identified, candidate genes will be screened for mutations in affected family members in whom breast cancer segregates with the locus on 17q21. The frequency of this gene has been calculated to be 0.0033; from this the incidence of carriers, i.e. those carrying such a predisposition, is one in 150 women. The isolation of BRCA1 and the elucidation of the mutations resulting in breast and ovarian cancer predisposition will allow identification of women who have inherited germ-line mutations in BRCA1. In families known to harbor a germ-line BRCA1 mutation, diagnosis of affected members will be rapid. It is possible that one will also be able to detect alterations of the second copy of this gene early in tumor development in individuals carrying a germ-line mutation. It is not yet known how frequently somatic BRCA1 mutations predispose to breast and ovarian carcinoma in the general female population. If, as in other genetic diseases, new germ-line mutations occur in some women and thus contribute to the development of breast cancer, it may be feasible to screen women in the general population for predisposing mutations. In addition, if acquired genetic mutations of the BRCA1 gene are involved as early events in the development of non-familial forms of the disease, early detection of possible breast carcinoma may become feasible in biopsy of breast tissue.

Introduction

Although the incidence of breast cancer is estimated to be 1/9 for a woman over her lifetime, certain women appear to be at an increased risk. These women harbor germ-line mutations that predispose to breast cancer susceptibility, and in general develop the disease at an earlier age. A characteristic of familial cancers is that besides being of earlier onset than normal, the

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cancer is often bilateral. Some of the women who are at increased risk of developing breast and ovarian cancer harbor a mutation in a gene termed BRCA1 (see Table 1 for list of abbreviations used). Over their lifetime, the likelihood that these women will develop breast or ovarian cancer is approximately 90%.

Tumor development and progression is accompanied by a series of events that occur in a single clone of cells as a result of molecular lesions in a specific set of genes. Breast cancer, like other cancers, is likely to occur as a result of aberrant gene expression. Some of this is due to loss of expression of genes such as those that normally regulate or suppress cell growth (tumor suppressor genes or anti-oncogenes), while some is due to an increase in gene expression, e.g. the activation of growth-promoting factors such as proto-onco-

Table 1. Table of abbreviations

Abbreviation	Definition
Alu-PCR:	(see IRS-PCR)
APC:	adenomatosis polyposis coli
BAC:	bacterial artificial chromosome
BRCA1:	gene for familial early onset breast and ovarian cancer
cDNA:	DNA copied off an mRNA template by reverse transcriptase
cM:	centimorgan, or 1/100th of a Morgan
COL1A1:	collagen, type I, alpha I
DCC:	deleted in colorectal carcinoma
DGGE:	denaturing gradient gel electrophoresis
D17S74:	74th single copy segment of DNA to be isolated from human chromosome 17 (term for a locus)
EDH17B2:	estradiol 17-beta dehydrogenase I
ERBB2:	avian erythroblastic leukemia viral v-erb-B2
FAP:	familial adenomatosis polyposis coli
FISH:	fluorescence in-situ hybridization
GIP:	gastric inhibitory polypeptide
kb:	kilo-base (1,000 bases)
HER2/*neu*:	See ERBB2
HOX2:	homeo box region 2
HTF islands:	HpaII tiny fragment islands
IRS-PCR:	inverse-repeated sequence PCR
LOH:	loss of heterozygosity
Mb:	mega-base (1,000,000 bases)
Morgan:	a unit of recombination (there are 33 Morgans in the human genome)
MEN1:	multiple endocrine neoplasia I
MEN2:	multiple endocrine neoplasia II
NF2:	neurofibromatosis 2 (bilateral acoustic neuroma)
NME1:	non-metastatic cells 1, expressing NM23 protein
NME2:	non-metastatic cells 2, expressing NM23 protein
PHB:	prohibitin
RARA:	retinoic acid receptor, alpha
RFLP:	restriction fragment length polymorphism
RB1:	retinoblastoma I (including osteosarcoma)
PCR:	polymerase chain reaction
SSCP:	single-strand conformation polymorphism
STS:	sequence tagged site
THRA1:	thyroid hormone receptor alpha 1 (avian erythroblastic leukemia viral (v-erbA) oncogene homolog 1, formerly ERBA1)
VNTR:	variable number of tandem repeats
YAC:	yeast artificial chromosome

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genes. Chromosomal rearrangements have identified several chromosomal regions or genes likely to be involved in either the development or the progression of breast cancer. One can also localize genes predisposing to disease on the basis of co-segregation with DNA markers in multiply affected families. The gene we are searching for was identified by Mendelian genetics, and localized to human chromosome 17q21.

Current molecular genetic technology is being used to isolate this gene; termed positional cloning, it relies on localizing the disease gene genetically, capturing the region physically, searching for the genes in the region, and identifying BRCA1 among them. Although one would expect women from a large proportion of families where breast cancer is segregating as a Mendelian trait to harbor an alteration at BRCA1, it is estimated that women with no family history may also harbor an altered BRCA1 gene. It has been estimated that 1/150 to 1/500 women are at increased risk of developing breast and ovarian cancer due to an alteration of BRCA1 in their germ-line DNA. This review describes our approaches to isolating BRCA1, and outlines positional cloning approaches in general.

