Characterization of a Breast Cancer Cell Line Derived from a Germ-Line BRCA1 Mutation Carrier


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Abstract

A tumor cell line, HCC1937, was established from a primary breast carcinoma from a 24-year-old patient with a germ-line BRCA1 mutation. A corresponding B-lymphoblastoid cell line was established from the patient’s peripheral blood lymphocytes. BRCA1 analysis revealed that the tumor cell line is homozygous for the BRCA1 5382insC mutation, whereas the patient’s lymphocyte DNA is heterozygous for the same mutation, as are at least two other family members’ lymphocyte DNA. The tumor cell line is marked by multiple additional genetic changes including a high degree of aneuploidy, an acquired mutation of TP53 with wild-type allele, and loss, an acquired homozygous deletion of the PTEN gene, and loss of heterozygosity at multiple loci known to be involved in the pathogenesis of breast cancer. Comparison of the primary tumor with the cell line revealed the same BRCA1 mutation and an identical pattern of allele loss at multiple loci, indicating that the cell line had maintained many of the properties of the original tumor. This breast tumor-derived cell line may provide a useful model system for the study of familial breast cancer pathogenesis and for elucidating BRCA1 function and localization.

Introduction

Mutation of the BRCA1 gene accounts for most families with an inherited predisposition to breast and ovarian cancer, approximately one-half of families with multiple cases of breast cancer only, and 8–10% of women with early-onset breast cancer unselected for family history (1–3). These observations suggest that inherited BRCA1 mutations may account for ~8,000–10,000 new cases of breast cancer in the United States each year. The inheritance of a germ-line mutation of the BRCA1 gene, although associated with a markedly increased incidence of breast cancer, is not solely responsible for the development of breast cancer in predisposed women. Multiple somatic genetic changes appear to be required in addition for the development of breast tumors in predisposed women (4).

Although the function of the BRCA1 protein is not yet clearly determined, evidence suggests that BRCA1 may play a role in DNA repair, function as a transcription factor, or possibly exist as a secreted granin-like molecule (5–7). If BRCA1 functions in DNA repair, then one would expect an accelerated accumulation of other genetic aberrations in tumors derived from BRCA1 mutation carriers. Controversy exists as to the cellular localization of BRCA1, either in the nucleus or cytoplasm, or both, according to different stages of the cell cycle and exposures to DNA-damaging agents. Some of the difficulties in determining the cellular localization and potential functions of BRCA1 are due to lack of evidence supporting antibody specificity. However, a major problem also has been the lack of available BRCA1 null cell lines to facilitate research studies in this area.

Somatic mutation of the BRCA1 gene is not thought to occur in sporadic breast tumors, although mislocalization of BRCA1 protein has been reported in sporadic breast tumors (8, 9). Although a number of breast cancer cell lines have been established, no breast cancer cell lines have been reported to date that derive from a heterozygous BRCA1 mutation carrier. The establishment of such a cell line would provide another method to study tumor growth regulation conferred by BRCA1 and could also conceivably serve as a substrate for genetic transfection studies. Reported here is the establishment and characterization of a breast cancer cell line homozygous for a germ-line-inactivating BRCA1 mutation.

Materials and Methods

Patient Material. The patient was a 24-year-old woman with a nonmetstatic infiltrating ductal carcinoma of the breast. She had had one child previously at the age of 22. Her identical triplet sister had developed breast cancer the previous year at the age of 23. The third identical triplet had a bilateral prophylactic mastectomy at age 24. The patient’s mother was reported to have had cancer of the uterine cervix at the age of 22. Both maternal grandparents had died of colon cancer in their sixties. The family is Caucasian and not of known Ashkenazi descent. A pedigree of the family is shown in Fig. 1. After obtaining informed consent for genetic studies, blood and tumor tissue were obtained from the patient and blood from her mother and two sisters. No adjuvant chemotherapy or radiation had been given prior to collection of tumor material.

