Advances in Breast Cancer: Pathways to Personalized Medicine

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Abstract

Breast cancer is a complex disease caused by the progressive accumulation of multiple gene mutations combined with epigenetic dysregulation of critical genes and protein pathways. There is substantial interindividual variability in both the age at diagnosis and phenotypic expression of the disease. With an estimated 1,152,161 new breast cancer cases diagnosed worldwide per year, cancer control efforts in the postgenome era should be focused at both population and individual levels to develop novel risk assessment and treatment strategies that will further reduce the morbidity and mortality associated with the disease. The discovery that mutations in the BRCA1 and BRCA2 genes increase the risk of breast and ovarian cancers has radically transformed our understanding of the genetic basis of breast cancer, leading to improved management of high-risk women. A better understanding of tumor host biology has led to improvements in the multidisciplinary management of breast cancer, and traditional pathologic evaluation is being complemented by more sophisticated genomic approaches. A number of genomic biomarkers have been developed for clinical use, and increasingly, pharmacogenetic end points are being incorporated into clinical trial design. For women diagnosed with breast cancer, prognostic or predictive information is most useful when coupled with targeted therapeutic approaches, very few of which exist for women with triple-negative breast cancer or those with tumors resistant to chemotherapy. The immediate challenge is to learn how to use the molecular characteristics of an individual and their tumor to improve detection and treatment, and ultimately to prevent the development of breast cancer. The five articles in this edition of CCR Focus highlight recent advances and future directions on the pathway to individualized approaches for the early detection, treatment, and prevention of breast cancer.

The past decade has witnessed an explosion in our understanding of the molecular mechanisms involved in breast cancer progression, and an evolution toward a central role for genetic alterations in the early detection, diagnosis, and treatment of breast cancer. It is now well accepted that among all populations, an estimated 5% to 10% of breast cancer cases arise in individuals who inherit highly penetrant mutations in breast cancer susceptibility genes such as the BRCA1 and BRCA2 genes (1–3). However, the nature of all the genetic modifiers of risk in BRCA1 and BRCA2 mutation carriers, as well as the additional genes conferring breast cancer risk, are just beginning to be revealed (Fig. 1). Furthermore, recent advances in DNA microarray technology and other methods of large-scale gene expression analysis have been adopted for both the biological characterization and the therapeutic planning of breast cancer. Further understanding of the molecular biology and gene expression signatures of breast cancer are critical for developing novel approaches to the prevention and management of the disease. Two of the earliest and best examples of targeted therapies were developed in breast cancer after progress in cancer biology. Targeting the estrogen receptor was possible after identifying estrogen receptor as a major factor in premenopausal women with hormone-sensitive breast cancer. Similarly, amplification of the human epidermal growth factor receptor 2 (HER2) oncogene has proved to be the major predictor of response to treatment with the humanized anti-HER2 monoclonal antibody trastuzumab. These examples stimulated the search for other validated anticancer targets in breast cancer. This issue of CCR Focus does not attempt to provide an exhaustive review of all the latest advances in breast cancer research related to estrogen receptor signaling, HER2 resistance, and breast cancer stem cell biology. Rather, we focus on the exciting genomic discoveries that are transforming the traditional approaches to breast cancer management.

A Genomic Approach to Primary Prevention of Breast Cancer

High-penetrance genes. A growing body of evidence documents the benefits of preventive measures with minimal risk to women with identifiable highly penetrant mutations in the BRCA1 and BRCA2 genes. Whereas other genes, such as TP53
in Li-Fraumeni syndrome and PTEN in Cowden syndrome, also contribute to a small fraction of hereditary breast cancer, mutations in these genes are rare and account for a relatively small percentage of inherited breast cancer risk (Fig. 1). Germline mutations in the BRCA1 or BRCA2 genes are strong predictors of breast and/or ovarian cancer development, and the contribution of these mutations to breast cancer risk within any specific population is a function of both their prevalence and penetrance as reviewed by Fackenthal and Olopade (4).

Mutation prevalence varies among ethnic groups and may be influenced by founder mutations as has been observed in Ashkenazi Jewish populations and Icelanders. Although estimates of both mutation prevalence and penetrance rates are inconsistent and occasionally controversial, understanding them is critical for providing individualized risk assessment. In a recent population-based study from Northern California, John and colleagues (5) estimated the BRCA1 mutation prevalence rates to be 3.5%, 1.3%, 0.5%, 8.3%, and 2.2% in Hispanic, African American, Asian American, Ashkenazi Jewish, and other non-Hispanic white breast cancer patients, respectively. Because the study is population-based, they also provided an estimate of BRCA1 mutation carrier prevalence in the general U.S. population with an assumption of 90% mutation detection rate to be 0.27% in Hispanics, 0.12% in African Americans, 0.04% in Asian Americans, 1.66% in Ashkenazim, and 0.22% in other non-Hispanic whites (5).

