Old and new concepts in histopathological characterization of familial breast cancer

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BRCA1- and BRCA2-deficient cells display genomic instability due to impaired DNA repair and may subsequently be predisposed to malignant transformation. Cancers arising in BRCA1 gene mutation carriers differ substantially from sporadic breast cancers of age-matched controls in their histopathological appearance. BRCA1-related breast cancers have been morphologically associated with poorly differentiated and medullary types, exhibiting triple negativity and ‘basal phenotype’. There are different types of mutations listed in Breast Cancer Information Core professional databases and most of them are small insertions or deletions. Moreover, the search for more pathological alterations has led to identification of missense mutations, intronic variant sequences and unclassified variants, reporting an unclear role in breast cancer susceptibility. We review the latest evidence regarding analysis of various mutations in BRCA1/2 genes and low-risk breast cancer susceptibility genes. Preliminary data from our laboratories indicate that biomarkers for invasiveness may lead to better characterization of familial breast cancers. Multivariate regression analysis has allowed us to select the best combination of markers to predict familial or hereditary breast cancers. We found that a marker signature comprising human epidermal growth factor receptor 2 (HER2) negativity, Na+/H+ exchanger regulatory factor 1 (NHERF1) negativity and BRCA1 positivity (designated ‘triple-biomarker’ signature) is frequently associated with familial breast cancer and promises to be a reliable test in its molecular characterization.

introduction

Breast cancer is still a major cause of death in women worldwide and represents an etiologically heterogeneous disease, with an intrinsic complexity in cellular–biomolecular profile and diversity in its responsiveness to treatment. Family history brings together genetic and environmental factors that may cause cancer [1]. Clinical evidence and large epidemiological studies have shown that up to 5%–10% of all breast carcinoma may be attributed to germline mutations in well-studied breast cancer susceptibility genes. These genes have been divided into high-risk and low- to moderate-risk susceptibility genes. BRCA1, BRCA2, PTEN, TP53, LKB1/STK11 and CDH1 belong to the high-risk breast cancer susceptibility genes. In contrast, a small group of tumors are secondary to mutations in the low- to moderate-risk breast cancer susceptibility genes, such as CHEK2, TGFβ1, CASP8 and ATM. The genes that have received a great deal of attention in breast cancer pathology are BRCA1 and BRCA2. Although these genes confer a high risk for breast and ovarian carcinoma. Most of the genetic variants described above have been identified in studies considering breast cancer as a single disease. It has been established that breast cancers arising in the carriers of BRCA1 and BRCA2 gene mutation differ from each other and from the sporadic breast cancers of age-matched controls in their histopathological and cytological appearance [2–4].

histopathological features

BRCA1 mutation-related breast carcinomas (i.e. arising in mutation carriers) have a distinct histopathological phenotype, probably due to expression of the basal-like phenotype [5]. Invasive ductal carcinoma not otherwise specified is the most common histological subtype in both hereditary and sporadic breast cancers, but certain subtypes do occur more frequently in hereditary breast tumors. For example, BRCA1 mutation-related tumors are often poorly differentiated medullary carcinomas [6]. Medullary carcinoma is characterized by the following features: (i) syncytial architecture; (ii) absence of tubular or glandular structures; (iii) complete histological circumscription; (iv) high nuclear grade characterized by vesicular nuclei with one or two conspicuous nucleoli and numerous mitotic figures; and (v) marked lymphoplasmacytic stromal infiltrate [7–8]. BRCA1 mutation-related breast cancers are known to have pushing or circumscribed margins, which
imparts an expansile growth pattern resulting in pushing, rather than infiltrating, the adjacent structures (Table 1). Moreover, vascular invasion is frequent in these BRCA1 germline mutation-related breast cancers, thereby accounting for the frequent hematogenous and lymphatic dissemination in these hereditary cancers [9]. This vascular peritumoral invasion could be a predictive factor for high risk, particularly in patients with familial BRCA1 and/or BRCA2-related tumors [10].