Tumor Cell Culture Establishment. The patient from whom the breast tumor cell line was derived underwent a mastectomy with gross resection of the primary tumor. A portion of the primary tumor tissue was placed in RPMI 1640 with 5% fetal bovine serum and antibiotics immediately after surgical removal. Tumor tissue was minced and scraped to release tumor cells into the medium. Cells were cultured in T-25 flasks at 37°C with 5% CO2. Medium was changed weekly, and cultures were observed for cell growth. Cultures were trypsinized and passaged when sufficient colonies of epithelial growth were noted. Estrogen and progesterone receptor studies on the cultured cells as well as the primary tumor were performed by Nichols-Coming Institute using a radioactive binding assay. HER2/neu expression was determined by a quantitative ELISA assay (Calgiocchem, Cambridge, MA). Telomerase assay was performed by the telomeric repeat amplification protocol assay (10). For cytogenetic evaluation, cells were cultured on coverslips. Standard methods of harvesting and chromosome banding were used (11). The cell line was designated HCC1937 (for Hamon Cancer Center).

For establishment of a corresponding B-lymphoblastoid cell line, peripheral blood was centrifuged through Histopaque (Sigma Biochemicals, St. Louis, MO), washed in RPMI 1640, and resuspended in initiation medium consisting of RPMI 1640 with 15% fetal bovine serum, 25 μM HEPES, and 1 mM sodium bicarbonate.
The patient as well as the affected and one unaffected sister was heterozygous for the BRCA1 mutation, 5382insC. The patient’s mother’s DNA demonstrated only wild-type BRCA1.

The cell line HCC1937 was compared with DNA from the peripheral blood cells as out the genome known to be commonly lost in breast cancer. DNA from the tumor cell line HCC1937, the B-lymphoblastoid cell line, and unprocessed peripheral mononuclear blood cells was prepared using standard methods (13).

Blastoid cell line, and unprocessed peripheral mononuclear blood cells was changed approximately weekly. Cultures were observed daily for approx

preparations of pyruvate and 5 ml EBV-conditioned medium from an EBV-producing marmoset cell line (12). Cultures were incubated at 37°C with 5% CO2. Medium was changed approximately twice weekly. Cultures were observed daily for approximately 2 weeks, when loose aggregates of nonadherent lymphocytes began to proliferate rapidly. DNA from the tumor cell line HCC1937, the B-lymphoblastoid cell line, and unprocessed peripheral mononuclear blood cells was prepared using standard methods (13).

Allelotyping. Using polymorphic dinucleotide and tetranucleotide microsatellite repeat markers, patterns of allelic losses were studied at loci throughout the genome known to be commonly lost in breast cancer. DNA from the cell line HCC1937 was compared with DNA from the peripheral blood cells as well as the B-lymphoblastoid cell line. Primer sequences were obtained from the Genome Database, and PCR amplification and electrophoresis were performed as described previously (14). For allelotype analysis of the primary tumor, areas were microdissected as described previously (14).

Mutation Analysis. SSCP analysis of genomic DNA was performed by a modification of the technique described by Orita et al. (15). Specific genes known to be involved in the pathogenesis of breast cancer were examined as possible secondary acquired changes in the cell line. Coding regions of exons 5–11 of the TP53 gene, the entire open reading frame of CDKN2A, the PTEN gene, and the BRCA1 gene were analyzed (16–21). Primers were designed to amplify fragments 150–200 bp in length. Sequence analysis of DNA fragments demonstrating abnormal mobility on SSCP gels was performed by cloning amplified PCR fragments into pCMV5 vectors and sequencing using Sequenase (United States Biochemical, Cleveland, OH) according to the manufacturer’s instructions. 35S-Labeled reactions were electrophoresed on 6% acrylamide gels. A minimum of 8 clones was sequenced for each region of interest. Direct sequence analysis of the entire coding region of the BRCA2 gene was done by Myriad Genetics (Salt Lake City, UT). Mimatched primer pairs were designed at mutation sites as described in “Results.”