Thus, these mutations are very rare in the general population, and a family history of breast cancer or young age of onset is the best way to identify mutation carriers. For example, based on findings from the population-based Australian Breast Cancer Family Study (6, 7), among women with two or more relatives with breast and/or ovarian cancer, about 1 in 2 have a BRCA1 or BRCA2 mutation and among women with at least one relative, about 1 in 18 have a detectable mutation (Fig. 2), leading to the conclusion that inherited susceptibility to breast cancer is multifactorial and may be due to yet unidentified genetic variants (Fig. 1). Interestingly, due to paternal transmission or small family structure, a large proportion of women who test positive for BRCA mutations have no family history of breast or ovarian cancer. To facilitate genetic counseling, there are now a number of carrier prediction models based on either a Mendelian approach (8, 9) or empirical data (10–13) that can be used in clinical practice for risk prediction. Using data from 292 minority families with at least one member tested for BRCA mutations through the Breast Cancer Family Registry and the University of Chicago Cancer Risk Clinic, we have recently validated the BRCAPRO risk prediction model in African American, Hispanic, and other

![Fig. 1. Genetic susceptibility to breast cancer. Familial breast cancer comprises approximately 20% to 30% of all breast cancers. BRCA1 and BRCA2 are two major high-penetrance genes associated with hereditary breast and ovarian cancer syndrome and explain less than 10% of all breast cancer cases. Mutations in CHEK2 contribute to a substantial fraction of familial breast cancer. Carriers of TP53 mutations develop Li-Fraumeni syndrome and are at high risk of developing early-onset breast cancer, but these mutations are very rare. Susceptibility alleles in other genes, such as PTEN, ATM, STK11/LKB1, and MSH2/MLH1, are also rare causes of inherited breast cancer. About half of the familial clustering of breast cancer is unexplained. The susceptibility to breast cancer in this group is presumed to be due to either additional high-penetrance susceptibility genes (which remain to be identified) or variants at many moderate or low-penetrance loci, each conferring a moderate risk of the disease (polygenic susceptibility; ref. 11). Eight low penetrance variants recently identified by whole genome association studies account for a small proportion (about 5%) of familial cases. The majority of women with breast cancer (so-called sporadic) do not have inherited mutations but may carry common low-penetrance genetic risk variants. The presence of such multiple genetic risk variants in the same individual may predispose to the development of breast cancer, independent of family history. Seven (FGFR2, TNRC9, MAP3K1, LSP1, 2q23, 5p12, 8q24) of the eight variants discovered thus far together account for 60% of breast cancer in the general population of women of European Ancestry (19). Adapted with permission from Lippincott, Williams & Wilkins (110).]
U.S. minority families (14). The model did reasonably well, especially among Hispanic families, but there is room for improvement in racial/ethnic groups other than Hispanics. Thus, whereas the contribution of other genes to early onset and hereditary breast cancer remains to be clarified, genetic testing for \textit{BRCA1} and \textit{BRCA2} has become standard of care and an important component of personalized breast cancer risk assessment and prevention.

**Moderate-penetrance genes.** In this issue of \textit{CCR Focus}, Garcia-Closas and Chanock (15) have provided a comprehensive review of recent work from large consortial studies that have led to the discovery of additional breast cancer susceptibility loci through candidate gene or whole genome approaches. These studies suggest that much of the genetic component of breast cancer risk remains uncharacterized and probably arises from combinations of low-penetrance variants that, individually, might be quite common in the population. Identifying these common single nucleotide polymorphisms (SNP) through genome-wide association studies in populations of European ancestry has uncovered eight common low-penetrance genetic variations in five candidate genes and three nongenic genomic regions, including \textit{CASP8}, \textit{FGFR2}, \textit{TNRC9}, \textit{MAP3K1}, \textit{LSP1}, and rs13387042 on 2q35, rs313281615 on 8q24, and rs10941679 on 5p12 (Fig. 1, refs. 16–22).

Under a polygenic model, seven of the eight low-penetrance genes were estimated to explain about 5% of the genetic risk for breast cancer with an additional small contribution from the eighth SNP in 5p12 not included in the original reports. Based on these studies, the deCODE Breast Cancer gene test, which includes the original seven SNPs, has been developed and is being marketed to the general population. The studies suggest that common genetic variants can influence the pathologic characteristics of breast cancer. Five of the SNPs identified from the genome-wide association studies (\textit{FGFR2}, \textit{TNRC9}, 8q24, 2q35, and 5p12) mostly confer risk of estrogen receptor–positive breast cancer in postmenopausal women of European ancestry, and it is estimated that these seven markers together account for 60% of breast cancer (based on population attributable risk and assuming the risk markers are independent; ref. 19). As genome-wide association studies are based on linkage disequilibrium, which is quite different among Caucasians, Asians, and Africans (23, 24), these novel SNPs and their association with tumor phenotypes will have to be replicated in other populations.

All genome-wide association studies to date started the discovery stage in women of European ancestry and confirmed their findings mainly in large consortia of women of European ancestry in the Cancer Genetic Markers Susceptibility Initiative of the U.S. National Cancer Institute, Breast Cancer Association Consortium, and the Consortium of Investigators of Modifiers of \textit{BRCA1}/2. The SNP in \textit{FGFR2} has been tested in Asian samples, but the effect was modest (17). The \textit{FGFR2} SNP could not be confirmed in one study with African Americans, but the study was not sufficiently powered (21). In another study, four SNPs on 5p, including rs4415084 (but not rs10941679), conferred nominally significant risk in a sample of 689 Nigerian breast cancer patients and 469 controls (22). However, these observations could not be replicated in a sample of 428 African Americans and 457 controls from the Multiethnic Cohort Study (22). This raises the question of whether these polymorphisms affect the risk of breast cancer among women of African ancestry in the same way as women of European ancestry. To examine the contribution of these novel loci to breast cancer in other populations, studies with enough power to detect small effects are needed. Of note, African Americans are more likely to develop breast cancer at a younger age and to have overrepresentation of estrogen receptor–negative breast cancer. It is plausible that yet unidentified genetic variants contribute to risk in women of African ancestry and underscores the urgent need for genome-wide association studies in larger cohorts of women of African ancestry, who remain understudied but have the highest risk of aggressive estrogen receptor–negative breast cancer.