Although some reports indicate more frequent occurrence of tubular, lobular and pleomorphic lobular carcinomas in BRCA2 carriers [4, 11], no similarly defined phenotype has been described for this group of familial breast cancer patients. BRCA2 gene-related tumors usually show a ductal phenotype of no special type, with the frequency of estrogen receptor (ER) positivity similar to sporadic cases [4]. Furthermore, BRCA2 mutation-related tumors may show a more prominent lymphocytic infiltrate, foci of necrosis and pushing margins than in sporadic cases [7]. These features, however, are less consistently exhibited than in the typical medullary carcinoma of BRCA1-related breast cancers.

BRCA1-related breast cancers are often poorly differentiated carcinomas (grade 3) and tend to have higher mitotic counts, a greater degree of nuclear pleomorphism and less tubule formation than age-matched sporadic tumors [3, 6]. In contrast, BRCA2 tumors are moderately or poorly differentiated carcinomas (grades 2 and 3) without being more pleomorphic or having necessarily higher mitotic counts and they exhibit less tubule formation [12].

**Steroid receptors and HER2**

ER has become one of the most important prognostic and predictive markers for breast cancer [13]. The expression of ER is inversely correlated with tumor grade [14]. Because BRCA1- and BRCA2-associated tumors are overall of higher grade than sporadic cancers, they would be predicted to be more often ER negative. A number of studies have evaluated hormone receptor status in familial breast cancers: those with a BRCA1 mutation or a known familial BRCA1 mutation more frequently had ER- and progesterone receptor (PR)-negative tumors than control participants [15–17]. Studies of women in several different ethnic groups, including Ashkenazi Jewish, Japanese and Swedish, have demonstrated that BRCA1 mutation carriers were more likely to have ER-negative breast cancer [18–21]. In contrast, BRCA2-related tumors, even if of higher grade than sporadic age-matched controls, tend to have a similar frequency of ER- and PR-positive tumors [5] to sporadic cases [16]. BRCA1-related cancers are most often ER negative, indicating that ER negativity is intrinsic to such BRCA1 cancers and reflects the cell of origin of these tumors [22]. Hosey and colleagues [23] showed that BRCA1 protein regulates the synthesis of ER through binding to the ER-α gene promoter, ESR1. Liu and colleagues [24] proposed that BRCA1 may actually be required in the differentiation of ER-negative stem/progenitor cells to ER-positive luminal cells. This finding indicated that loss of BRCA1 may result in the accumulation of ER-negative breast stem cells, which are genetically unstable and more likely to undergo carcinogenesis.

A large number of studies have reported a lower percentage of human epidermal growth factor receptor 2 (HER2) positivity in BRCA1-related cancers compared with sporadic tumors unselected for family history [16]. HER2 expression in these tumors is lower than would be predicted on the basis of their histologic grade [21]. Similarly, HER2 overexpression is very infrequent in BRCA2-related carcinomas, with reported frequencies ranging from 0% to 3.7% [25].

**p53 and proliferative markers**

BRCA1- or BRCA2-deficient cells display genomic instability due to deficiency in the repair of DNA double-strand breaks by the conservative mechanism of homologous recombination [26]. A defective cell cycle checkpoint is then likely to further contribute to cancer predisposition and to facilitate growth of cells carrying genomic mutations [27].

The tumor protein 53 (p53), also known as transformation-related protein and guardian of genome stability and integrity, is an important key factor in the cellular response to DNA damage and its loss may, therefore, be a prerequisite for development of BRCA1-associated breast tumors [28]. Ample evidence has demonstrated that homozygous BRCA loss induces cell death by activating a p53-dependent checkpoint both in mouse embryos and in mammary epithelial cells [29] and that impairment of this checkpoint by p53 loss overcomes the cell-lethal effects of BRCA loss [30]. Tumorigenesis in carriers of germ-line mutations in BRCA1 and BRCA2 is frequently accompanied by the loss of the wild-type allele [31] implying that these proteins are tumor suppressors, and have a ‘caretaker’ role in the maintenance of genomic stability rather than acting as ‘gatekeepers’ in the regulation of cellular proliferation [32].

