REVIEW

Inherited and acquired alterations in development of breast cancer

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Abstract: Breast cancer is the most common cancer among women, accounting for about 30% of all cancers. In contrast, breast cancer is a rare disease in men, accounting for less than 1% of all cancers. Up to 10% of all breast cancers are hereditary forms, caused by inherited germ-line mutations in "high-penetrance," "moderate-penetrance," and "low-penetrance" breast cancer susceptibility genes. The remaining 90% of breast cancers are due to acquired somatic genetic and epigenetic alterations. A heterogeneous set of somatic alterations, including mutations and gene amplification, are reported to be involved in the etiology of breast cancer. Promoter hypermethylation of genes involved in DNA repair and hormone-mediated cell signaling, as well as altered expression of micro RNAs predicted to regulate key breast cancer genes, play an equally important role as genetic factors in development of breast cancer. Elucidation of the inherited and acquired genetic and epigenetic alterations involved in breast cancer may not only clarify molecular pathways involved in the development and progression of breast cancer itself, but may also have an important clinical and therapeutic impact on improving the management of patients with the disease.

Keywords: breast cancer, inherited susceptibility, acquired alterations, epigenetics

Introduction

Breast cancer is currently the most common cancer among women, accounting for about 30% of all cancers.[1] In contrast, breast cancer is a rare disease in men, accounting for less than 1% of all cancers.[2] The age-specific incidence rates for breast cancer in women increase rapidly until the age of 50 years, and then increase at a slower rate for older women, while incidence rates for breast cancer in men increase linearly and steadily with age. Overall, current epidemiologic and pathologic data, such as age-frequency distribution, age-specific incidence rate patterns, and prognostic factor profiles, suggest that male breast cancer is similar to postmenopausal female breast cancer.[2] It is generally accepted that breast cancer may represent the same disease entity in both genders, and common hormonal, genetic, and environmental risk factors are involved in the pathogenesis of breast cancer in women and men. Hormonal changes, such as increased estrogen exposure due to diabetes, obesity or liver disease, and environmental and lifestyle factors, such as carcinogen exposure or alcohol intake, are associated with risk of developing breast cancer.[3] However, the major predisposition factor for breast cancer is a positive family history of the disease. Patients of both genders with a positive first-degree family history have a twofold increased risk, which increases to more than fivefold with the number of affected relatives and early onset relatives, thus suggesting a relevant genetic component in

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breast cancer risk.[4] It is estimated that up to 10% of all breast cancers are hereditary forms, caused by inherited germ-line mutations in breast cancer susceptibility genes. Commonly, inherited mutations are loss-of-function mutations that occur in tumor suppressor genes involved in DNA repair and cell cycle checkpoint activation.[1] The remaining 90% of breast cancers are due to acquired somatic, genetic, and epigenetic alterations.[5] Genetic alterations include gain-of-function mutations, amplification, deletions, and rearrangements occurring in genes which stimulate cell growth, division, and survival.[6] Epigenetic deregulation, mainly due to promoter methylation, may also contribute to the abnormal expression of these genes.[7] In addition, the involvement of micro RNAs (miRNAs) in modulating gene expression in the development of breast cancer has been recently reported.[8] The focus of this review will be on the most relevant inherited and acquired alterations in the development of breast cancer in both genders. We did a systematic literature search using PubMed to provide a synopsis of the current understanding and future directions of research in this field. We selected original articles and reviews published up to April 2011. The following search key terms were used to query the PubMed website: "inherited breast cancer," "breast cancer AND susceptibility," "breast cancer AND somatic alterations," "breast cancer AND epigenetic," "breast cancer AND miRNA." The abstracts resulting from these queries were individually analyzed for relevance.

Inherited susceptibility to breast cancer

To date, 5%–10% of all breast cancers are caused by inherited germ-line mutations in well identified breast cancer susceptibility genes.[1] According to their mutation frequency and the magnitude of their impact in breast cancer susceptibility, these genes can be divided into "high-penetrance," "moderate-penetrance," and "low-penetrance" genes (Figure 1).[9] Variants in the two major high-risk breast cancer genes, ie, BRCA1 and BRCA2, occur rarely in the population, but confer a high risk of breast cancer to the individual.[1] P53 and PTEN, two genes involved in rare syndromes (Li-Fraumeni and Cowden syndromes, respectively), also confer a high risk of breast cancer.[1] However, P53 and PTEN germ-line mutations are very rare, and it is unlikely that these mutations would account for a proportion of breast cancers in the absence of their respective syndromes.[10,11]

Overall, high-risk genes account for about 25% of inherited breast cancers (Figure 1).[12] Variants in genes function-

