BRCA1 Mutations in Primary Breast and Ovarian Carcinomas

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Loss of heterozygosity data from familial tumors suggest that *BRCA1*, a gene that confers susceptibility to ovarian and early-onset breast cancer, encodes a tumor suppressor. The *BRCA1* region is also subject to allelic loss in sporadic breast and ovarian cancers, an indication that *BRCA1* mutations may occur somatically in these tumors. The *BRCA1* coding region was examined for mutations in primary breast and ovarian tumors that show allele loss at the *BRCA1* locus. Mutations were detected in 3 of 32 breast and 1 of 12 ovarian carcinomas; all four mutations were germline alterations and occurred in early-onset cancers. These results suggest that mutation of *BRCA1* may not be critical in the development of the majority of breast and ovarian cancers that arise in the absence of a mutant germline allele.

All familial tumor suppressor genes identified to date incur somatic mutations at some frequency in tumors of the same type that arise in genetically predisposed individuals. For example, germline mutations in the *APC* gene, which predisposes individuals to adenomatous polyposis coli, dramatically increase the risk of colon cancer. The *APC* gene is frequently inactivated by somatic mutations of both alleles in the majority of sporadic colon tumors (*1*). This observation fits the classical two-hit model for inactivation of tumor suppressor genes formulated first by Knudson (*2*). A hallmark of somatic inactivation of tumor suppressor genes is loss of one copy of the gene in tumors [detected by loss of heterozygosity (LOH) for markers in or near the gene] with the remaining copy generally being inactivated by point mutations.

Mutations in the *BRCA1* gene predispose carriers to early-onset breast cancer and ovarian cancer (*3*). The gene has been localized to chromosome 17q12-q21 by linkage analysis of kindreds that segregate *BRCA1* susceptibility alleles (*4*, *5*). Tumors from affected *BRCA1* carriers show LOH in the *BRCA1* region that invariably involves loss of the normal *BRCA1* allele (*6*). The *BRCA1* region also displays LOH in 30 to 70% of sporadic breast and ovarian cancers (*7*–*11*). Collectively, these observations support the hypothesis that *BRCA1* is a tumor suppressor gene that has a role in both inherited and sporadic breast and ovarian tumors.

If *BRCA1* is the critical gene deleted by LOH in sporadic tumors, the remaining allele would be expected to contain inactivating mutations in *BRCA1*. The identification of *BRCA1* by positional cloning (*12*) allowed us to test this hypothesis directly. We typed primary breast and ovarian carcinomas for LOH using three highly polymorphic simple tandem repeat markers: *D17S1323* (Fig. 1) and *D17S855*, which are intragenic to *BRCA1*, and D17S1327, which lies approximately 100 kb distal to *BRCA1* (*13*). Thirty-six of 72 breast carcinomas (50%) and 12 of 21 ovarian carcinomas (57%) showed LOH, frequencies consistent with previous measurements (*7*–*11*). A panel of 32 breast and 12 ovarian tumors exhibiting LOH were examined for *BRCA1* mutations. This panel represents Caucasian and African American patients of varying ages (Table 1). We screened the complete coding region of 5589 base pairs (bp) and intron-exon boundary sequences of *BRCA1* in this tumor set by direct sequencing alone or by a combination of single-strand conformation analysis (SSCA) and direct sequencing (*14*).

A total of four mutations were found, one in an ovarian tumor and three in breast tumors (Table 2). One mutation, Glu1541Stop, introduced a stop codon that would create a truncated protein missing 323 amino acids at the COOH-terminus (Fig. 2). The other three were missense mutations, two of which [Ala1708Glu and Met1775Arg (Fig. 3, A and C)] involve substitutions of small, hydrophobic residues by charged residues, and the third (Pro1637Leu), found in an ovarian tumor, involves a more conservative substitution (Fig. 3, B and D).

Several lines of evidence suggest that all four mutations represent *BRCA1* susceptibility alleles. (i) All mutations found are present in the germ line. (ii) All mutations are absent in appropriate control populations, suggesting that they are not common polymorphisms (*15*). (iii) The mutant alleles are always retained in the tumor, as is

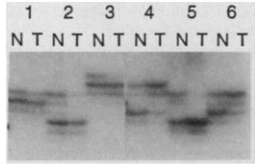

Fig. 1. Loss of heterozygosity (LOH) at the *BRCA1* intragenic *D17S1323* simple tandem repeat marker in breast and ovarian carcinomas. N, normal (blood) DNA; T, tumor DNA. Lanes 1 to 3, breast carcinomas showing loss of the upper allele in each case; lanes 4 to 6, ovarian carcinomas showing loss of the lower, upper, and lower alleles, respectively. LOH analysis was done as described (*7*).