Breast tumors from human BRCA carriers have an increased frequency of TP53 mutations with respect to those found in
tumors from non-BRCA1/2 carriers [33, 34]. In particular, studies that sequenced the TP53 gene have found that 30%–68% of BRCA1-related tumors have TP53 mutations at the DNA level [15, 35, 36]. Usually, TP53 mutation can be evaluated indirectly by immunohistochemical analysis, where tumoral cells with p53 positivity represent cells with accumulation of dominant-negative mutant p53 protein [37]. However, cells that show p53 negativity by immunohistochemistry have either wild-type p53 or TP53 mutations that do not give rise to accumulation of mutant p53 [38]. Studies that analyze p53 immunohistochemically have found that 60%–77% of BRCA1 tumors stain positive for p53 [15, 35, 38]. The presence of deleterious TP53 mutations in most BRCA1-related breast cancers indicates that p53 loss of function is essential for BRCA1-associated tumorigenesis and this could be an explanation for their high tumor grade and high proliferation index [39].

With regard to the proliferation markers, high expression of Ki67, high mitotic index and c-myc amplification characterize BRCA1-associated tumors. The increased proliferative activity in cancers with reduced BRCA1 function seems to indicate that BRCA1 may have a negative role in the regulation of cell proliferation. This is consistent with observations that expression of normal BRCA1 decreases the rate of cell proliferation [40] and that the reduction of normal BRCA1 expression increases the rate of cell proliferation [41]. In fact, Ki67 has recently been suggested as positively predictive of BRCA1-related status.

BRCA1-associated tumors, often ER negative, are usually negative for bcl-2 and cyclin D1, two ER-associated genes [42]. In contrast, they overexpress proteins that promote cell cycle progression, such as cyclin E, A or B1 [22, 43, 44]. In BRCA2 mutation-related tumors, the expression of cell-cycle-related proteins is similar to what is observed in sporadic ER-positive tumors with respect to cyclins A, B1, D and E, which promote cell cycle progression [44]. Expression studies on apoptosis markers have demonstrated that BRCA1-mutated tumors have increased expression of apoptosis-inducing genes and decreased expression of apoptosis-suppressing genes. Specifically, BRCA1 mutation-related tumors showed down-regulation of bcl2 but high levels of caspase 3 [25]. In contrast, overexpression of bcl-2 and BAX were present in BRCA2 mutation-related tumors, concordant with the correlation between these markers and ER status [45, 46].

**basal phenotype**

Recent gene expression profiling of breast cancer has identified specific subtypes with clinical, biological and therapeutic implications. The basal-like group of tumors is characterized by an expression signature similar to that of the basal/myoepithelial cells of the breast and is reported to have transcriptomic characteristics similar to those of tumors arising in BRCA1 germline mutation carriers [47]. This approach resulted in the identification of four to six molecular subtypes of breast cancer (two or three luminal types, HER2-overexpressing, normal breast-like and basal-like). Basal-like breast carcinomas (BLBCs) differ from those expressing ER and HER2, which represent two major determinants of breast cancer molecular subgroups. Sørlie et al. [48] using unsupervised hierarchical clustering, demonstrated the establishment of a breast cancer classification system identifying five distinct breast cancer molecular subgroups. These five subtypes are luminal A, luminal B, normal breast-like, HER2-overexpressing and basal-like subtypes. Each subtype expresses a distinct set of genes, imparting in the last two subtypes an aggressive clinical course and a poor outcome [48–50]. Subsequently, conventional histopathological and molecular analyses have demonstrated that familial BRCA1 mutation-related tumors have a basal-like phenotype [49, 51].

Morphologically, BLBCs often show increased mitotic count, geographic necrosis, stromal lymphocytic infiltrate and pushing margins. However, there is a great deal of morphologic overlap with other subtypes of breast cancer. Some investigators defined BLBC as expressing at least one of the basal cytokeratins (CK5, CK14 and CK17) [52, 53]. Others have included only lack of expression of ER, PR and HER2 and expression of one basal CK, epidermal growth factor receptor (EGFR) and/or c-kit [54].

**BRCA1** mutation-related breast cancers show a triple-negative phenotype that is ER negative, PR negative and HER2 negative [55]. Recent data indicate a model whereby BRCA1 and c-myc form a repressor complex on the promoters of specific basal genes, thus representing a potential mechanism to explain the overexpression of key basal markers observed in BRCA1-deficient tumors [56]. The proportion of BRCA1-related cancers with basal phenotype has been estimated to be 88% by Foulkes [57] and 57% by Lakhani et al. [12]. Conversely, triple-negative breast cancer has been reported to occur in only ~15% of sporadic breast cancers [58]. Basal-like phenotype is only occasionally found in BRCA2 carcinomas—these tend to be ER/PR positive at a frequency similar to that of sporadic cancers [2].