ally related to BRCA1/2 in DNA repair pathways confer an intermediate risk of breast cancer. These variants are rare, occurring in less than 1% of the population, and their contribution to the risk of breast cancer is less than 5% (Figure 1).[13] Recently, a third class of low-penetrance susceptibility alleles has been identified. These alleles, which may occur in genic or nongenic regions, confer a lower risk but are very common in the population.[13] Due to their low penetrance, the real contribution of these common variants to breast cancer risk is not entirely clear (Figure 1). Overall, this scenario suggests that the majority of genetic factors involved in breast cancer susceptibility are still unknown.
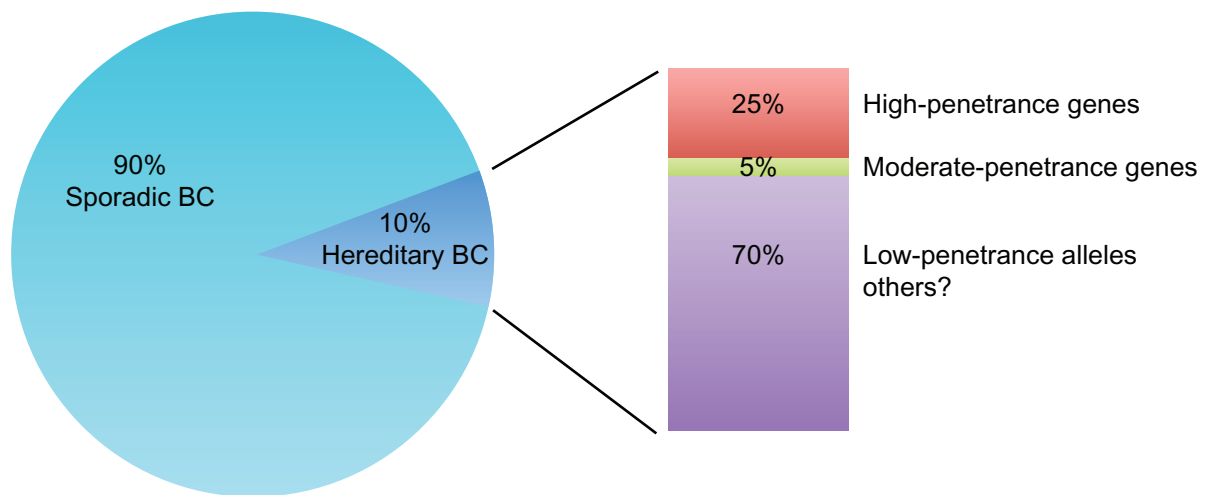
High-penetrance breast cancer genes

BRCA1 and BRCA2 are the most important breast cancer susceptibility genes in high-risk families (Table 1). BRCA1/2 mutations are considered to be responsible for approximately 30% of breast cancer cases with a family history of breast/ ovarian cancer, and it has been estimated that inherited BRCA1 and BRCA2 mutations account for 5%–10% of the total percentage of breast cancer.[14–16]

In women, germ-line BRCA1 and BRCA2 mutations confer a high risk for developing breast cancer by age 70 years. Initial studies based on multiple-case families, reported a female breast cancer risk at age 70 years in BRCA1 and BRCA2 mutation carriers of 85% and 84%, respectively.[17] Later meta-analyses showed that the average cumulative female breast cancer risk in BRCA1 mutation carriers by 70 years of age, unselected for family history, was 46%–65% and the corresponding estimates for BRCA2 were 43%–45%.[18,19]

In male breast cancer cases, BRCA2 mutations are much more common than BRCA1. Mutations in the BRCA2 gene are estimated to be responsible for 60%–76% of male breast cancers occurring in high-risk breast cancer families, whereas the BRCA1 mutation frequency ranges from 10% to 16%.[17,20] In a large population-based male breast cancer series, we reported a mutation frequency of about 7% and 2% for BRCA2 and BRCA1, respectively.[21] Interestingly, a founder effect was observed in BRCA1-associated male breast cancer cases.[22]

All known BRCA1/2 mutations are recorded in the Breast Information Core database (http://www.nhgri.nih. gov/Intramural_research/Lab_transfer/Bic/). To date, 1643 distinct germ-line BRCA1 mutations and 2015 BRCA2 mutations have been reported in the database. The great majority of BRCA1/2 mutations in breast cancer are predicted to truncate

Figure 1 Genetic susceptibility in hereditary breast cancer. Up to 10% of all breast cancers are caused by inherited germ-line mutations in breast cancer susceptibility genes. High-penetrance genes (*BRCA1* and *BRCA2*) contribute to 25% of hereditary breast cancer, moderate-penetrance genes (*CHEK2, ATM, PALB2, BRIP1, RAD51C*) contribute less than 5% to the risk of breast cancer. The great majority of hereditary breast cancer may be due to common low-penetrance alleles or other still unknown genetic factors. **Abbreviation:** BC, breast cancer.

the protein product. The most common type of mutations are small frameshift insertions or deletions, nonsense mutations, or mutations affecting splice sites resulting in a deletion of complete or partial exons or insertion of intronic sequences. The Breast Cancer Linkage Consortium has reported that approximately 70% of *BRCA1* mutations and 90% of *BRCA2* mutations in linked families are truncating mutations.[4] In addition to truncating mutations, an elevated number of missense variants has been identified. The most frequent are the *BRCA1* G61C in the RING-finger codon and the *BRCA2* I2490T in exon 15.

Some studies also indicate that *BRCA1/2* polymorphic variants could be associated with an increased risk of breast and ovarian cancer.[23,24] Association between the *BRCA2* N372H variant and increased breast cancer risk in particular has been reported from population-based studies.[25] Interestingly, we observed an association between the *BRCA2* N372H variant and risk of male breast cancer in young patients.[26] Breast cancer risk in women is influenced by the position of the mutation within the gene sequence. Women with a mutation in the central region of *BRCA1* were shown to have a lower breast cancer risk than women with mutations outside this region. *BRCA2* mutations located in the central region, referred to as the ovarian cancer cluster region, also appear to be associated with a lower breast cancer risk and a higher ovarian cancer risk than other *BRCA2* mutations.[27,28]