Segregation analysis to dissect diseases genetically

One risk factor for the development of breast cancer is a family history of the disease [26]. One can perform a "segregation analysis" to determine the best genetic model for a disease. These studies require the ascertainment of large numbers of affected individuals from a single population and provide information on the hypothetical genetic component of the disease, e.g. whether the disease is likely to be inherited in a recessive or dominant fashion, and the penetrance of the gene (percent of members with the defective gene who will develop the disease).

A large study of 4,730 histologically confirmed breast cancer cases between the ages of 20 and 54 along with 4,688 controls has provided evidence for the existence of a rare autosomal dominant allele with a frequency of 0.0033, that leads to an increased susceptibility to breast cancer [10]. The lifetime risk for a woman with such a susceptibility allele is predicted to be 92%, in contrast to the cumulative lifetime risk of non-carriers which is estimated to be approximately 10%. This study agreed with smaller, earlier ones such as that by Newman et al [35], who studied 1,579 cases of breast cancer and predicted that women with the susceptibility allele had a lifetime risk of developing breast cancer of 82%, versus 8% for the general population. The study by Newman et al suggested that 4% of cases are due to an inherited predisposition. Other studies suggest that more than one locus may predispose to familial breast cancer. It has been shown that < 1% of women, who develop breast cancer at a very young age and who often have children who develop sarcomas, carry a germ-line mutation in the tumor suppressor gene p53 [30].

17q21 linkage

Since segregation analyses suggested that some forms of breast cancer predisposition can be accounted for by a single gene, it was reasonable to attempt to map it. Gene mapping is currently performed by linkage analysis with DNA markers in multiply affected families followed by positional cloning approaches to isolate the gene subsequently.

Linkage analysis relies on the identification of a marker or markers that segregate with disease predisposition. Markers were originally protein polymorphisms, but have been replaced by DNA markers such as RFLPs (restriction fragment length polymorphisms) and VNTR (variable number of tandem repeat polymorphisms). There are many different classes of repeats that differ in the number of copies present at any one site. Some of the most useful are variable numbers of "di, tri and tetra-nucleotide repeats". Commonly called "polymorphic microsatellites", they have revolutionized linkage analysis since they are

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ubiquitous, with a microsatellite occurring approximately every 40kb. Polymorphic loci containing microsatellites are highly variable since most individuals are heterozygous. This is indispensable for linkage analysis since nearly every individual is informative and one can determine which allele is inherited with the disease gene most of the time, with the result that little information is lost from the rare but important families in which breast cancer segregates as a Mendelian trait. Microsatellites can be typed with the polymerase chain reaction, which is fast and requires approximately 100 times less DNA than RFLP-based linkage analysis (e.g. 30ng instead of 3-5μg). One additional advantage of microsatellites for a disease such as breast cancer, is that since affected members have often died at the time the family is genotyped, archival tissue such as microscope slides or paraffin blocks containing the patients' normal tissue can act as an invaluable source of DNA with which to reconstruct their genotypes. This can be performed with PCR-based typing; we routinely obtain sufficient DNA from a 10μm section for approximately 100 PCR reactions. We have also obtained sufficient DNA from tumor DNA scraped off microscope slides for approximately 25 PCR reactions.

In 1990, it was shown that a VNTR marker on chromosome 17q (D17S74, or cMM86) segregated with breast cancer predisposition in seven out of 23 families (40%) where the onset of the disease occurred before the age of 46 [22]. Here the two-point lod score in the early-onset families was high enough above the threshold value of 3.0 to be strong evidence for linkage. This analysis also demonstrated that the disease is genetically heterogeneous (i.e. that breast cancer predisposition did not always segregate with this marker and may be linked to other susceptibility genes in other families). Subsequent analyses showed that a confounding influence in the "late-onset" families is the co-occurrence of the disease in relatives due to non-germ-line alterations [31]. In the case of this predisposing gene BRCA1, D17S74 was initially shown to lie at a distance of 10% recombination from it. This represents a map distance of approximately 10cM (1% recombination corresponds to a map distance of approximately 1cM). In terms of physical distance, 1cM represents 1,0000kb, on average, although this distance can vary widely (for example, 1cM may correspond to 100kb in recombination hot spots, where there is more recombination than the average, and to 10,000kb in recombination cold spots, where it is less than the average).

The results of Hall et al [22] were quickly confirmed by Narod et al [34] who studied five families where both breast and ovarian cancer was segregating and who demonstrated that breast/ovarian cancer predisposition was linked to D17S74 in three families. Here the combined lod score was 2.20 at a recombination fraction Θ of .20 (or approximately 20cM).