Although much work remains to better understand how each of the newly identified loci contributes to increased risk and how multiple high-risk alleles interact to further modulate risk in diverse populations, the findings of genome-wide association studies open up new areas of research on the causes of breast cancer. New molecular pathways have been identified that will further contribute to our understanding of breast cancer genetics. With this information, we can begin to develop personalized risk reduction strategies as has been pursued for women at the highest risk due to inheritance of highly penetrant mutations in the \textit{BRCA1} and \textit{BRCA2} genes.

**Risk-reducing interventions.** Individuals carrying mutations in the \textit{BRCA1} and \textit{BRCA2} genes have a 40% to 80% chance of developing breast cancer, making these mutations the strongest breast cancer predictors known (1–3). Genetic testing for \textit{BRCA} mutations is offered in clinical practice because mutation carriers now have the option of choosing from a spectrum of evidence-based and potentially life-saving prevention strategies, including intensive breast cancer surveillance using magnetic resonance imaging (MRI), chemoprevention, risk-reducing bilateral mastectomy, and salpingo-oophorectomy. Studies have shown that \textit{BRCA1} and \textit{BRCA2} mutation carriers who undergo bilateral salpingo-oophorectomy before menopause reduce their risk of breast cancer by 53% to 75% (25–27), and peritoneal or ovarian cancer by 80% to 96% (28, 29). Likewise, genetic testing for low- to moderate-risk genetic variants will be offered in the clinic once the clinical utility is established. An argument can be made that these variants could help to stratify women whose lifetime risk of breast cancer warrants additional interventions than what is currently recommended for women in the general population.

Based on the high sensitivity of MRI in large randomized clinical screening studies of high-risk women, the American Cancer Society has developed new guidelines for breast cancer screening with MRI (30–35). Screening MRI is recommended for women with a moderate lifetime risk of breast cancer (>20%), as well as women with mutations in highly penetrant breast cancer susceptibility genes (e.g., \textit{BRCA1}, \textit{BRCA2}, \textit{TP53}, \textit{PTEN}, etc) and women who received mantle field radiation for...
Hodgkin’s disease (36). Mammography has been the mainstay of population-based breast cancer screening for several decades, leading to decreased mortality from breast cancer for women between the ages of 50 and 69 years. However, mammography has not been shown to reduce mortality in younger women, likely due to lower rates of breast cancer incidence, increased breast density making lesions harder to find, and higher rates of fast-growing tumors.

MRI has shown significantly better sensitivity and comparable specificity than mammography in high-risk populations (30–35). Overall, these studies have found that MRI sensitivity ranges from 77% to 100%, whereas mammography only has a sensitivity of 16% to 40%. Specificity for mammography ranges from 93% to 99% and 81% to 99% for MRI. Because the specificity of MRI in all studies to date is significantly lower than that of mammography and results in a higher rate of recall and biopsy, it is not recommended for screening moderate- or low-risk women. Although MRI screening is not perfect, it is more likely to identify smaller interval cancers than mammography without the added cumulative risk of radiation exposure. MRI thus provides a personalized strategy for early detection, with the potential to improve outcomes for moderate- to high-risk women. Thus, personalized genomics approach to intensive surveillance and chemoprevention using the newly discovered SNPs could allow better stratification of women who are likely to benefit from early use of intensive surveillance as reviewed by Pharoah et al. (19).

Fig. 2. Who has BRCA1 and BRCA2 mutations? Women with breast cancer diagnosed before age of 40 y, categorized according to the number of affected first- or second-degree female relatives (0, 1, 2, or >2) from the Australian Breast Cancer Family Study (6). Sporadic cases have no family history of breast cancer. Familial cases have one or more affected first- or second-degree relatives and make up about 30% of all breast cancer cases. Hereditary cases have a germline mutation in either BRCA1 or BRCA2 and are more likely to occur within the familial cohort; however, in absolute numbers, 60% of cases testing positive for a deleterious mutation have no family history and are in the “sporadic” group. The proportion of mutation carriers identified in each category of family history, in terms of 1 in x, is shown in the right side of the pyramid. The proportion of mutation carriers for each category of family history, in terms of a percentage of all identified mutation carriers, is shown on the right side of the figure. Only 1 in 2 cases from families with ≥2 affected relatives have identifiable BRCA1/2 mutation and they make up <1% of mutation carriers. Adapted with permission from J Natl Cancer Inst Monogr (6).