Southern blotting was performed to confirm the presence or absence of the PTEN coding sequence DNA in the tumor cell line as well as constitutional DNA. Genomic DNA was digested overnight with restriction enzymes EcoRI, HindIII, KpnI, BamHI, and MboI. Digested DNA was blotted on Hybond (Amersham, Arlington Heights, IL) membranes according to directions pro-vided by the manufacturer. DNA probes were prepared by amplification of the coding region(s) of exons 2–8 of the PTEN gene as described previously (22). Hybridization with 32P-labeled probe was carried out using standard techniques (13).

**Results**

**Cell Line Establishment.** A breast cancer cell line, designated HCC1937 (Hamon Cancer Center), was established from a grade III infiltrating ductal primary breast tumor from a 24-year-old breast cancer patient with a germ-line BRCA1 mutation. On histological evaluation of the primary tumor, large vacuoles were observed in many of the cells suggestive of a secretory variant of infiltrating intraductal carcinoma (Refs. 23 and 24, Fig. 2a). The cultured tumor cells also contained similar vacuoles and demonstrated a striking resemblance to the primary tumor (Fig. 2b). The vacuoles failed to stain with periodic acid-Shiff (with and without diastase treatment), alcin blue, mucicarmine, or oil red O (not shown). These results indicate that the vacuoles lacked glycogen, mucins, or neutral fat. The appearance of these cells was similar to the cytological appearance of cells of secretory carcinoma (25).

The cultured cells grew as an adherent monolayer. During growth phase they had the appearances of small to medium epithelioid cells with finely granular eosinophilic cytoplasm and nuclei demonstrating moderate atypia and occasional mitoses. However, at heavy cell density, a progressively increasing number of the larger vacuolated cells appeared. Approximately 11 months after initiation, it was apparent that a cell line had been established, as evidenced by continuous growth even after recovery from cryopreservation. Immortalization was further demonstrated in that the cells have grown continuously for over 30 months, have undergone multiple passages, and have demonstrated telomerase activity (data not shown).

Progesterone and estrogen receptor radiobinding assays demonstrated no significant levels of progesterone or estrogen binding in
either the primary tumor or HCC1937 cultured cells. Only very low levels of HER2/neu expression were observed.

**Molecular Analysis.** SSCP analysis of BRCA1 revealed an abnormality in exon 20 in both DNA derived from peripheral blood as well as the cultured cells (Fig. 3). DNA from cells derived from peripheral blood revealed a normal pattern as well as an extra band, whereas SSCP analysis of the tumor cell line revealed an absence of a normal band present in the peripheral blood DNA. The extra abnormal band was also observed in DNA from each of the patient’s triplet sisters, but not in the mother. The father’s DNA was not available for analysis. Sequence analysis of the PCR product amplified from exon 20 from cell line DNA revealed an inserted C residue at nucleotide 5382. All cloned sequences obtained from HCC1937 DNA contained this mutation. No wild-type sequences were observed. Sequence analysis of microdissected archival tumor tissue also revealed the presence of the 5382insC mutation and lack of normal wild-type BRCA1 sequence. To provide an alternative rapid method of detecting this mutation without the use of radioactivity, mismatched primers flanking the 5328insC mutation were designed, which resulted in an amplicon of 131 and 132 bp in the wild and mutant type alleles, respectively. The primer sequences are as follows: sense, 5’-CAAAGCCGAGCAAGAATGCTTACAAATTGAG-3’; and antisense, 5’-GTAATAAGTCTTACAAATTGAG-3’. The mismatched base in the sense sequence is underlined. The mismatched primer abolishes a restriction site (CCNNNG) in the wild-type allele, but not the mutant allele, for the enzyme BsaJI (New England Biolabs, Beverly, MA; Fig. 3). The coding sequence of the BRCA2 gene demonstrated no abnormality. DNA from HCC1937 repeatedly failed to amplify with primers designed to amplify exons 1–8 of the PTEN gene, suggesting the presence of a homozygous deletion, but did amplify exon 9 of this gene. To confirm whether this observation represented a true deletion of the PTEN gene, Southern blotting was performed. A Southern blot of DNA from HCC1937, lymphocyte DNA from the patient, as well as DNA from other cell lines, were digested with HindIII and hybridized with a 32P labeled PTEN coding sequence probe (20). An absence of bands corresponding to the PTEN coding sequence in HCC1937, with a normal pattern observed in the lymphocyte DNA, was demonstrated (Fig. 5). Similar results were obtained when DNA was digested with EcoRI, Kpnl, BamHI, XbaI, and MboI. The PTEN pseudo-gene, PTEN2 (22), localized to chromosome 9, was seen in all DNAs and provided an internal control for the PTEN homozygous deletion.