Specific *BRCA1* and *BRCA2* mutations show a high frequency in specific countries or ethnic groups, particularly in genetically isolated populations. These mutations descend from a single founder. For example, two founder mutations in *BRCA1* (185delAG and 5382insC) and one in *BRCA2* (6174delT) account for the majority of all *BRCA1/2* mutations (>90%) in the Ashkenazi Jewish population.[29] *BRCA1* 185delAG is present in about 1% of Ashkenazi Jews and in 20% of Ashkenazi women affected by breast cancer before

Table 1 Classes of genetic susceptibility and comparison of their different features

	High-penetrance	Moderate-penetrance	Low-penetrance
Genes	*BRCA1, BRCA2*	*CHEK2, ATM, PALB2, BRIP1, RAD51C*	10q26.13 (FGFR2), 2q33 (CASP8), 5q11.2 (MAP3K1), 11p15.5 (LSP1), 16q12.1 (TNRC9), 6q25 (ESR1), 14q24 (RAD51L1), 2q35, 8q24, 5p12, 1p11
Population frequency	<0.1%	MAF < 2%	MAF > 10%
Cancer risk (odds ratio)	>10.0	>2.0	1.1–1.6
Population attributable risk	Small	Individually small	High
Functional effect	Direct effect of mutation	Direct effect of variant	Linkage disequilibrium with causal variants
Strategy for identification	Linkage and positional cloning; resequencing of candidate genes	Resequencing of candidate genes	Case-control studies; genome-wide association study

the age of 50 years.[18,30] A single *BRCA2* mutation (999del5) has been found in the majority of multiple-case breast cancer families in the Icelandic population.[31,32] The *BRCA2* 999del5 accounts for about 8% of female breast cancer, rising to 24% of female breast cancers before the age of 40 years, and for about 38% of male breast cancers.[32,33] In Italy, a historically and genetically heterogeneous country, *BRCA1/2* founder mutations are found in small isolated geographic areas. The *BRCA1* 5083del19 was found in a geographically homogeneous population from Calabria, a region of Southern Italy, where it accounts for 33% of overall gene mutations.[34] A high frequency of *BRCA1* 5083del19 mutation has also been identified in the population of Sicily, a region near to Calabria.[35] Other regional founder mutations have been reported in Tuscany (*BRCA1* 1499insA, *BRCA1* 3347delAG), a region in Central Italy of ancient settlement.[21,22,36]

In addition to point mutations, small deletions and insertions, large-scale *BRCA1/2* rearrangements, including insertions, deletions, or duplications of more than 500 kb of DNA, have been identified in both male and female breast cancer.[37–40] Large genomic rearrangements may account for 3%–15% of all *BRCA1* and *BRCA2* mutations.[38] The higher density of *Alu* repeat sequences in *BRCA1* and both *Alu* and non-*Alu* repetitive DNA in *BRCA2* are thought to contribute to the large number of deletions and duplications observed in these genes.[37,40–43] The frequency of large *BRCA1* genomic rearrangements in families with a strong family history of breast and/or ovarian cancer, varies greatly (0%–36%) in different populations.[37–39] The frequency of large *BRCA2* genomic rearrangements seems to be lower (1%–2%) in comparison with *BRCA1*.[41,44] Interestingly, large genomic rearrangements in *BRCA2* are more frequent in families with male breast cancer.[38,43]

Moderate-penetrance breast cancer genes

Overall, fewer than 10% of breast cancers are attributable to known mutations in the breast cancer susceptibility genes, *BRCA1* and *BRCA2*.[13] Recently, direct interrogation of candidate genes involved in *BRCA1/2*-associated DNA damage repair pathway has led to the identification of other breast cancer susceptibility genes, classified as moderate-penetrance genes (Table 1). Variants found in this class of genes confer a smaller risk of breast cancer than *BRCA1/2* and, because of their rarity, are very difficult to detect in the population. Overall, mutations in moderate-penetrance genes account for less than 3% of the familial risk of breast cancer.[13]

CHEK2 1100delC was the first moderate breast cancer risk allele identified and was associated with a twofold risk among breast cancer cases unselected for family history and fivefold among familial breast cancer cases.[45,46] The *CHEK2* 1100delC mutation has also been shown to confer approximately a tenfold increase in breast cancer risk in men lacking *BRCA1/2* mutations, and was estimated to account for 9% of familial high-risk male breast cancer cases.[45] However, this association is not so evident in male breast cancer series unselected for family history, in which it was reported that the *CHEK2* 1100delC is unlikely to account for a significant proportion of male breast cancer cases.[47–50] The contribution of the *CHEK2* 1100delC mutation to breast cancer predisposition in both genders varies by ethnic group and from country to country. A decreased frequency of the 1100delC allele in North to South orientation has been observed in Europe both for male and female breast cancer.[50–53] Identification of the *CHEK2* 1100delC mutation as a breast cancer-associated allele induced mutational screening of the whole *CHEK2* gene sequence. However, at present, only a small number of rare truncating mutations and missense variants have been reported in breast cancer cases.[54,55]

ATM was first proposed as a breast cancer predisposition gene by epidemiological studies that reported an increased breast cancer risk in relatives of patients with ataxia telangiectasia, a recessive syndrome caused by mutation in the *ATM* gene.[56,57] However, molecular data corroborating this observation were provided after 20 years.[58] To date, many truncating splice site mutations and missense variants for *ATM* have been found and associated with a relative risk of breast cancer of about 2.4.[58] Currently there are no data about the role of *ATM* in men predisposed to breast cancer.

