Molecular Diagnosis for Breast Cancer

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Abstract: Within the past few years a number of genes whose mutated forms are associated with a high risk of breast cancer have been identified, including BRCA1, BRCA2, c-myc, erbB2, and p53. The identification of these genes, together with rapid advances in techniques for molecular genetic analysis, should improve the diagnosis and therapy of this group of diseases. Allelic losses at other specific loci in breast tumors also may serve as prognostic factors. This article reviews the genetic basis of hereditary and sporadic breast cancers and discusses the clinical application of new molecular knowledge with regard to diagnostic testing, surveillance, and prognostic diagnosis for women with hereditary predispositions or who are at high risk for recurrence of breast cancer.

Key words: Breast neoplasms; Oncogenes; Tumor suppressor genes; Loss of heterozygosity

Introduction

It is now widely accepted that cancer is a complex disease that results from the accumulation of numerous genetic aberrations within the tissue in question. The isolation of two breast cancer-susceptibility genes, BRCA1 and BRCA2, has opened the door to early detection and pre-emptive treatment of familial breast cancers. Furthermore, because over-expression of another gene, erbB2, is highly correlated with shortened disease-free survival and with overall survival of node-positive patients, a bioengineered product called Herceptin may be indicated for the treatment of patients with metastatic breast tumors that over-express the erbB2 product, HER-2 protein. Other highly specific cancer therapies based on results of molecular diagnoses will be available in the near future.

Familial Breast Cancer

A family history of breast cancer has long been recognized as an important risk factor, contributing as it does to about 10% of all breast cancer cases. BRCA1 and BRCA2 have been identified already as tumor suppressor genes associated with familial breast cancer.
1. **BRCA1 and BRCA2**

In 1990 Hall et al.\(^1\) performed linkage analyses of pedigrees exhibiting a high incidence of breast cancer, and found linkage between polymorphic DNA markers in region 17q21 of the long arm of chromosome 17 and early-onset breast cancer. They assumed the existence of a familial breast cancer susceptible gene in this region, naming it *BRCA1*, and in 1994 Miki et al.\(^2\) identified the gene itself. Then, by conducting shotgun linkage analyses of pedigrees with familial tendencies to breast cancer that did not show linkage to *BRCA1*, Wooster et al.\(^3\) localized *BRCA2*, another breast-cancer susceptibility gene, to an interval of approximately 6cM in region 13q12–13 on the long arm of chromosome 13; in 1995 they succeeded in identifying the gene in the same manner as *BRCA1* had been, that is, by localizing parts of *BRCA2* using detailed linkage analysis and positional cloning techniques. Both *BRCA1* and *BRCA2* encode potential tumor-suppressing proteins. Murine embryos carrying a *BRCA1* null mutation are developmentally retarded and hypersensitive to gamma-irradiation, suggesting a failure in DNA damage repair.\(^4\) Both genes are large; each spans approximately 80kb of genomic DNA, and both have extremely large central exons encoding >50% of the protein. Both proteins are large as well: 1863 and 3418 amino acids respectively. BRCA1 protein and BRCA2 protein maintain genomic stability through their involvement in homologous recombination and in repair of transcription-coupled and double-strand breaks.

2. **Genetic diagnosis before onset**

Germline mutations in *BRCA1* or *BRCA2* together account for only 15–20% of breast cancers known to cluster in families and less than 5% of breast cancer overall in the US and Western Europe. However, mutations in these two genes are assumed to be actually responsible for up to 45% of familial breast cancers in Western countries. Germline mutations in *BRCA1* are observed in 1–2% of all patients with breast cancer in Japan.\(^5\) Both *BRCA1* and *BRCA2* are transmitted through autosomal dominant inheritance. The onset of breast cancer tends to occur at younger ages, and more often with concomitant onset of ovarian cancer, in pedigrees that transmit alterations in the *BRCA1* gene than in pedigrees that do not carry mutations in *BRCA1*.

The discovery of the breast cancer-susceptibility genes has defined carriers of mutations in *BRCA1* and *BRCA2* as a high-risk group before onset, and management guidelines are now available for women who carry germline mutations of either gene. Pre-emptive approaches based on these guidelines appear to reduce the risk of breast and ovarian cancer by at least 60% and 90%, respectively.\(^6\) Because both *BRCA1* and *BRCA2* are large genes, however, with no specific “hot spots” of mutation, searching for mutations in individual cases involves considerable expense and time. Hacia et al.\(^7\) have designed high-density arrays consisting of over 96,600 oligonucleotides, each 20 nucleotides long, to screen for a wide range of heterozygous mutations within the 3.45 kilobases constituting exon 11 of *BRCA1*. Such DNA chip-based assays may represent a valuable new technology for high-throughput, cost-efficient detection of other genetic alterations as well.