A Genomic Approach to Treatment of Breast Cancer

Schneider et al. (37), in this issue of CCR Focus, discuss triple-negative breast cancer, a distinct molecular subtype of breast cancer that has emerged from DNA microarray gene expression studies. Human mammary glands contain two distinct subtypes of epithelial cells, basal (myoepithelial) and luminal, which can be easily distinguished by the pattern of expression of certain cytokeratins. Basal cells lie closest to the basement membrane and stain positive for cytokeratin 5/6. Luminal cells compose the upper, more differentiated, layer of the mammary gland epithelium and express cytokeratin 8/18. The cytokeratin pattern is largely conserved after transformation of epithelial cells, allowing determination of the cell-type origin of primary cancers. Most breast cancers originate from the luminal epithelium and express luminal cell-specific cytokeratins. It is estimated, however, that 3% to 15% of all breast cancers seem to originate from basal-like epithelium and express basal-specific cytokeratins as discussed by Schneider et al. (37). Perou and colleagues, using complementary DNA microarrays, first proposed a molecular classification of breast cancers based on variations in global gene expression patterns (38–40). Following these original reports, several other groups have confirmed that individual cancers could be categorized, based on their gene signature, to at least five distinct subtypes: luminal A, luminal B, normal-like, HER2-like, and basal-like. Normal-like tumors resemble normal breast tissue, HER2-like are characterized by HER2 overexpression, luminal A and B are estrogen receptor positive, and basal-like are triple negative (estrogen receptor negative, progesterone receptor negative, and HER2 negative). BRCA1-associated breast cancers cluster within the basal-like subtype (41), which suggests that host germline genetics may determine the tumor subtype. These subtypes have been shown to correlate with clinical outcomes (40, 42, 43). The luminal A and B subtypes have the best prognosis, whereas HER2-like and basal-like have the worst prognosis, although the inclusion of trastuzumab in primary therapy for...
HER2-positive breast cancer has led to vast improvements in outcomes for patients with HER2-positive breast tumors. Immunohistochemical markers have been used to define these subtypes with similar prognostic value (44, 45), which allows for breast cancer subtype assignment in epidemiologic studies and clinical practice.

As illustrated in Fig. 3, the distribution of breast cancer subtypes has been reported to vary across populations (46–49). Estrogen receptor–positive, luminal A breast cancers were predominant in Asian, white, and postmenopausal African American populations, about 40% in premenopausal African Americans, and only 27% in indigenous Africans. In contrast, the proportion of the estrogen receptor–negative, basal-like subtype was 27% in indigenous Africans and premenopausal African Americans, about 15% in postmenopausal African Americans and premenopausal European Americans, and only about 10% in other populations. There is also a clear gradient in the proportion of estrogen receptor–negative unclassified breast tumors across populations, with Africans having the highest proportion. Interestingly, the proportion of HER2-positive tumors (luminal B and HER2-positive/estrogen receptor–negative subtypes combined) was about 15% in all populations except the Japanese. As discussed by Garcia-Closas and Chanoèk (15), genomic risk factors for these subtypes are also likely to vary across populations, which suggests that an integrative epidemiology (50) approach must be applied to drug development at both the population and individual levels. Breast cancer clinical trials must be conducted in populations in which the drugs are to be used, as one can no longer assume that drugs that are developed in predominantly European populations will produce similar outcomes in populations of Asian or African ancestry.

**Genomic Biomarkers**

In their review in this issue of *CCR Focus*, Dowsett and Dunbier (51) discuss the important role of biomarkers in the optimal selection of treatment for breast cancer. For example, two multigene expression profiles have shown the ability to outdo the traditional prognostic and predictive factors. Oncotype Dx, a 21-gene reverse transcription-PCR-based assay (52), has already proved successful in identifying subsets of node-negative, estrogen receptor–positive patients who will benefit from the addition of chemotherapy to adjuvant antiestrogen therapy and those who will not. MammaPrint, a 70-gene signature, was developed from studying tumors of women with node-negative disease, unselected for estrogen receptor status (53, 54). Tumors from patients who remained disease-free for 10 years were found to have distinct profiles compared with patients who experience early relapse. Both Oncotype Dx and MammaPrint have the potential to identify patients with node-negative tumors who do not require additional therapy, thus sparing these women from unnecessary and potentially toxic therapy. Both of these assays are Food and Drug Administration–approved and are currently undergoing prospective validation to more clearly define their roles in the optimization of therapy for node-negative breast cancer patients.

The next decade will surely witness the further explosion of predictive and prognostic genomic markers, and older biomarkers such as Ki67 will assume more prominence. Expanding on the success of Oncotype Dx and MammaPrint, investigators are trying to identify signatures that predict response to specific therapies. Hess and colleagues (55) have used transcriptional profiling to identify genomic signatures, which predict for response to the T-FAC adjuvant chemotherapy regimen. An important advance in the last decade is the incorporation of trastuzumab into adjuvant therapy for HER2-positive breast cancer. Trastuzumab, a monoclonal antibody to HER2, in combination with standard adjuvant chemotherapy, has decreased the risk of recurrence by more than 50% (56). Targeting HER2 is a true success story and shows how identifying and inhibiting a target can transform a very aggressive phenotype into one with a favorable outcome. Prior to the use of targeted therapy for HER2-positive breast cancer, this form of the disease was associated with a high risk of relapse and a uniformly poor prognosis. Understanding the biology of HER2-positive breast cancer and the development of a tailored therapeutic approach has led to a cure for many women with this form of the disease.