Table 1. Age of breast cancer onset and race of patients studied. MSKCC, Memorial Sloan-Kettering Cancer Center; SEER, Surveillance, Epidemiology, and End Results program; Cau, Caucasian; Af Am, African American.

	Age		Race	
	45 or under	Over 45	Cau	Af Am
Breast tumors				
MSKCC	8(44%)	10(56%)	16(89%)	2(11%)
Duke	5(36%)	9(64%)	6(43%)	8(57%)
USA SEER*	(14%)	(86%)		
Ovarian tumors				
Duke	3(25%)	9(75%)	9(75%)	3(25%)
USA SEER*	(13%)	(87%)		

*See (*24*).

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the case in tumors from patients belonging to kindreds that segregate *BRCA1* susceptibility alleles (6) (if the mutations represented neutral polymorphisms, they should be retained in only 50% of the cases). (iv) The age of onset in the three breast cancer cases with mutations varied between 24 and 42 years of age, consistent with the early onset of breast cancer in individuals with *BRCA1* susceptibility; similarly, the ovarian cancer case was diagnosed at age 44, an age that falls in the youngest 13% of all ovarian cancer cases (Table 1). (v) Two of the four cases had positive family histories of breast or ovarian cancer found retrospectively in their medical records, although the tumor set was not selected with regard to this criterion. Patient BT106 was diagnosed at age 24 with breast cancer. Her mother had ovarian cancer, her father had melanoma, and her paternal grandmother also had breast cancer. Patient MC44, an African American, had bilateral breast cancer at age 42. This patient had a sister who died of breast cancer at age 34, another sister who died of lymphoma, and a brother who died of lung cancer. Her mutation (Met1775Arg) had been detected previously in kindred 2099, an African American family that segregates a *BRCA1* susceptibility allele (*12*), and was absent in African American and Caucasian controls. Patient MC44 is to our knowledge unrelated to kindred 2099. The detection of a rare mutant allele, once in a *BRCA1* kindred and once in the germ line of an apparently unrelated early-onset breast cancer patient, suggests that the Met1775Arg change may be a common predisposing mutation in African Americans. Collectively, these observations indicate that all four *BRCA1* mutations in tumors represent susceptibility alleles; no somatic mutations were detected.

The absence of somatic mutations in *BRCA1* is unexpected, given the frequency of LOH on 17q and the usual role of susceptibility genes as tumor suppressors in cancer progression. There are several possible explanations for this result. One possibility is that some *BRCA1* mutations were missed by our screening procedures. However, selection of a tumor panel with demonstrable LOH in the region should have enhanced our ability to detect point mutations that might otherwise be obscured by normal cell contamination. Indeed, our analysis identified all five coding region polymorphisms found in the 17q-linked kindreds (*12*), as well as two intron polymorphisms that are common in the general population (*16*) and are therefore unlikely to have functional significance. Complete deletions of specific polymerase chain reaction (PCR) targets may have escaped detection. The selection of the tumor set on the basis of LOH would have eliminated any tumors that had homozygous deletions, as informative cases would have appeared as having retained both alleles as a result of normal tissue contamination.

A second possibility is that *BRCA1* somatic mutations may fall primarily in noncoding sequences. Such mutations could affect mRNA levels, as appears to occur with one of the *BRCA1* germline susceptibility mutations (*12*). To test for altered expression of *BRCA1*, we used reverse transcriptase–PCR to monitor *BRCA1* mRNA in nine breast cancers and 12 ovarian cancers. In all samples the *BRCA1* transcript levels were comparable to those in normal breast and ovarian tissue controls, and there was no evidence of aberrantly sized PCR products (*17*). The *BRCA1* transcript undergoes complex splicing (*12*), and it is conceivable that subtle alterations in splice site usage may modulate function, a point that will require further investigation.

Finally, it is possible that 17q LOH may not reflect somatic inactivation of *BRCA1*. The presence of other tumor suppressor genes on chromosome 17, such as *TP53*, may contribute to LOH that incidentally includes the *BRCA1* region. Deletion units on 17q adjacent to the *BRCA1* locus have been detected in several breast and ovarian

Table 2. Germline mutations of the *BRCA1* gene in breast and ovarian cancer patients.

Patient	Codon	Nucleotide change	Amino acid change	Age of onset	Family history
BT098	1541	GAG→TAG	Glu→Stop	39	−
OV24	1637	CCA→CTA	Pro→Leu	44	−
BT106	1708	GCG→GAG	Ala→Glu	24	+
MC44	1775	ATG→AGG	Met→Arg	42	+

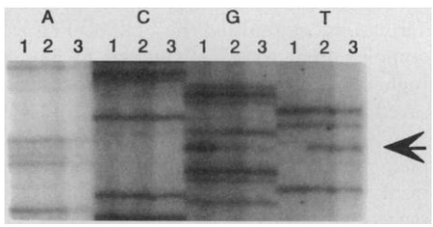

Fig. 2. Germline *BRCA1* mutation in patient BT098. Sequence analysis shows a nonsense germline mutation in patient BT098. Lane 1, normal (blood) DNA control; lane 2, patient's blood DNA; and lane 3, patient's tumor DNA. The arrow indicates a G→T transversion creating a GAG→TAG nonsense mutation on the sense strand in the patient DNA samples. This alteration appears in the germline DNA of this individual and is increased in relative intensity in her tumor DNA, reflecting LOH for the normal allele in the tumor.