**Sporadic Breast Cancer**

Cancerous solid tumors generally result from the accumulation of mutations in numerous genes. Genes that are involved in the development and progression of tumors can be divided into three major categories: oncogenes, tumor-suppressor genes, and mismatch-repair genes.

1. **Amplification of oncogenes**

Oncogenes encode products that facilitate cell growth, but they are termed proto-oncogenes in normal DNA. Once the oncogene has been activated by point mutation or gene amplifications, an affected cell may produce unusually large amounts of the normal gene product (or
an aberrant protein). This transforms the cell into a cancer cell that looks very different from its former self.

1. C-myc amplification

The c-myc gene, located at 8p24.3, encodes a nuclear transcription factor that is involved in gene expression. To clarify the clinical significance of c-myc amplification, Deming et al.\(^8\) conducted 29 studies in which the weighted average frequency of c-myc amplification in breast tumors was 15.7%. Amplification was significantly associated with risk of relapse and death. Harada et al.\(^9\) reported that c-myc was amplified in 28% of the 279 breast cancers they examined in Japan. Amplification of c-myc is relatively common in breast cancers, where it correlates with poor prognoses, e.g. inflammatory carcinoma, progression of postmenopausal tumors, or metastasis to lymph nodes. However, the relationship between c-myc amplification and breast-cancer progression or the extent of malignancy remains to be fully clarified.

2. ErbB2 amplification

ErbB2 was cloned by virtue of its homology to the avian erythroblastosis virus oncogene (v-erbB). ErbB2 is located at 17q22.1 and its product (known as HER-2, human epidermal growth factor receptor 2) is a receptor-type tyrosine kinase found on the surface of cells; when functioning normally, it is a key component for regulating cell growth. However, when the HER-2 protein is over-expressed, extra HER-2 receptors may be produced. This situation increases cell growth and reproduction, often resulting in more aggressive breast-cancer cells. Harada et al.\(^9\) reported that erbB2 amplification had occurred in 19% of 457 breast-cancers they examined in Japan. Clear relationships exist between erbB2 amplification and breast-cancer progression or the extent of malignancy remains to be fully clarified.

2. Tumor suppressor genes

The genes in this category normally control cell proliferation or differentiation. Mutations that inactivate one or more of these genes lead to abnormal cell growth.

1. p53 and breast cancer

p53, located at 17p13.1, was the first gene identified as a mutant in human tumors. Its normal protein product participates in regulation of the cell cycle and in apoptosis. Mutations of p53 have occurred in 17–40% of sporadic breast cancers examined,\(^11\) and most of them are missense mutations concentrated in a core region that encodes the sequence-specific DNA-binding. Mutant forms of p53 protein interfere with the growth-suppressing effects of wild-type p53, indicating that the gene product is actually a tumor suppressor (dominant negative). Many investigators have examined mutations of p53 in detail and have correlated them with the prognosis and with the sensitivity to anti-tumor drugs. A statistically significant association has been noted between p53 mutations that occur in conserved domains, and poor prognosis. As p53 mutations are found most frequently in advanced breast cancers, it appears that aberrant p53 is involved in the progression stages of such tumors.

2. LOH and breast cancer

A high frequency of somatically occurring losses of heterozygosity (LOH) at specific chromosomal sites in tumor cells usually suggests that one or more tumor suppressor genes should be present in those regions. Isolation of the APC, RB, and WT1 genes on the basis of LOH analyses has supported this premise. LOH is also common in sporadic breast cancers. A pioneering study on breast-cancer allelotypes was conducted by Sato et al.\(^12\) who searched for LOH on the short and long arms of all autosomal chro-
mosomes in breast cancers from 79 women. The results indicated high frequencies of deletions on the short arm of chromosome 3 (46%), the long arm of chromosome 13 (25%), the long arm of chromosome 16 (51%), and the short arm of chromosome 17 (58%), suggesting the existence of tumor suppressor genes in those regions whose loss is associated with the onset and development of breast cancer. Correlations found among common chromosomal deletions and clinicopathological factors suggest that some chromosomal alterations are specific for different aspects of development and progression of breast cancer, e.g., differentiation of tumor cells, progression in situ, metastasis to lymph nodes, and hormonal dependency.