**Triple Negativity and Fanconi Anemia-BRCA DNA Repair Pathways**

As discussed by Schneider et al. (37), women with triple-negative breast cancer pose a major challenge to the oncology community as many are young, and a significant proportion have BRCA1 mutations. As the discovery of BRCA1 and BRCA2 has led to individualized approaches for the screening and prevention of breast cancer in high-risk women, an understanding of BRCA1 and BRCA2 biology is leading to tailored approaches for the treatment of BRCA-associated breast cancers. The association of BRCA1 with DNA repair was first established by the seminal observation that BRCA1 colocalizes with the homologous recombinase RAD51 in subnuclear foci (57). Subsequent studies indicate that BRCA1 associates in protein complexes that participate in DNA repair pathways. Cells lacking BRCA1 or BRCA2 are unable to sense DNA damage properly, transmit and process the damage response signal, or repair DNA damage by homology-directed recombination. Instead, such cells utilize nonconservative, error-prone, and potentially mutagenic mechanisms of nonhomologous end joining and single-strand annealing, leading to genomic instability (58). BRCA1 is an integral part of the repair process itself. A significant impairment of homology-directed recombination and increase in frequency of nonhomologous end joining has been observed in Brca1-deficient mouse embryonic stem cells and conditional mutants (59, 60) and can be corrected by reexpression of wild-type Brca1. Mouse Brca1-deficient cells and human BRCA1-deficient tumor cells both exhibit significant genomic instability, gross chromosomal aberrations, and centrosome amplifications.

Fanconi anemia (FA) is a rare hereditary disorder characterized by bone marrow failure, marked genomic instability, and increased incidence of cancer. BRCA1 and BRCA2 are part of the Fanconi anemia protein complex. In fact, BRCA2 is Fanconi
anemia protein D1 (FANCD1). In response to DNA damage, a nuclear complex of five FA proteins (A, C, E, F, and G) interact with FANCL and cause ubiquitinilation of FANCD2. Ubiquitinilated FANCD2 colocalizes with BRCA2/RAD51/BRCA1 complex in nuclear foci after DNA damage. The disruption of the FA-BRCA pathway due to defects in participating proteins results in an impaired response to DNA damage and increased cancer susceptibility (61).

Because tumor cells deficient in BRCA and FA genes repair DNA damage by utilizing single-strand annealing and nonhomologous end joining mechanisms and are particularly sensitive to interstrand cross-links following treatment with interstrand cross-link–generating drugs (e.g., mitomycin C, platinum and its analogues), these pathways have been targeted for treatment (61). DNA cross-links caused by mitomycin C and the platinum family of drugs block DNA replication and lead to stalled replication forks. Poly(ADP-ribose) polymerase 1 (PARP1) functions as a DNA damage sensor for both single- and double-strand breaks. PARP2 is a similar protein that plays an important role in base excision repair by homodimerization and heterodimerization with PARP1. Thus, both PARP1 and PARP2 play critical roles in the maintenance of genomic stability by regulating DNA repair mechanisms. PARP1 inhibitors dramatically reduce repair of single-strand breaks and double-strand breaks in BRCA-deficient tumors, resulting in increased tumor sensitivity to DNA damaging agents such as cisplatin (62).

In normal cells heterozygous for BRCA, the wild-type BRCA allele is active and its protein product can repair double-strand breaks by error-free, homology-directed recombination. As a result, treatment of BRCA mutation carriers with PARP inhibitors is expected to be highly specific for cancer cells and yet nontoxic to healthy tissues. A number of clinical trials with PARP inhibitors have been initiated, including a phase II study of the efficacy and safety of the PARP inhibitor KU-0059436 for the treatment of BRCA-mutant breast cancer. As they target cells deficient in DNA repair, they represent a personalized approach to the treatment of BRCA-associated breast cancer.

Another novel compound in development that targets cells impaired in DNA repair is trabectedin (Ecteinascidin, Et-743, Yondelis). Trabectedin has recently been approved in Europe and North Korea for the treatment of soft tissue sarcomas and is undergoing clinical trials for the treatment of breast, ovarian, prostate, and other solid tumors. Trabectedin is a new chemical entity with a unique, multicomponent mechanism of action. It is the only chemotherapy agent that binds to the minor groove of the DNA, bends the DNA toward the major groove, and exerts its therapeutic effect by interfering with cellular transcription-coupled nucleotide excision repair mechanism to induce cell death (63, 64). These agents target DNA repair pathways, and therefore, have the potential to be useful in BRCA-associated breast or ovarian cancer.

### A Genomic Approach to Drug Development

The study of pharmacogenomics is particularly attractive in oncology as most chemotherapy agents have a narrow therapeutic window, with severe drug toxicities that can be...
potentially life-threatening. Although advances in adjuvant chemotherapy for breast cancer have led to marked reductions in recurrence and mortality, women are still concerned about the short- and long-term toxicities associated with treatment. Moreover, improvements in chemotherapy have effects that are much more striking in women with estrogen receptor–negative tumors than in women with estrogen receptor–positive tumors, some of whom can now be spared chemotherapy. For example, the risk of death for dose-dense doxorubicin/cyclophosphamide followed by dose-dense paclitaxel (as in INT C9741) when compared with low-dose cyclophosphamide/doxorubicin/5-fluorouracil was reduced by 55% (38-69%) in women with estrogen receptor–negative tumors versus only 23% (-17% to 49%) in estrogen receptor–positive tumors (65). Thus, it is conceivable that, given the right combination of highly effective and less toxic chemotherapy, women with hormone receptor–negative breast cancer might be expected to have a more favorable outcome. In addition, hormonal therapies might be more effective if they are given in appropriate doses, taking into account the genetic variants that affect metabolizing enzymes as discussed in the review by Tan et al. (66) in this issue of CCR Focus.