**Allelotyping Data.** Allelotyping results comparing HCC1937 and peripheral blood DNA at 51 informative and 10 uninformative markers are summarized in Table 1. A LOH was observed in the majority of loci examined including chromosomal regions 1p21, 1p36, 3p21, 3q26, 4q33–35, 5p15.3, 5q11–5q22, 6q13, 6p21.3, 8p21, 9p23–24, 10q23–21, 11p12–22, 13q12.2–13q21, 17p13.1, and 17q21, whereas retention of heterozygosity was observed at 3p25, 3q26, 4q33–35, 5p15, 7q31, 8q11.2, 9p12–13, 9q21–33, 11p15.5, 13q14, and 19p12–3. Using comparisons of the mother’s DNA, the parental origin of allele loss could be determined at most loci. Both paternal and maternal allele loss was observed. No acquired

![SSCP Diagram](image_url)

Fig. 3. Molecular analysis of BRCA1. Single-strand conformation analysis (left) revealed an aberrant band in lymphocyte DNA from the patient (BC260-002) and each of her two sisters analyzed (BC260-001 and BC260-000). The tumor cell line demonstrated the mutant band as well as the absence of a wild-type band observed in the constitutional DNA. Sequence analysis (middle) revealed an inserted C residue at position 5382. No wild-type sequence at position 5382 was detected in any of the clones analyzed from HCC1937-amplified DNA. Designed restriction fragment length polymorphism analysis using mismatched repair primers as described in “Results” is demonstrated at right. Both uncut (131) and cut (122) fragments are detected in the B-lymphoblastoid cell line (BL), whereas in the HCC1937 tumor cell line (CL), only the cut fragment (122 bp) is observed. SM, size marker, 100-bp ladder.
extraneous bands suggestive of microsatellite instability were noted at any of the loci examined. At selected loci, allelotyping of microdissected archival material was also performed with results identical to the cell line DNA in all loci examined (Table 1). Not all loci examined in the tumor cell line were examined in microdissected archival tissue because of limited archival material.

Cytogenetics. Cytogenetic analysis revealed an extremely complex abnormal karyotype. Of 19 metaphases, no 2 revealed the exact same karyotype. An approximately equal number of metaphases were observed with modal numbers of 51-56 and 92-110 chromosomes, consistent with the evolution of a clone of cells with a near-tetraploid karyotype in addition to a clone of near-diploid cells. Double minute chromosomes were observed rarely in some passages. Numerous marker chromosomes were observed of unknown derivation. The karyotype of the cell line DNA in all loci examined (Table 1). Not all loci examined sected archival material was also performed with results identical to any of the loci examined. At selected loci, allelotyping of microdissected archival material was also performed with results identical to the cell line DNA in all loci examined (Table 1). Not all loci examined in the tumor cell line were examined in microdissected archival tissue because of limited archival material.

Discussion

In this study, we report the establishment and characterization of breast carcinoma cell line HCC1937, derived from a germ-line BRCA1 mutation carrier. Histologically, the tumor is characterized as an invasive ductal carcinoma with features of secretory carcinoma. Like many of the mutant BRCA1-associated tumors described to date, the tumor and the corresponding cell line lacked estrogen or progesterone receptors (4, 26, 27). Like the majority of disease-associated BRCA1 mutations, the mutation present in this breast cancer cell line causes a truncated protein product. The inserted C at nucleotide 5382 results in erroneous translation of the protein distal to codon 1755 and termination at codon 1829, whereas wild-type BRCA1 consists of 1863 amino acids. Evidence suggests that the COOH terminus of BRCA1 is essential for function in that patients with a germ-line truncating mutation at codon 1853 are susceptible to early-onset breast cancer, and in vitro studies demonstrate that the COOH terminus of BRCA1 is active in transcriptional activation (6, 20). This particular BRCA1 mutation has been observed in multiple families and is the second most common BRCA1 mutation reported (28).