The involvement of *BRCA2* in the Fanconi anemia pathway promoted mutation screening of other Fanconi anemia genes functionally linked to *BRCA2*, such as *PALB2*, *BRIP1*, and, more recently, *RAD51C*.[59] *PALB2* truncating mutations were estimated to be associated with a 2.3-fold increased risk.[60] *PALB2* mutations have now been identified in many countries, with frequencies varying from 0.6% to 2.7% in familial breast cancer cases.[61–69] Two founder *PALB2* mutations, 1592delT and 2323C > T, were respectively identified in 1% of Finnish and 0.5% of French-Canadian breast cancers unselected for family history.[70,71] Interestingly, *PALB2* mutations were found in families with both female and male breast cancer cases, suggesting that *PALB2* may be involved in male breast cancer risk.[60,67] To date, five studies

have reported on the frequency of *PALB2* mutations in male breast cancer.[70,72–75] Overall, *PALB2* seems to have a role as a moderate-penetrance gene in male breast cancer to a comparable extent as for female breast cancer. Recently, it has been reported that *PALB2* heterozygote mutation carriers were four times more likely to have a male relative with breast cancer.[76]

Deleterious *BRIP1* mutations were initially estimated to confer a twofold increased breast cancer risk and to account for about 1% of *BRCA1/2* negative familial/early-onset breast cancer cases, but further studies suggested that the *BRIP1* contribution to breast cancer susceptibility might be more limited than initially reported.[77–80] Indeed, a total of only eight *BRIP1* truncating mutations in 11 *BRCA1/2* mutation-negative breast cancer cases from three independent studies have been identified worldwide.[77,81,82] Several studies in diverse populations failed to detect truncating mutations.[78–80] To date, only one study has investigated the role of *BRIP1* in male breast cancer susceptibility, and no evidence was found that germ-line variants in *BRIP1* might contribute to male breast cancer predisposition.[83] Taken together, these data suggest that the contribution of *BRIP1* to breast cancer predisposition in both females and males is less consistent compared with other moderate breast cancer susceptibility genes, such as *CHEK2* and *PALB2*.

Recently, mutations in *RAD51C*, another gene associated with Fanconi anemia, were identified as breast cancer susceptibility alleles, accounting for 1.3% of female patients from families with at least one case each of breast and ovarian cancer.[84] However, further studies did not confirm this frequency.[85,86] At present, there is no evidence that *RAD51C* mutations contribute to male breast cancer susceptibility.[87]

Low-penetrance breast cancer genes

A polygenic model, in which many genes that confer low risk individually act in combination to confer much larger risk in the population, has been suggested for susceptibility to breast cancer and other common cancers.[88] Breast cancers unaccounted for by currently known high-penetrance and moderate-penetrance breast cancer susceptibility genes can be explained by this model. This hypothesis, speculated for years, has only recently been confirmed by multigroup collaborations working in genome-wide association studies performed in a very large series of cases and controls from different countries, in order to increase the power to detect small effects on risk.[89,90] Common low-penetrance breast cancer susceptibility single nucleotide polymorphisms have

thus far been reported in regions that cover known protein-coding genes, including *CASP8*, *FGFR2*, *TNRC9*, *MAP3K1*, *LSP1*, *RAD51L1*, and *ESR1* and in regions such as 8q24, 2q35, 5p12, and 1p11 with no known protein-coding genes (Table 1).[90–95] The relative risk conferred by these alleles ranges from 1.07 to 1.26. Overall, these single nucleotide polymorphisms are estimated to account for less than 4% of the familial risk of breast cancer in women.[90] Interestingly, many of the alleles are in intronic portions of genes, and often are noncoding regions that may confer susceptibility. This might be explained by the observation that some of these loci are located in regions of linkage disequilibrium that cover different genes, but it is very difficult to establish which of a set of variants in linkage disequilibrium is the most functionally relevant.[96] Furthermore, some of these single nucleotide polymorphisms, including *CASP8*, *FGFR2*, *TOX3*, and *MAP3K1*, could act as modulators of the risk conferred by mutations in the high-penetrance breast cancer susceptibility genes, *BRCA1* and *BRCA2*.[97] Recently, a subtle regulatory effect of one allele in the prostate/breast cancer-associated 8q24 block was also demonstrated, which acts as a *cis* enhancer of the *MYC* promoter.[98] Interestingly, different haplotype blocks within 8q24 were specifically associated with risk of different cancers.[99] In particular, four blocks were site-specific (one for breast cancer and three for prostate cancer), and a fifth was a multicancer susceptibility marker because it was associated with a risk of various cancers, including prostate, colon, ovarian, kidney, thyroid, and laryngeal cancer, but not breast cancer.[99–101] None of the presently identified loci is directly linked to the DNA repair pathway. Instead, many of the coding loci are in genes somatically mutated in diverse cancers, including breast cancer. Recently, it was observed that genetic germ-line variations in genes encoding for "driver kinases" may influence breast cancer risk, thus suggesting that low-penetrance alleles might be a link between germ-line and somatic alterations in breast cancer.[102]

Specific single nucleotide polymorphisms seem to be associated with specific clinicopathological features. In particular, loci at *FGFR2*, *MAP3K1*, and 2q35 were found to associate specifically with estrogen receptor-positive breast cancer.[92,103,104] Data are still limited for less common tumor subtypes, such as estrogen receptor-negative or basal-like breast tumors. Whether these loci are associated with the risk of breast cancer in males has not yet been investigated, but an involvement of low-penetrance alleles in male breast cancer susceptibility cannot be excluded and warrants ad hoc studies.