### Integrative Epidemiology

There are interindividual and interethnic variability of drug pharmacokinetics and pharmacodynamics, which may be contributed by commonly occurring genetic polymorphisms of drug-metabolizing enzymes and transporters (66). Spitz et al. (50) recently proposed a unifying premise of integrative epidemiology and suggested that the same genes that are implicated in cancer risk may also be involved in a person’s propensity to carcinogenic exposure and/or to modulation of therapeutic outcome. Examples include glutathione-related transporter genes and the cytochrome P450 enzymes, such as CYP3A4 and CYP191A variants. Variants in these genes have been implicated in cancer risk and are important pathways for metabolizing antineoplastic drugs.

Tamoxifen, a selective estrogen receptor modulator, is commonly used for both the chemoprevention of breast cancer and the treatment of early and advanced stage estrogen receptor–positive breast cancer. Failure of tamoxifen therapy has long been attributed to intrinsic or acquired resistance of the tumor to the effects of estrogen receptor blockade. Tan and colleagues (66) show that interindivudual genetic variability plays a critical role not only in determining toxicity from therapy but also in determining benefit, in some cases. Tamoxifen undergoes extensive metabolism via the CYP pathway to several primary and secondary metabolites, some of which exhibit more potent antiestrogenic effects than tamoxifen itself on breast cancer cells. CYP2D6 is one of the key enzymes in this pathway that metabolizes tamoxifen to a more active metabolite, endoxifen. There are several variants in the CYP2D6 gene that result in the poor metabolizer phenotype, which in turn has been shown to correlate with worse outcomes.

The majority of variations in drug-metabolizing enzymes identified to date have been through a “candidate gene” approach. It is unlikely, however, that drug efficacy and toxicity is a polygenic trait not attributable to a single gene. Genome-wide association studies are making the study of multiple genes and SNPs involved in drug toxicity and efficacy possible. Several groups have developed genome-wide approaches to identify germline polymorphisms that correlate with cytotoxicity. Huang and colleagues (67) have developed a preclinical model that uses the International HapMap Project lymphoblastoid cell lines. The HapMap Project contains lymphoblastoid cell lines from four distinct ethnic populations: Caucasians (Utah, United States), Africans (Yorubas from Nigeria), Japanese (Tokyo), and Chinese (Han from Beijing). These cell lines are an invaluable resource because they have been extensively genotyped. When lymphoblastoid cell lines are treated in vitro with a chemotherapeutic agent, individual cytotoxicity phenotypes are identified, which can then be combined with cell line–specific genotype data to do genetic association studies. This information can then be correlated to gene expression data, the so-called “triangular” approach, to allow the identification of SNPs that may explain variation in drug sensitivity. Importantly, the approach allows for simultaneous discovery of multiple SNPs involved in susceptibility, without bias for any particular gene.

### Genomic Approach to Disparities in Breast Cancer Outcomes

Population differences in the distribution of variants in drug-metabolizing enzymes and transporters might be relevant in addressing differences in outcomes in diverse populations and specifically in addressing health disparities. Women of African ancestry have a lower overall incidence of breast cancer, but a higher overall mortality as compared with white women (68, 69). The disparity in outcomes may be partly related to lower tolerance for side effects of treatment. Recent studies focus on disparities in treatment outcomes because differences in socioeconomic factors and tumor biology do not entirely account for the disparity in clinical outcomes. Several studies have documented differences in the receipt of cancer treatment by race (70–73), and large clinical trials have established that dose delays, reductions, or early termination of chemotherapy greatly reduce treatment benefit (74–76). Despite these data, undertreatment is common especially among African American women. In a retrospective study of over 20,000 women treated in community practices, Lyman and colleagues (77) found that 36.5% of patients received <85% of their planned chemotherapy. In a study of 472 women with early-stage breast cancer, Hershman et al. (78) found that a substantial fraction of women terminated their chemotherapy prematurely and that early termination was significantly associated with both poorer survival and black race. Suboptimal delivery of chemotherapy dose and intensity has been associated with decreased efficacy and poorer survival (79–81). For these reasons, future drug development in breast cancer should incorporate genomic markers to identify interindividual and interethnic variability of drug pharmacokinetics and pharmacodynamics. With the development of more efficacious and less toxic drug regimens, one can expect to see a reduction in
health disparities for the most vulnerable patients, especially women of African ancestry.

The Epigenome and Breast Cancer

Much of the focus in breast cancer research over the past few decades has been on breast cancer genetics: identifying mutations and characterizing their roles in carcinogenesis. In the last several years, studies have shown that epigenetics plays a key role in tumor progression. Epigenetic changes are defined as heritable and reversible changes in gene expression that are not accompanied by changes in DNA sequence.