**new biological markers**

In contrast to germline mutations in BRCA1, BRCA2, TP53 and PTEN genes, mutations in other genes such as STK11, CHEK2 and ATM account for a small proportion of hereditary breast cancer syndromes, often with distinct clinical features. However, scanty and contradictory data exist on the morphological phenotype of tumors from carriers of these low-risk genes. For example, while some studies report a higher frequency of unfavorable features such as larger size and higher grade in patients carrying CHEK2 mutations [59], others have found a lower grade but positive association with higher risk for contralateral breast cancer and worse disease-free but not overall survival in these patients [60]. The histopathology of breast cancers in ATM variant-only carriers is not significantly different from controls, and known features of BRCA1 mutation-associated cancer are rarely seen, except for the small group of cases with a pathogenic BRCA1 mutation.

Several studies have found that the frequency of mutations in ATM, BRIP1, PALB2 and CHEK2 are many times greater for cases with a strong family history than for controls. For ATM, BRIP1 and PALB2, this relative frequency was higher by about sevenfold or more [61–63] and the CHEK2 1100delC mutation
is found in 5.1% of familial cases compared with 1.1% of controls [64]. The search for more pathological alterations led to the analysis of more mutations in \textit{BRCA1/2} genes particularly in unclassified variants, single-nucleotide polymorphisms (SNPs) and intronic variants. Recently, Pilato et al. [65] reported that the risk of SNP \textit{BRCA1 K1183R} for breast cancer is significantly more frequent in mutated families; conversely, \textit{BRCA2 N372H} is more frequently present in breast cancer relatives of families without pathological \textit{BRCA} mutations. Furthermore, specific haplotypes are transmitted to all relatives such as \textit{BRCA1 871Len-1038Gly}, which is present in both \textit{BRCA}-mutated and unmutated families. In contrast, \textit{BRCA2 289His-991Asp-IVS14+53 C→T} is present only in BRCA\textit{X} families indicating a harmful role for this SNP. The study of Pilato underscores that the genetic screening of patients with familial breast cancer should also analyze for polymorphisms and intronic variants in all families because of their possible role as susceptibility markers [65].

An emerging role for \textit{BRCA1} is in regulating hypoxia-inducible factor-1\(\alpha\) (HIF-1\(\alpha\)) stability and in modulating expression of its downstream targets [66]. About 90\% of \textit{BRCA1}-related breast cancer show a significantly higher expression of HIF-1\(\alpha\) than found in sporadic controls.

Overexpression of HIF-1\(\alpha\) in hereditary breast cancer seems to be caused by hypoxia rather than by activation of oncogenes or inactivation of tumor suppressor genes [67], HIF-1\(\alpha\) is considered to be a central initiator of angiogenic activity in tumors by activating transcription of the \textit{VEGF} gene. The biological effects of VEGF are mediated by two related receptor tyrosine kinases, vascular endothelial growth factor receptors (VEGFRs) 1 and 2. VEGFR1 was originally thought to be a receptor specifically expressed in vascular endothelial cells, but some studies have shown that it is present in breast cancer cells, and it is significantly correlated with tumor cell survival and high metastasis risk [68, 69]. Also, we previously showed the switching of \textit{Na}+/\textit{H}+ exchanger regulatory factor 1 (NHERF1) from the membrane into cytoplasm in breast carcinogenesis [70]. The strong correlation with low vascularization and HIF-1\(\alpha\) indicates that NHERF1 expression may play an important role in driving metastatic progression by changing the tumor microenvironment [71].