Acquired alterations in breast cancer

Breast cancer development and progression is a multistep process resulting from the accumulation of genetic alterations, such as mutations and copy number variations, and also epigenetic alterations, such as promoter methylation, resulting in aberrant gene expression. The increasing number of deregulated genes subsequently affects important cellular networks, such as cell cycle control, DNA repair, cell adhesion or migration, and differentiation, driving normal breast cells into highly malignant derivatives with metastatic potential. Such alterations can result either in inactivation of tumor suppressor genes (eg, *TP53*, *BRCA1*) or activation of protoncogenes (eg, *PIK3CA*, *MYC*), both of which contribute to the malignant state of a transformed cell. Recent landmark studies have shed new light on the genomic landscape of breast cancer. Within a breast tumor there are many infrequently mutated genes and a few frequently mutated genes, resulting in incredible genetic heterogeneity.[105,106] The great majority of somatic mutations frequently lie in hotspot regions that might represent targets in cancer therapy. Both genetic and epigenetic alterations are also frequently associated to specific biological and clinicopathological tumor characteristics, allowing the identification of personalized therapies targeting the associated molecular pathways.[107–116]

Genetic alterations in breast cancer

A number of gene and chromosome alterations have been identified in sporadic breast carcinomas. Indeed, the great majority of breast cancer cases are due to solely somatic genetic alterations without germ-line ones.[117,118] A heterogeneous set of somatic alterations, including gene amplification, deletion, mutations, and rearrangements, were reported to be involved in the etiology of breast cancer.[6] The amount of information on these alterations has been dramatically increased by the introduction of high-throughput molecular cytogenetic approaches. Using large-scale approaches, the sequence of about 18,000 genes has been analyzed in breast cancer cases, and it has been reported that about 10% of these had at least one nonsilent mutation.[105] The great majority of alterations are single base substitutions (about 90%), with a prevalence of missense changes (60%, Figure 2). The remainder are somatic mutations resulting in stop codon or splice site alterations, and only a few of these are insertions, deletions, or duplications[105] (Figure 2). Somatic mutations found in cancers can be subdivided mainly into two biological classes, ie, "driver" and "passenger" mutations. Driver mutations confer proliferative advantage to tumor cells and
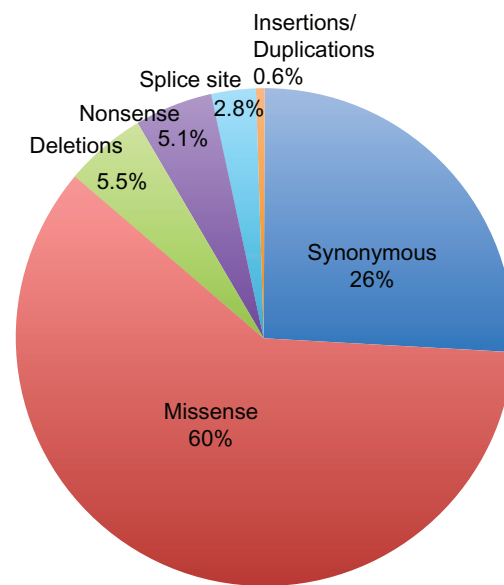
Figure 2 Somatic mutations in breast cancer. The great majority of somatic mutations are single base substitutions, mainly missense mutations; missense changes account for about 60% of somatic alterations; the remaining somatic mutations result in stop codon (5.1%) or in alterations of splice site (2.8%) and only a few percentages of these are insertions, deletions, or duplications (5.6%).

are positively selected during cancer development. Passenger mutations are present in the tumor progenitor cells, are biologically neutral, and do not confer a growth advantage.[105]

Several studies have shown a bimodal distribution of mutations in breast cancer. It has been proposed that the genomic landscape of breast cancer consists of "mountains" and "hills," where the mountains correspond to the most frequently mutated genes, specifically *PIK3CA* and *TP53*, and the hills consist of a much larger number of less frequently mutated cancer-associated genes (<5%).[105,106] Additional to mutations in *PIK3CA* and *P53*, alterations in several genes implicated in pathways involved in breast tumorigenesis, including the phosphatidylinositol 3-kinase/Akt and NF-kB pathways, have been also identified (eg, *IKBKB*, *IRS4*, *NFKBIA*, *NFKBIE*, *NFKB1*, *PIK3R1*, *PIK3R4*, *RPS6KA3*, *MAP3K1*, *AKT1*, and *GATA3* genes).[105,119,120] These genes could be considered the hills of the mutational landscape of breast cancer, because their mutation frequencies are lower than for genes considered to be the mountains.

Mutations of the *PIK3CA* gene are observed in 16%–40% of female breast cancers and in about 18% of male breast cancers.[109,121–124] *PIK3CA* mutations are associated with a positive estrogen receptor and progesterone receptor status, nodal involvement, and high histological grade, suggesting that they could be strong prognostic factors in breast cancer.[107–110] The great majority (85%) of *PIK3CA* mutations are in exons 9 and 20, encoding the helical and kinase

domains, respectively. The majority of mutations are located in two hot spot regions, including the central helical domain and the COOH terminal kinase domain. The three most common hot spot mutations lead to amino acid changes in the helical domain (E542K and E545K) and in the kinase domain (H1047R).[125] In particular E542K, E545K, and H1047R represent 3.6%, 6.2%, and 14.8% of the total *PIK3CA* mutations in breast cancer, respectively.[125]