In an attempt to confirm the previously published linkage results, to localize the disease locus more definitively, to examine the extent of genetic heterogeneity, and to estimate the penetrance of the BRCA1 gene, a joint analysis of data from 13 groups was performed with a total of 214 families with apparent hereditary predisposition to breast and/or ovarian cancer [15]. This localized BRCA1 to an 8.3cM interval (18 cM in females) between D17S588 and D17S250, with odds of 66:1. When families with only breast cancer were considered, breast cancer predisposition was linked to this locus in approximately 45% of the families. When families with both breast and ovarian cancer were considered, cancer predisposition was linked in nearly all cases. This suggests that a gene(s) on chromosome 17q accounts for most families with both early-onset breast and ovarian cancer, but that there exist other genes predisposing to breast cancer. In the linked families, the risk associated with inheritance of the defective gene was estimated to be 59% at age 50 and 82% at age 70 [15].

An example of a hypothetical family in which breast cancer predisposition in females segregates with a highly informative DNA marker is shown in Figure 1.

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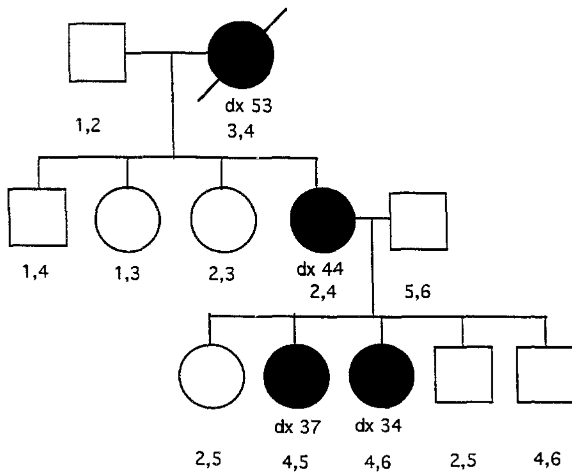

Figure 1. Example of family with early onset breast cancer in which breast cancer predisposition is segregating with allele 4 of a linked locus. Genotypes at the linked locus are shown underneath the pedigree symbols. Circles: females; squares: males; shaded circles: affected females; Dx: age at which breast cancer was diagnosed. In this pedigree, males with allele 4 are unaffected. This resembles the situation for BRCA1, where males harboring a linked allele are unaffected.

Evidence that BRCA1 is a tumor suppressor gene

There is some evidence that the BRCA1 gene is a tumor suppressor gene [54]. A hallmark of tumor suppressors is the finding of nearby allele losses, reflecting regions of chromosomal loss at the suppressor gene locus in tumor DNA [44]. In the case of a suppressor gene involved in inherited predisposition, these allele losses would be expected to occur on the chromosome containing the wild-type allele, thereby inactivating this allele (inactivation of the first allele having been inherited). When loss of heterozygosity studies are performed in tumors of affected members in multiply affected breast and ovarian cancer families shown by linkage analysis to harbor a germ-line BRCA1 mutation, it has consistently been observed by us and others [54], that the chromosome 17 which is lost is the one which carries the wild-type BRCA1 gene. The chromosome 17 retained in the tumors is the one containing the mutant BRCA1. This suggests that tumor predisposition in these cases is due to loss of a normal BRCA1 gene, and provides evidence that BRCA1 is a tumor suppressor. In the family in Figure 1, one would expect that tumors exhibiting LOH of human chromosome 17 would retain allele 4.

Familial vs sporadic forms of breast cancer

Breast cancer attributable to lesions at BRCA1 may be similar to other malignancies that occur both as a sporadic form and a familial form: e.g. renal-cell carcinomas and Von Hippel Lindau disease [51], colon tumors and familial adenomatous polyposis (FAP) and Gardner's syndrome [2, 21,23,36], and acoustic neuromas which can be found sporadically or in individuals with a genetic predisposition due to neurofibromatosis type 2 (NF2) [16].

In addition to the linkage of breast cancer predisposition to BRCA1 in some families (approximately 60% of families with three or more members with breast cancer), there is also some evidence that alterations of a gene at 17q21 occur in tumors of women with no family history. This is based primarily on studies of sporadic breast tumors. In one instance, 40.8% of premenopausal and 32.5% of postmenopausal breast carcinomas had undergone LOH at 17q21.3 [49]. Similar results have also been observed by Futreal et al [18], who describe a common region of deletion that lies between D17S250 and D17S579 at 17q11.2-a21. Other chromosomal regions implicated in the etiology of breast cancer are 3p13-14.3 (the segment where breakpoints are often seen in renal cell carcinomas [11,27,43], 11p, 13q, 16q22-q23, and 17p13 [47,48]. An association has also been demonstrated between LOH on 17p and 17q and amplification of the erbB2 oncogene [48] which has previously shown to have predictive value for recurrence of breast cancer [53]. Interestingly, tumors which had lost chromosome 17p, the locale of p53, had also lost chromosome 13q, the locale of RB1. These

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