In cancer, the main epigenetic mechanisms underlying abnormal gene expression include aberrant CpG-island-promoter methylation of specific tumor suppressor genes, global changes in genomic DNA methylation, and alterations in histone modification (deacetylation and methylation; Fig. 4). These abnormalities can be reversed by inhibitors of both DNA methyltransferases and histone deacetylases (82). It has been postulated that promoter methylation may serve as the second “hit” in the Knudson two-hit model in sporadic cancer through inactivation of the normal allele of a tumor suppressor gene, implicated in hereditary cancer syndrome such as BRCA1 (83). Hypermethylation of BRCA1 at promoter CpG islands occurs in 15% to 31% of sporadic breast tumors and may play a critical role in sporadic breast cancer by inactivating one BRCA1 allele followed by loss of the wild-type BRCA1 (84). BRCA1 methylation in sporadic breast cancer seems to result in a similar tumor phenotype to that seen in BRCA1 mutation carriers (83–86). These findings are consistent with our study of C-MYC amplification in BRCA1-deficient breast cancers (BRCA1-mutated hereditary and BRCA1-methylated sporadic), in which we showed that the pattern of C-MYC amplification in BRCA1-methylated cases resembles that of BRCA1-mutated cases rather than that of sporadic cancers (87).

We and others have offered a model of breast carcinogenesis in which BRCA1 promoter methylation serves as a “first hit,” much like an inherited germline mutation, and the “second hit” results in reduced BRCA1 copy number and/or chromosome 17 aneusomy. In this model, BRCA1 promoter hypermethylation occurs early and, when complete, causes defects in chromosome structure, cell division, and viability (84). A BRCA1-deficient cell must acquire additional alterations, such as TP53 mutations or MYC amplification, that overcome these problems and force tumor progression down the same limited set of molecular pathways, similar to the progression of hereditary BRCA1 mutated tumors as illustrated in Fig. 4. Because the majority of BRCA1-mutated breast cancers are basal-like, Foulkes (88) has hypothesized that the key function of BRCA1 is to be a stem cell regulator and promote the differentiation of glandular epithelium. For this reason, in a BRCA1-deficient cell, this transition can fail or abort, and the basal cell phenotype gene expression pattern would be retained. Interestingly, Wicha and colleagues (89) have recently confirmed that inactivation of BRCA1 in breast epithelial stem cells restricts subsequent progenitor cells to a basal-like cell subtype. Collectively, these observations suggest that inactivation of BRCA1 by mutation or methylation promotes breast cancer with basal tumor phenotype. In contrast, loss of BRCA2 expression via aberrant promoter methylation does not seem to occur in sporadic cancers.

Functional equivalency between the effect and significance of the epigenetic silencing of BRCA1 in sporadic breast cancer and genetic suppression of the gene in BRCA1 mutation carriers has implications for clinical practice. Lafarge and colleagues (90) recently showed that decreased expression of BRCA1 in the HBL100 breast cancer cell line led to increased sensitivity to the DNA-damaging agent cisplatin. As previously discussed, a number of studies have also correlated loss of BRCA1 with defects in DNA repair and cell cycle checkpoints. Tumors

![Fig. 4. The epigenetic progenitor model of cancer.](https://example.com/image.png)

Fig. 4. The epigenetic progenitor model of cancer. Cancer arises in three steps: (a) polyclonal epigenetic disruption of progenitor cells; (b) an initiating monoclonal mutation, and (c) acquisition of genetic and epigenetic plasticity. First is an epigenetic alteration of stem/progenitor cells within a given tissue, which is mediated by aberrant regulation of tumor-progenitor genes (TPG). For example, in breast cancer, the first step could be methylation of the BRCA1 promoter as a result of events within the stem cells themselves, the influence of the stromal compartment, or environmental damage or injury. Second step is a gatekeeper mutation (GKM) in a tumor suppressor gene (TSG) such as TP53 or oncogene amplification (ONC) such as MYC amplification. Although these gatekeeper mutations are themselves monoclonal, the expanded or altered progenitor compartment increases the risk of cancer when such a mutation occurs and the frequency of subsequent primary tumors (shown as separately arising tumors). Third is genetic and epigenetic instability, which leads to increased tumor evolution. Many of the properties of advanced tumors (invasion, metastasis, and drug resistance) are inherent properties of the progenitor cells that give rise to the primary tumor and do not require other mutations (highlighting the importance of epigenetic factors in tumor progression). Adapted by permission from MacMillan Publishers Ltd: Nature Reviews Genetics (ref 111), copyright 2006.
lacking functional BRCA1 protein show a high frequency of chromosomal aneuploidy, characteristic of a defective G2-M checkpoint. Sudo and colleagues (91) have shown that paclitaxel sensitivity is dependent on an intact checkpoint function, thus implying that any interference with the spindle assembly checkpoint would generate paclitaxel resistance. BRCA1-deficient cells secondary to mutation or epigenetic regulation may confer a unique chemosensitivity profile. Thus, BRCA1 deficiency secondary to promoter methylation may represent a novel therapeutic target for the management of a subset of basal-like or triple-negative breast cancers.

Both inherited and sporadic breast cancers can also exhibit variable estrogen receptor-α expression. Interestingly, estrogen receptor-α coding region mutations seem to be quite rare (95, 96), although there is convincing evidence that estrogen receptor-α is an epigenetically regulated gene that can undergo promoter methylation in a significant proportion of breast cancers (97) and can strongly associate with BRCA1 promoter methylation (98). An alternative epigenetic mechanism underlying loss of estrogen receptor-α expression has been suggested by the results of cell-based assays analyzing histone function as a determinant of gene expression (99). Restoration of estrogen receptor-α expression by histone deacetylase inhibitors suggests that reorganizing the heterochromatin-associated proteins, without demethylation per se, can restore functional estrogen receptor-α expression (99). This possibility is being explored clinically in an ongoing phase II trial of a new generation histone deacetylases inhibitor, vorinostat.7 The investigators of this trial will determine whether or not, following vorinostat treatment, a tumor becomes sensitive to hormone therapy and/or exhibits increased expression of estrogen receptor-α. In addition, there have recently been a number of phase I/II trials initiated to investigate combining different classes of histone deacetylases inhibitors with traditional therapies for the treatment of breast cancer (100).