Although several series of breast carcinoma cell lines have been reported, no previously established cell line is known to be associated with mutation of BRCA1. Yuan et al. (29) reported an ovarian cancer cell line that carries a mutation of BRCA1, causing a truncation at the
Table 1 Allelotyping of HCCI937 cell line DNA and corresponding primary tumor

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Markers that were examined that were not informative included D1S161 (p31–q21), D3S1577 (p13), D3S1313 (3p14), KICA (3p21.3), ROI1.2 (3q21–q24), mfd122 (5q31–33.3), D3S177 (8p1–21), D6S300 (6q13–14), D6S126 (9p22), and D19S253 (19p13.1).

ND, not determinate; RH, retention of heterozygosity; NI, not informative.

COOH-terminal portion of the protein. It is not known whether this BRCA1 mutation is germ line, although it is quite possible that this line derived from a BRCA1 mutation carrier because of a separate report of the same germ line mutation in a breast-ovarian cancer family (30) and because sporadic mutations in ovarian cancer are rare (8, 31).

The cell line HCCI937 demonstrated a considerable degree of aneuploidy as demonstrated by multiple karyotypic abnormalities, a high incidence of LOH at loci involved in breast cancer pathogenesis, and a high DNA index. Of 19 cell lines examined, this tumor demonstrated the highest incidence of LOH. At multiple loci, the corresponding archival tumor tissue was allelotype as well, with identical findings of allele loss or retention at each locus examined. Marcus et al. (32) reported, in a series of hereditary breast cancers using archival

4. A. Gazdar, unpublished data.

BRCA1 MUTANT BREAST CANCER CELL LINE

The breast cancer risk associated with the BRCA1 5382insC mutation is ~55% by age 70 according to one study (33). This risk increases with age, and although the risk at all ages is greater than that of noncarriers at all ages, the observed incidence of breast cancer in the early twenties as observed in this patient and her sibling suggests that other factor(s), either genetic or environmental, may have influenced the development of breast cancer in this family. The question arises as to whether an additional genetic predisposition factor is carried by this family. However, no additional germ-line mutations were found in BRCA2, PTEN, or TP53. In the rarely observed families in which more than one breast cancer predisposing germ-line mutation occurs in the same individual, the phenotypes are not markedly different with respect to age of onset or number of tumors (34, 35). Perhaps other yet unidentified genetic predisposition genes, genetic modifiers, or environmental factors contributed significantly to early onset of tumor development in this family. The fact that both the patient from whom the cell line derived, as well as her affected sister, had very early-onset breast cancers, and both previously bore children at an early age, suggests that in this family, early child-bearing was not a protective factor. This observation, along with the estrogen and progesterone receptor-negative status, suggests that factors other than hormonal stimulation had stimulated tumor development.

Considerable controversy has existed over the localization of the BRCA1 protein in both normal and malignant tissue. One of the technical challenges in determining the cellular localization of BRCA1 is the specificity of antibodies for the BRCA1 protein. The establishment of a cell line that is null for any COOH-terminal BRCA1 should be useful in sorting out antibody specificity and cellular localization issues. In addition, studies comparing localization of BRCA1 in its mutant form compared with wild-type BRCA1 will be useful in elucidating the role of BRCA1. Likewise, transfection studies with wild-type BRCA1 have only been done with breast cancer cells that already contain wild-type BRCA1 (36). It will be of interest to see the effect on cell growth and tumorigenicity of replacing wild-type BRCA1 into the HCCI937 cell line.