Mutations of the *TP53* gene in breast cancer range from 15% to 71% among different populations.[126] More than 90% of *TP53* mutations reported in breast cancer are located in conservative regions within exons 5 to 8.[126,127] More than 2% of all *TP53* mutations are represented by three *TP53* hot spot mutations, ie, 273 (CGT > CAT), 158 (CGC > CTC), and 248 (CGG > CAG).[128] However, an overrepresentation of codon 163 (TAC > TGC) mutation has been observed in breast cancer. Indeed, this codon, rarely mutated in most cancers, accounts for over 2% of all breast cancer mutations. Interestingly, codon 163 is a hot spot for *TP53* mutation in breast cancer among *BRCA1/2* carriers.[127] Overall, *TP53* mutations are strongly associated with high histological grade, negative estrogen receptor status, increased global genomic instability, and germ-line *BRCA1* mutations.[111–113] At least 14 different common polymorphisms have been described in the *TP53* gene (IARC p53 data base www.p53.iarc.fr/p53main.html). The most common *TP53* polymorphic variants are the16 bp duplication in intron 3 (TP53PIN3) and the *TP53* G215C (Arg72Pro). There is some evidence of an association between TP53PIN3 and Arg72Pro variants and elevated breast cancer risk, although some studies suggest a neutral or protective effect for these polymorphisms.[129–131] Genotype and haplotype analyses of these two *TP53* polymorphisms also revealed that the presence of a specific haplotype carrying the consensus sequence for TP53PIN3 (allele without the 16 bp insertion), and the variant allele for Arg72Pro (72Pro) is associated with an earlier age at onset of breast cancer in *BRCA2* mutation carriers.[132,133]

In addition to nonsynonymous mutations arising from single nucleotide substitutions, several splice variants specific to breast cancer have been reported.[134,135] Interestingly, breast cancer-specific alternative splicing is not restricted to splicing defects resulting in loss of protein functions, and may also include modifications that generate proteins with new functions.[134]

Alternative splicing events can involve breast cancer-specific genes, such as *BRCA1*, *ESR2*, and *HER2*, or genes involved in cell cycle progression, DNA damage response, and spliceosome assembly.[134,136–138] The number of known

BRCA1 mRNA variants representing aberrant splicing products is relatively high.[136] The four predominant mRNA variants with a molecular weight lower than full length BRCA1 are Δ(9,10), Δ(9,10,11q), Δ(11q), and Δ(11). The variants that would be expected to differ the greatest at the functional level from the full length species are those lacking the largest exon 11, containing many functional domains involved in protein–protein interaction.[136]

Different *ESR2* (estrogen receptor β) splice variants have been identified, and studies on the function of some of these suggested that they might act as a dominant negative receptor in the estrogen receptor α and β pathways.[135,139]

A specific splicing variant of *HER2* (ΔHER2), which causes lack of exon 16 encoding the extracellular domain, has been identified in 9% of breast cancers overexpressing HER2 protein, suggesting that HER2 proteins carrying splicing variants may represent the oncogenic receptor population.[137,140]

Different alternative splicing events have been identified, in which changes in splicing correlate with estrogen receptor status and histological tumor grade.[134] Thus, analysis of alternative splicing might provide information about the biology of the tumor.

Genomic instability, such as gene copy number alterations and DNA amplifications, has also been observed frequently in breast cancer. The most commonly amplified regions in breast cancer include 8q24, 11q13, 12q14, 17q11, 17q24, and 20q13, with amplification of genes such as *HER2* (15%–20%), *EGFR* (14%), *MYC* (15%–20%), *CCND1* (15%–20%), *ESR1* (20%), and *EMSY* (7%–13%).[114,141,142] Amplification of these regions increases genetic instability in breast cancer and is generally associated with poor prognosis.[114]

HER2 and *EGFR* are members of the epidermal growth factor receptor family, and both genes are targets for copy number amplification in breast cancer.[143] Amplification of the *HER2* gene causes HER2 protein levels that are 10–100 times greater than normal. *HER2*-positive breast cancers are associated with a worse prognosis and resistance to hormonal therapy.[144,145] *EGFR* upregulation in breast cancer is not only due to gene amplification but often results from either high polysomy of chromosome 7 or transcriptional induction by the transcription factor YBX1 (Y box binding protein 1).[146] *EGFR* amplification is frequently associated with poor prognosis parameters in breast cancer patients, such as large tumor size, high histological grade, high proliferative index, and negative estrogen receptor status.[147,148] Moreover, increased *EGFR* gene copy numbers are observed

in triple-negative (estrogen receptor, progesterone receptor, and HER2 negative) breast cancers together with decreased *BRCA1* mRNA expression.[149]

MYC functions as a transcription factor, regulating up to 15% of all human genes. Although the relationship between amplification and overexpression is not clearly delineated, *MYC* amplification is significantly correlated with aggressive tumor phenotypes and poor clinical outcomes. *MYC* amplification is emerging as an important predictor of response to HER2-targeted therapies, and its role in *BRCA1*-associated breast cancers makes it an important target in basal-like/triple-negative breast cancers.[150]

Other two genes frequently amplified in breast cancer are *ESR1* and *CCND1*. Amplification of *ESR1* is associated with the expression of the estrogen receptor in breast cancer.[151] Overall, higher *ESR1* gene amplification is found in tumors with *CCND1* gene amplification in comparison with tumors without *CCND1* gene amplification.[151] *CCND1* amplification occurred preferentially in estrogen receptor-positive breast cancer and is associated with reduced overall survival.[152–155] Tumors which are sufficiently genetically unstable to develop one gene amplification have increased probability of developing multiple gene amplifications. The coamplification of one or several oncogenes, such as *EGFR*, *ErbB2*, *CCND1*, or *ESR1*, occurs commonly in breast cancer, and is reported in up to 30% of *CCND1*-amplified and up to 40% of *ErbB2*-amplified tumors.[114]