Preliminary data from our laboratories evaluated the possibility of including invasiveness biomarkers for a better characterization of familial breast carcinomas, utilizing a prospective monoinstitutional series of familial and sporadic tumors. Among them, we found of interest the NHERF1 protein, which has been a long-standing subject of study by our laboratory [70, 72, 73]. On the basis of univariate analysis of all breast cancer cases, the absence of membranous NHERF1 (Figure 1) is demonstrated to be the only independent variable that predicted family history of breast cancer. These findings confirmed that NHERF1 cytoplasmic overexpression is associated with aggressive clinical parameters and unfavourable prognosis, as we previously reported [70]. Generali et al. [74] have shown that HIF-1\(\alpha\) overexpression plays a critical role in sporadic breast carcinogenesis correlating with poor prognosis. HIF-1\(\alpha\) overexpression is seen at a much higher frequency in \textit{BRCA1}-related cancers than in sporadic cancers [67]. However, in contrast to van der Groep et al. [67] and Yan et al. [75], we did not observe a substantial difference between familial and sporadic cancers in HIF-1\(\alpha\) expression, perhaps attributable to differences in antibodies and a low presence of \textit{BRCA1-mutated} tumors in our series.

Multivariate regression analysis allowed us to select the best combination of markers to predict familiarity (Table 2). A highly predictive three-marker signature, out of five, has been found including HER2- and NHERF1-negative and \textit{BRCA1}-positive expression, identified as ‘triple biomarkers’. The triple-biomarker signature is frequently associated with patients with familial breast cancers, and promises to be a reliable identifier of a familial risk for breast cancer.

Although triple negativity is an aggressive phenotype of breast cancer, as previously reported [48, 49, 76], no significant difference has been identified in the behavior of breast cancer between the familial and sporadic settings. Interestingly, in agreement with Sørlie et al. [49] and Foulkes [57], we show that the subgroup of triple-negative phenotype present in familial breast cancers is significantly associated with \textit{BRCA1-mutated} cancers. However, triple negativity per se has not provided an improvement in identifying familial breast cancer. We hypothesize that the expression of biomarkers of invasiveness such as NHERF1, HIF-1\(\alpha\) and VEGFR1 may help to more reliably identify cases of familial breast cancer.

**conclusions**

We propose that prognostication of familial breast cancer can be further improved by utilizing the ‘triple biomarker’
consisting of negative HER2 and NHERF1 and positive BRCA1 expression that is more frequently associated with familial breast cancer. Therefore, this evaluation of invasiveness may also be useful in identifying and further classifying familial breast cancers. Furthermore, we are evaluating the possibility of utilizing the ‘triple-biomarker’ signature to select patients for BRCA1/BRCA2 gene sequencing.

Histopathological and molecular studies are defining special features that are particularly more prevalent in women who are mutation carriers of special features that are particularly more prevalent in women. 

Table 2. Multivariate analysis with breast cancer familiality (i.e. positive family history) as dependent variable from 187 microarrayed breast carcinomas

<table>
<thead>
<tr>
<th>Independent factors</th>
<th>OR</th>
<th>(95% CI)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>HER2: negative versus positive</td>
<td>4.486</td>
<td>(1.732–11.623)</td>
<td>0.002</td>
</tr>
<tr>
<td>Membranous NHERF1: negative versus positive</td>
<td>6.995</td>
<td>(1.692–28.916)</td>
<td>0.007</td>
</tr>
<tr>
<td>BRCA1: negative versus positive</td>
<td>0.387</td>
<td>(0.163–0.918)</td>
<td>0.031</td>
</tr>
<tr>
<td>ER: negative versus positive</td>
<td>2.710</td>
<td>(0.9303–7.895)</td>
<td>0.068</td>
</tr>
<tr>
<td>MIB1: negative versus positive</td>
<td>0.675</td>
<td>(0.291–1.567)</td>
<td>0.360</td>
</tr>
</tbody>
</table>

By multivariate regression analysis including HER2, membranous NHERF1, BRCA1, ER and MIB1 as independent factors, the best combination of predicting ‘familiality’ has been found to be the ‘triple biomarkers’: negative HER2 status (OR 4.486; 95% CI 1.732–11.623), negative membranous NHERF1 (OR 6.995; 95% CI 1.692–28.916) and positive nuclear BRCA1 (OR 0.387; 95% CI 0.163–0.918) significantly correlated with family history of breast cancer.

*P*-values were determined by the use of the logistic regression multivariate analysis.

OR, odds ratio; CI, confidence interval; ER, estrogen receptor; human epidermal growth factor receptor 2 (HER2); NHERF1, Na+/H+ exchanger regulatory factor 1.

disclosure

The authors declare no conflict of interest.

references


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