Although the tumor from which our cell line derives is distinctive in terms of its histology and very early age of onset, the acquired TP53 mutation, the estrogen receptor/progesterone receptor negativity, and the marked aneuploidy observed may prove to be characteristic of BRCA1-associated tumors. Thus, cell line HCCI937 may serve as a very useful reagent in studying breast cancer pathogenesis in BRCA1 families.

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Note Added in Proof
The cell line HCC1937 has been deposited with the American Type Culture Collection.

References
1. Easton, D., Bishop, D., Ford, D., Crockford, G., and Breast-Cancer-Linkage-Consor-
2. Fitzgerald, M. G., MacDonald, D. J., Kramer, M., Hoover, I., O'Neil, E., Unsal, H.,
Silva-Arrieto, S., Finkelstein, D. M., Beer-Romero, P., Enghert, C., Scro, D. C.,
Smith, B., Younger, J. W., Garber, E. D., Duda, R. B., Mayzel, K. A., Isselbacher,
K. J., Friend, S. H., and Leshner, A. D. Germline BRCA1 mutations in Jewish and
non-Jewish women with early-onset breast cancer. N. Engl. J. Med., 334: 143-149,
1996.
3. Langston, A., Malone, K., Thompson, J., Daling, J., and Ostrander, E. BRCA1
4. Tirkkonen, M., Johansson, O., Arngran, B. A., Olsson, H., Ingvarsson, S., Karhu,
synergistic genetic changes associated with tumor progression in carriers of BRCA1
5. Scully, R., Chen, J., Ochs, R., Keegan, K., Hoekstra, M., Jeunteun, J., and Livingston,
M., Kamb, A., and Wiseman, R. BRCA1 mutations in primary breast and ovarian
7. Kamb, A., Gruis, N., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S.,
and Park, J. Germline mutation of BRCA1 gene in Korean breast and ovarian
8. Merajver, S. D., Pham, T. M., Caduff, R. F., Chen, M., Pay, E. L., Cooney, K. A.,
Gregory, S., Gumba, C., Micklem, Y., Barfoot, R., Hamoud, R., Patel, S., Rice, C.,
Biggs, P., Hashim, Y., Smith, A., Connor, F., Arason, A., Gudmundson, J., Florene,
D., Kelsell, D., Ford, D., Tonin, P., Bishop, D. T., Spurk, N., Ponder, B. A. J.,
Ecles, R., Peto, J., Deviolee, P., Corneliwse, C., Lynch, H., Narod, S., Lenoir, B.,
Gillinis, V., Barkdottir, R. B., Easton, D. F., Bentley, D. R., Futreal, P. A.,
10. Hung, J., Kishin, Y., Sugii, K., Kuzuma, M., Minna, J. D., Morrison, P., Rosteck, P.,
11. Kamb, A., Gruis, N., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S.,
and Park, J. Germline mutation of BRCA1 gene in Korean breast and ovarian
12. Couch, F., and Weber, B. Breast Cancer Information Core. Mutations and polymor-
phisms in the familial early-onset breast cancer (BRCA1) gene. Hum. Mutat., 8: 8-18,
1996.
Weber, B. L., Collins, F. S., Johnston, C., and Frank, T. S. Somatic mutations in the
Weber, B. L., Collins, F. S., Johnston, C., and Frank, T. S. Somatic mutations in the
15. M., Kamb, A., and Wiseman, R. BRCA1 mutations in primary breast and ovarian
Weber, B. L., Collins, F. S., Johnston, C., and Frank, T. S. Somatic mutations in the
17. Merajver, S. D., Pham, T. M., Caduff, R. F., Chen, M., Pay, E. L., Cooney, K. A.,
Weber, B. L., Collins, F. S., Johnston, C., and Frank, T. S. Somatic mutations in the
18. Merajver, S. D., Pham, T. M., Caduff, R. F., Chen, M., Pay, E. L., Cooney, K. A.,
Weber, B. L., Collins, F. S., Johnston, C., and Frank, T. S. Somatic mutations in the
19. M., Kamb, A., and Wiseman, R. BRCA1 mutations in primary breast and ovarian
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