EMSY amplification in sporadic breast tumors has been shown to be associated with a poor prognosis.[141] Amplification of *EMSY* has been reported in sporadic breast cancer but not in *BRCA2*-associated breast cancer, suggesting that *BRCA2* mutations and *EMSY* gene amplification may be mutually exclusive.[156] Indeed, EMSY protein interacts with the transactivation domain of BRCA2, reducing its activity, and it has been suggested that amplification of *EMSY* can explain somatic *BRCA2* inactivation.[156]

Recently it has been reported that male breast cancers have a lower frequency of gene copy number alterations than female breast cancers.[157] Moreover, different chromosomal regions were found to be altered in male and female breast cancer. Male breast cancer alterations targeted Xp11.23 and 14q13.1 regions in more than 50% of cases. Shared amplified regions between male and female breast cancers are 8q24 (53%), 11q13 (50%), and 17q24 (30%), mapping for *MYC*, *EMSY*, and *HER2*, respectively.[117,157] Furthermore, *HER2* and *CCND1* gene amplification is observed in about 8% and 12% of male breast cancer cases, respectively.[158,159]

Epigenetic alterations

Epigenetic changes, in particular DNA methylation, are emerging as one of the most important events involved in breast cancer initiation and progression, and there is evidence that DNA methylation may serve as a link between genome and environment. Interestingly, factors that can be modulated by the environment, such as estrogens, elicit epigenetic changes (such as DNA methylation) and this could contribute to breast cancer risk. Furthermore, tumor-specific CpG island hypermethylation profiles are now emerging in breast cancer.[7] Tumor-related genes that become hypermethylated may play a significant role in breast cancer, including *BRCA1* and hormone response genes, such as estrogen, progesterone, androgen, and prolactin receptors.[160] Epigenetic silencing is one of the mechanisms by which mammary epithelial cells repress estrogen receptor expression, leading to the estrogen receptor-negative molecular subtypes of breast cancer.[161] *ESR1* methylation is more common in estrogen receptor-negative than in estrogen receptor-positive tumors, but there is no clear link between *ESR1* methylation and estrogen receptor status. It has been suggested that a heterogeneous *ESR1* gene methylation pattern may evolve during breast cancer progression and play a role in estrogen receptor-negative recurrences or metastases in patients with estrogen receptor-positive tumors.[162] Interestingly, breast tumors with *BRCA1* methylation show a high frequency of *ESR1* promoter methylation. *BRCA1* somatic mutations are extremely rare in sporadic breast cancer, but 9%–13% of these tumors reveal aberrant *BRCA1* methylation, especially when loss of heterozygosity occurs at the *BRCA1* locus.[160] *BRCA1*-associated breast cancers are generally basal-like tumors, and promoter methylation is one mechanism of *BRCA1* gene silencing in sporadic basal-like breast cancers. However, there is no significant difference in *BRCA1* methylation between sporadic basal-like breast cancers (14%) and matched sporadic nonbasal-like breast cancers (11%). *BRCA1* methylation also appears to be similar across distinct breast cancer molecular subtypes (14%–17%) including ductal, mucinous, and lobular breast cancers.[163,164]

Tumor-specific CpG island hypermethylation profiles are emerging, and the growing list of genes inactivated by promoter hypermethylation in breast cancer include genes involved in evasion of apoptosis (*RASSF1A*, *HOXA5*, *TWIST1*), in cell cycle control (*CCND2*, *p16*, *RARβ*), and tissue invasion and metastasis (*CDH1*). Tumor suppressor genes, such as *GSTP1*, *RIL*, *HIN-1*, *CDH13*, *APC*, and *RUNX3*, are frequently methylated in breast cancer tissues.[115,161,165] These genes are not only hypermethylated

in tumor cells, but show increased epigenetic silencing in normal epithelium surrounding the tumor site. Thus, methylation frequently represents an early event in breast cancer tumorigenesis. For example, *CCND2*, an important regulator of the cell cycle, has been frequently found to be methylated in breast cancer and is also methylated in ductal carcinoma in situ, suggesting that it may represent an early event in tumorigenesis.[161] Another gene frequently hypermethylated in breast cancer is *RASSF1A*. *RASSF1A* methylation is also an early epigenetic event in breast cancer and is found in ductal carcinoma in situ and in lobular carcinoma in situ.[160] Its diverse functions include regulation of apoptosis, growth regulation, and microtubule dynamics during mitotic progression.[161]

There is some evidence that DNA hypermethylation patterns can identify breast cancer subgroups having distinctive biological properties that could be used for prognostication and for prediction of response to therapy.[115,166,167] An association between methylation in five genes, including *RARb*, *CDH1*, *CCND2*, *p16*, and *ESR1*, and poor histological differentiation of breast cancer is frequently reported.[115,161] Furthermore, distinct epigenetic profiles can be identified when dividing breast tumors into groups based on hormone receptor status.[166,168] Differences in methylation status of the promoter region CpG islands of major breast cancer tumor-related genes, such as *RASSF1*, *CCND2*, *GSTP1*, *TWIST*, *RARb*, and *CDH1*, have been found relating to estrogen receptor and HER2 status.[115] In particular, methylation of these tumor-related genes resulted in significantly higher estrogen receptor-positive and HER2-positive breast tumors.[115,161] On the other hand, double-negative (estrogen receptor-negative, HER2-negative) breast cancers have significantly lower frequencies of *RASSF1*, *GSTP*, and *APC* methylation. Interestingly, epigenetic differences between estrogen receptor-positive and estrogen receptor-negative breast cancer arise early in cancer development and persist during cancer progression.[115]