Molecular Diagnosis for Postoperative Prognosis

1. LOH for prognosis of breast cancers

We have investigated and reported the significance of LOH as a prognostic factor in breast cancers. To examine whether specific allelic losses might correlate with postoperative survival, in a 5-year prospective follow-up we tested tumors from a cohort of 264 breast-cancer patients for allelic losses of 18 microsatellite markers representing either a known tumor suppressor gene or a region where genetic alterations are frequent in breast tumors. Patients whose tumors had lost an allele at 1p34, 13q12, 17p13.3, or 17q21.1 sustained significantly higher risks of postoperative mortality than those whose tumors retained both alleles at those loci at the time of initial surgery. Figure 1 shows our analysis of postoperative survival with regard to LOH status at 1p34. Kaplan-Meier analysis of overall survival revealed that postoperative mortality risk was increased in patients whose tumors showed LOH at this locus, compared with patients whose tumors retained both alleles (log-rank test, \( p = 0.0047 \)). We conclude that allelic losses at these four loci can serve as negative prognostic indicators to guide postoperative management of patients.

2. Postoperative prognosis and LOH in node-negative breast cancer

Although the prognosis for patients whose breast cancers have not metastasized to lymph nodes (node-negative breast cancer) is better than that for patients with metastasis, about 10% of node-negative patients in Japan experience relapse within 10 years of initial surgery. With this discrepancy in mind we examined tumors from a cohort of 228 node-negative breast cancer patients for allelic losses at 18 microsatellite loci representing either known tumor suppressor genes or regions where breast cancers frequently exhibit allelic losses. We followed the patients clinically for 5 years or until death. Patients whose tumors had lost an allele at 1p34–36 bore significantly higher risks of postoperative recurrence than those whose tumors retained both alleles in that region. The 5-year recurrence rate was 15% among patients with losses versus 2% among patients with retention (Fig. 2). Multivariate analysis demonstrated that allelic loss at 1p34–36 was an independent postoperative predictor of shorter disease-free survival (hazard ratio, 5.8; \( p = 0.0117 \)). Thus, allelic loss at 1p34–36 in a tumor may have the potential of serving as a negative prognostic indicator to guide postoperative management of many breast cancer patients, especially as a
means of selecting the women who will benefit most from adjuvant chemotherapy and/or endocrine therapy.

3. Clinical application of genetic diagnosis for postoperative prognosis in breast cancer

As mentioned above, the postoperative recurrence rate among patients with breast cancer whose tumors had lost one allele at 1p34–36 was statistically greater than among patients whose tumors had retained both alleles. On the basis of these findings, we are now attempting to provide genetic diagnosis for LOH at the 1p34–36 locus in clinical settings. Samples from tumors and peripheral blood of breast-cancer patients undergoing surgery are transported from hospitals in Nagano and the Cancer Institute in Tokyo to our laboratory, where we analyze the tumor DNAs for LOH. The analyses are completed within a week and reports are returned to doctors in less than two weeks. We expect that the results of 1p34–36 LOH diagnosis, combined with St. Gallen’s guideline and/or histopathological diagnosis, will assist postoperative therapeutic planning for high-risk breast-cancer patients.

Conclusion

Genes that are known to be implicated in sporadic and familial breast cancers, and the significance of molecular diagnosis for those genes, have been reviewed in this article. In western countries, where breast cancer is the most common cancer among women, prophylactic mastectomy or administration of antiestrogen are occasionally undertaken for prevention of breast cancer in high-risk individuals. Although prophylactic mastectomy seems to be associated with considerable reduction in the risk of breast cancer, such aggressive intervention is not the general rule in Japan where both morbidity and mortality are relatively low. However, postoperative molecular diagnosis is valuable for Japanese patients, to guide decisions regarding aggressive adjuvant therapy in malignant cases. It is also helpful for avoiding excessive administration of anticancer drugs in cases where prognosis is better. New technologies growing out of the Genome Project are contributing to the development of diagnostic methods that should allow even more rapid and accurate diagnosis. Even so, the protection of privacy for individual patients presents an ethical problem, and we recognize also the potential emotional consequences to a patient regarding genetic diagnosis. Therefore, it is necessary to develop appropriate policies for release of genetic information.

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REFERENCES