DNA methylation of certain genes (e.g., RASSF1A, CYP26A1, KCNAB1, SNCA, HIN-1, TWIST, and Cyclin D2) occurs in both premalignant lesions, such as atypical hyperplasia, and carcinoma of the breast (101, 102). These findings suggest that epigenetic changes occur early in breast tumorigenesis and may serve as potential markers for early detection or risk assessment. Moreover, specific epigenetic changes may have prognostic and/or predictive value (103). These observations are being translated into clinical care. For example, the National Cancer Institute is sponsoring a study of women at high risk of developing breast cancer who, following surgical resection for ductal carcinoma in situ or stage I, II, or III invasive breast cancer, are treated with simvastatin. Simvastatin belongs to the statin family of 3-hydroxy-3-methylglutaryl CoA reductase inhibitors, drugs that lower cholesterol in patients with cardiovascular disease; these agents have a theoretical role in chemoprevention through down-regulating Ras, up-regulating p27, and altering estrogen receptor levels. The change in methylation status across a panel of genes (estrogen receptor-α and estrogen receptor-β, Cyclin D2, RAR-β, Twist, RASSF1A, and HIN-1) that are known to be frequently and specifically hypermethylated in breast cancer will be evaluated and correlated with changes in hsCRP, lipid profile, contralateral breast density, and estrogen concentration.

Conclusion and Future Directions

Whereas the past decade has witnessed significant advances in the prevention and treatment of breast cancer by targeting the estrogen receptor and HER2 oncogene, the options for women with triple-negative disease remain suboptimal. The triple-negative molecular subtype is characterized by marked heterogeneity, which has further complicated clinical trial design. Biomarkers of response and precise classification of tumors will be required so that subsets of triple-negative tumors that respond to chemotherapy can be identified and this knowledge incorporated into future trials that incorporate molecularly targeted biological agents. With the availability of novel technologies, such as microRNA profiling and genome-wide association studies, we are just beginning to better understand the role of host genetics in the optimization of therapy as well as in susceptibility.

The recent development of microRNA microarray technology yields new insights in breast cancer. MicroRNAs are a class of short noncoding RNAs that are found in animals, plants, and viruses. They seem to alter their effects through the posttranscriptional repression of gene expression. Although more than 500 microRNAs have been identified in the human genome, their function is not yet well understood. MicroRNA profiling has revealed that they are frequently deregulated in human tumors. MicroRNAs have been shown to affect oncogenes, tumor suppressor genes, and several processes important in cancer, including angiogenesis, apoptosis, cell cycle, and cell migration (104–106). Given the emerging data that microRNAs can be associated with either “oncogenic or antioncogenic” tumor suppressive activities, interference with microRNAs may represent a novel therapeutic approach for certain subsets of breast cancer in the future.

Personalized approaches for the prevention and treatment of breast cancer will not be realized by any one approach, but rather through multiple approaches acting in concert. The oncology workforce in the next decade will evolve novel technologies to deliver personalized medicine at cheaper costs as most hospitals and physician practices develop electronic medical records. Adverse drug interactions and reactions can be documented and shared with other providers to improve the patient’s care. The next decade will usher in novel targeted therapies for other subtypes of breast cancers because we recognize that breast cancer is not one disease but a heterogeneous group of diseases. We will learn why there is differential response to new drugs so that patients who are unlikely to respond to a particular dosage of a therapy based on their tumor profile and individual genotypes will be spared unnecessary exposure to the agent. This is because we know that there is heterogeneity in drug metabolism, leading to
variability in drug efficacy and toxicities. Drugs will be dosed based on a better understanding of pharmacogenetics and pharmacodynamics. In a similar fashion, there is heterogeneity in cancer risk. A key component of cancer control will be identifying high-risk individuals to initiate cancer prevention interventions that will ultimately reduce overall mortality. Women who need MRI screening will get it based on individual risk, not population risk. There is a strong argument for individualizing breast cancer surveillance based on individualized estimates of cancer risk, such as currently provided with predictive genetic testing for BRCA1 and BRCA2. Such individualized screening approach has been suggested in the American Cancer Society recommendations for breast cancer screening for high-risk women, emphasizing shared decision-making in considering the risks and benefits of available screening technologies especially for younger women (107–109). These personalized approaches are likely to be cost-saving in the long run by eliminating unwarranted toxicities and streamlining early detection and prevention.

Identification of novel breast cancer susceptibility loci will further increase our understanding of alternate pathways that contribute to cancer risk and offer new therapeutic targets. The pathway to personalized medicine is dependent on advances in a broad range of disciplines, and a transdisciplinary systems biology approach to breast cancer will be needed to characterize novel susceptibility loci, understand the role of epigenetics in carcinogenesis and tumor progression, develop predictive and prognostic biomarkers, and identify novel therapeutic targets that are more efficacious and less toxic to the patient.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**Toward Personalized Medicine in Breast Cancer**


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