Micro RNA

miRNAs are small noncoding, double-stranded RNA molecules involved in post-transcriptional regulation of target genes. Aberrant expressions of miRNA are associated with cancer progression, by acting either as tumor suppressor genes or oncogenes.[8,169] Much attention has been paid to deregulation of gene expression through the action of specific miRNA in breast cancer. Microarray studies demonstrated that overall miRNA expression could clearly separate normal versus cancerous breast tissue, with the most significantly

deregulated miRNAs being *mir-125b*, *mir-145*, *mir-21*, *mir-155*, and *mir−335*.[170–172] Interestingly, a large number of miRNAs, overexpressed or underexpressed in breast cancer, are predicted to regulate expression of key breast cancer proteins, such as BRCA1/2, ATM, PTEN, CHEK2, MLH1, P53, and ER.[169] Moreover, specific miRNAs, including miR-NAs that regulate genes involved in cell proliferation, such as *MAPK*, *RAS*, *HER2*, *HER3*, and *ESR1*, have been shown to play a direct role in male breast cancer development.[173,174] Indeed, cluster analysis of miRNA expression profiles reveals cancer-specific alterations of miRNA expression in male breast cancer distinct from female breast cancer, such as downregulation of miRNAs that suppress HOXD10, a protein involved in cell proliferation and migration, and vascular endothelial growth factor.[173]

Significant differences in miRNA expression profiles associated with molecular subtypes of breast cancer and correlated with specific clinicopathological factors, such as estrogen receptor, progesterone receptor, and HER2 status, emerged in breast cancer.[170] Thus, evaluation of the associations between miRNA expression profiles and clinicopathological characteristics may be important to identify distinct breast cancer subgroups and may lead to improvements in the clinical management of breast cancer patients. Indeed, some miRNAs, including *mir-21* and *mir-145*, have been shown to have potential clinical applications as novel biomarkers in the diagnosis and prognosis of breast cancer.[175,176] Moreover, miRNAs may act as strong inhibitors of cellular pathways via regulation of entire sets of genes, thus suggesting a possibly great potential for miRNAs in breast cancer prevention and therapeutics.[116]

Future research directions

Recent advances in technology have shed more light on the complexity of breast cancer biology and have provided data that allow risk estimation for patients with inherited mutations, prognostic and predictive determinations for patients with sporadic breast cancer, and targets for therapies. Recently, loci identified by genome-wide association studies have greatly expanded the list of genes associated with breast cancer risk.

However, evaluation of the functional consequences of low-penetrance alleles in breast cancer risk and their association with breast cancer molecular subtypes and clinicopathological characteristics are still challenging, but are needed for clinical application. Moreover, exploration of the polygenic model proposed for low-penetrance alleles requires further research in diverse and large populations. Overall, despite the

remarkable efforts made in recent years, much of the complex landscape of familial breast cancer risk remains unknown, suggesting the need for ongoing efforts in this field.[96]

The introduction of high-throughput molecular approaches has greatly increased the amount of information on the genomic landscape of breast tumors. Serial analysis of the cancer genome in different phases of its evolution might lead to improved management of the individual breast cancer patient.[118]

Moreover, studying the global methylation status as well as miRNA expression profiles of different types of tumors will allow the development of profiles unique for breast cancer and its subtypes, staging, and prognostic categories, leading to diagnostic applications and identification of new therapeutic targets.[116,171]

Conclusion

The identification of breast cancer susceptibility genes, in particular *BRCA1* and *BRCA2*, has changed the management of breast cancer patients with a family history of breast cancer. Several models have been developed, and are currently used to assess the pretest probability of identifying *BRCA1/2* germ-line mutations in individuals at risk for hereditary breast and ovarian cancer. Moreover, novel therapeutic strategies specific for *BRCA1* and *BRCA2* cancers are emerging, including crosslinking agents and poly ADP ribose polymerase inhibitors.[156]

Both genetic and epigenetic acquired alterations are frequently associated with specific biological and clinico-pathological tumor characteristics, allowing identification of personalized therapies targeting specific molecular pathways. In particular, a number of compounds, including trastuzumab, lapatinib, and pertuzumab, are currently under clinical evaluation for HER2-targeted therapy.[177] However, the majority of HER2-overexpressing breast cancers do not respond to HER2-targeted therapy alone.[178] There is evidence showing that combination therapy involving the use of HER2 and endothelial growth factor receptor inhibitors, such as trastuzumab and lapatinib, may have promising results in breast cancer treatment.[178] Crosstalk between the endothelial growth factor receptor/HER2 and phosphatidylinositol 3-kinase/Akt pathways provides a rationale for combining anti-endothelial growth factor receptor/HER2 agents and inhibitors of phosphatidylinositol 3-kinase/Akt/mTOR in breast cancer. In addition, DNA methylation as well as miR-NAs are currently emerging as interesting candidates for the development of therapeutic strategies against breast cancer. In conclusion, elucidation of the inherited and acquired

genetic and epigenetic alterations involved in breast cancer has not only clarified the molecular pathways involved in development and progression of breast cancer itself, but may also have important clinical and therapeutic implications in the management of patients with breast cancer.

Disclosure

The authors report no conflicts of interest in this work.

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