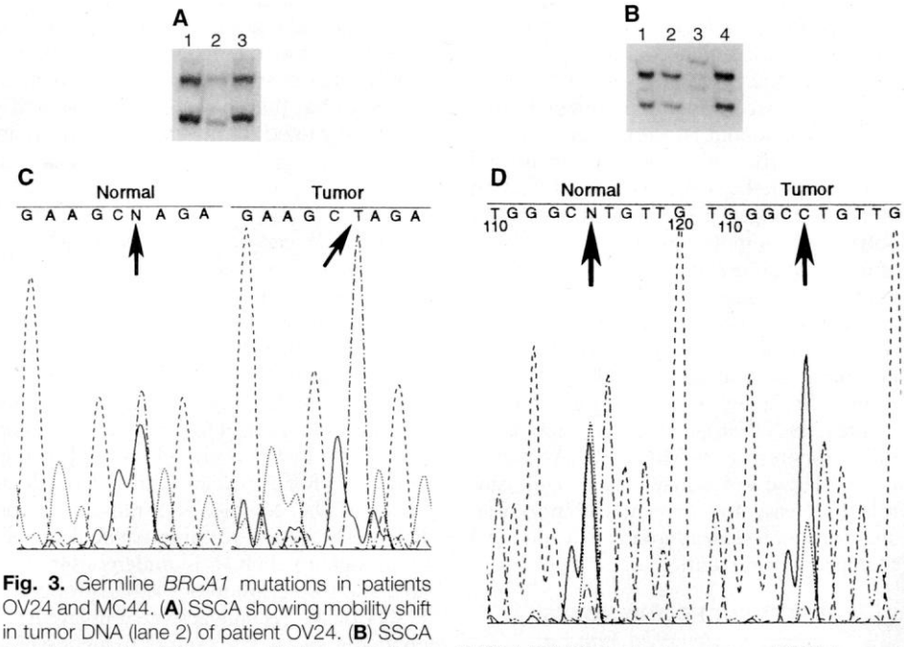

Fig. 3. Germline *BRCA1* mutations in patients OV24 and MC44. (**A**) SSCA showing mobility shift in tumor DNA (lane 2) of patient OV24. (**B**) SSCA showing mobility shift in tumor DNA (lane 3) of patient MC44. (**C**) ABI electropherograms of OV24 normal and tumor DNA sequences. The arrow indicates the heterozygous germline C/T (denoted by N), and the T allele is retained in the tumor. This C→T transition results in a Pro1637Leu alteration. (**D**) ABI electropherograms of MC44 normal and tumor DNA; the antisense strand is shown. The arrow indicates the heterozygous germline A/C (denoted by N). This A→C transversion results in an ATG→AGG (Met1775Arg) change on the sense strand.

tumor sets (*8*, *11*, *18*, *19*). The proportion of incidental *BRCA1* LOH attributable to linked genes awaits isolation and characterization of these putative 17q tumor suppressor loci. Also, a proportion of the allelic losses observed may be explained by the background of random aneuploidy in breast tumors, which averages as much as 15% (*20*).

If confirmed by further studies, the absence of somatic *BRCA1* mutations implies that there may be a fundamental difference between the genesis of sporadic tumors and those in genetically predisposed *BRCA1* carriers. For example, mutations in *BRCA1* may only have an effect on tumor formation when present at a specific stage early in breast and ovarian development, a possibility consistent with a primary role for *BRCA1* mutations in premenopausal breast cancer. However, no clinical or pathological differences in familial versus sporadic breast and ovary tumors, other than age of onset, have been described (*21*). On the other hand, the finding of an increased frequency of *TP53* mutation and microsatellite instability in breast tumors from patients with a family history of breast cancer (*22*) is consistent with an etiologic difference between familial and sporadic tumors. The absence of somatic *BRCA1* mutations may reflect the existence of multiple genes that function in the same pathway of tumor suppression as *BRCA1* but that collectively represent a more favored target for mutation in sporadic tumors.

The data from primary tumors support a role for *BRCA1* in early-onset breast cancer and ovarian cancer and raise the possibility that *BRCA1* may have only a minor role in sporadic breast and ovarian tumor formation. Of paramount concern is the function of wild-type BRCA1 protein in breast and ovarian epithelial cells. The identification of a zinc finger domain in the BRCA1 polypeptide, a motif found in several DNA binding proteins, indicates that *BRCA1* may regulate gene expression (*12*). For example, the function of *BRCA1* in hormone-sensitive tissues suggests that *BRCA1* may mediate hormonal signals in the breast and ovary. Ultimately, it will be important to identify the other genes in the pathway of tumor suppression in which *BRCA1* participates. These genes may play a significant role in breast and ovarian carcinogenesis and may represent alternative avenues for therapeutic intervention.

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14. Tumor DNA samples from Memorial Sloan-Kettering Cancer Center (MSKCC) were sequenced with a Cyclist cycle sequencing kit (Stratagene, La Jolla, CA) (*12*) and those from Duke University were sequenced with an ABI 373 automated fluorescent sequencer with PRISM dye terminators according to the manufacturer's suggestions. Sequences were analyzed with the Sequence Navigator software package (ABI, Foster City, CA). PCR-generated templates from genomic DNAs of the Duke University tumor samples as well as complementary DNA (cDNA) templates from 9 of the breast and all 12 of the ovarian tumors were examined by SSCA as described (*23*). Sequences of intron-based PCR primers used to amplify each of the 23 exons of *BRCA1* and PCR conditions are available by anonymous FTP at the following internet address: Morgan.Med.utah.edu in the directory pub/BRCA1 or by fax at the following number: 801-584-3650. The GenBank accession number for the *BRCA1* sequence is U14680.
15. The mutations found in BT098 and BT106 were absent from 162 Caucasian control chromosomes, the mutation found in OV24 was absent in 128 Caucasian chromosomes, and the mutation in MC44 was absent in 116 African American and 48 Caucasian control chromosomes.
16. Two intronic polymorphisms were detected. PM4 is located 143 bp upstream of exon 12 in intron 11. The A and C alleles were detected by allele-specific oligonucleotide hybridization in 116 and 56 chromosomes, respectively. PM5 is located 49 bp downstream of exon 18 in intron 18. The G and A alleles were detected in 123 and 55 chromosomes, respectively.
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25. We thank H. Brownlee, R. Bell, S. Neuhausen, S. Bayer, E. Harvey, and N. Glover for technical support; P. Vojta and T. Devereux for reviewing the manuscript; and F. Bartholomew for assistance in the preparation of the manuscript. Supported in part by NIH grants CA48711 and CA55914 (to M.H.S.), CA56749 (to J.D.I.), CA55640 (to A.B.), and the NIH Office of Minority Health.

2 September 1994; accepted 14 September 1994

Phosphate-Regulated Inactivation of the Kinase PHO80-PHO85 by the CDK Inhibitor PHO81

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A complex consisting of the cyclin-dependent kinase (CDK) PHO85 and the cyclin PHO80 phosphorylates and is thought to inactivate the transcription factor PHO4 when yeast cells are grown in medium containing high concentrations of phosphate. The CDK inhibitor PHO81 inhibits the kinase activity of the PHO80-PHO85 complex when *Saccharomyces cerevisiae* cells are grown in medium depleted of phosphate. A region of PHO81 with similarity to the mammalian CDK inhibitor $p16^{INK4}$ is sufficient for inhibition in vitro. These studies demonstrate that CDK inhibitors are used to regulate kinases involved in processes other than cell cycle control and suggest that the ankyrin repeat motif may be commonly used for interaction with cyclin-CDK complexes.

The *PHO5* gene encodes a secreted acid phosphatase in *Saccharomyces cerevisiae* whose transcription is regulated in response to extracellular concentrations of inorganic phosphate (*1*). *PHO5* expression is dependent on the activity of the transcription factor PHO4. When yeast cells are grown in medium containing a high concentration of phosphate, PHO4 is phosphorylated by the PHO80-PHO85 cyclin-cyclin–dependent kinase (CDK) complex and transcription of *PHO5* is repressed (*2*). In medium depleted of phosphate, PHO4 is underphosphorylated and transcription of *PHO5* is induced. PHO4 might become activated upon phosphate starvation as a result of induction of a PHO4 phosphatase or inactivation of the PHO80-PHO85 complex.

To investigate the mechanism of PHO4 activation, we purified the PHO80-PHO85 complex from yeast cells grown in low or high concentrations of phosphate and assayed its kinase activity in vitro with PHO4 as a substrate. The PHO80-PHO85 complex was isolated by immunoprecipitation from a yeast strain expressing PHO80 tagged with three copies of the hemagglutinin epitope (HA_3-PHO80) (*3*). The kinase activity of the PHO80-PHO85 complex isolated from yeast cells grown in medium containing a high concentration of phosphate was approximately five times higher than that of the complex isolated from yeast cells grown in medium depleted of phosphate (Fig. 1, lanes 1 and 2). Protein immunoblotting indicated that the observed difference in kinase activity was not

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