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Pathology of hereditary breast cancer

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Patients with germline mutations in BRCA1 or BRCA2 genes are predisposed to breast cancer. The BRCA1-associated breast cancers have distinct morphology, being more often medullary-like, triple negative and showing a 'basal' phenotype. On the other hand, BRCA2 and BRCAX cancers are a heterogeneous group without a specific phenotype. When incorporated into risk assessment models, pathology data improves prediction of carrier status. The role of BRCA1 and BRCA2 in DNA repair is being exploited to develop novel therapies, for example, using the poly-ADP-ribose polymerase inhibitors. A number of low-to-moderate-penetrant genes/loci have also been identified, but their role and contribution in breast cancer development is still under investigation.
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Breast cancer is the commonest malignancy in women and it is estimated that a million women worldwide will develop breast cancer each year. A number of risk factors have been identified including early menarche, late menopause, nulliparity and a positive family history.[1] First-degree relatives have an approximately twofold increase in risk of developing the disease. A number of highly penetrant breast cancer susceptibility genes have been identified and include BRCA1 and BRCA2.[2,3] These genes confer a high risk of breast and ovarian carcinoma. Two genes associated with rare cancer syndromes, P53 (Li–Fraumeni syndrome)[4] and PTEN (Cowden syndrome)[5] also confer a very high risk of breast cancer. Although all these genes confer a very high risk, they account for a relatively small proportion of inherited breast cancers. It has become increasingly clear that overall susceptibility to breast cancer is likely to be mediated through variants in many genes, each conferring a small-to-moderate risk of the disease. A number of such genes have been reported in the literature and include CHEK2, ATM, NBS1, RAD51, BRIP1 and PALB2.[6–10] It is not known how many more genes that confer a small risk are yet to be identified or how these genes come together or interact with each other or with environmental factors to increase the breast cancer risk.

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BRCA1, BRCA2 and BRCAX

The first high-penetrance gene, BRCA1, was isolated in the year 1994[2,11] and a year later, BRCA2 was localised and cloned.[3,12] Over the last decade, it has been shown that breast cancer arising in patients harbouring a germline mutation in BRCA1 and BRCA2 genes differs from age-matched sporadic breast cancer cases and from familial breast cancers arising in non-BRCA1/2 patients. These differences are in morphology, immunophenotype and molecular characteristics.[3,12–29] These differences tell us something about the biology of familial breast cancer, but could also potentially be used in cancer clinics to predict which patients may harbour BRCA1 germline mutation.

Cellular Functions of BRCA1 and BRCA2

BRCA1 has several cellular roles. It has been implicated in DNA repair, cell-cycle regulation, transcriptional regulation and chromatin remodelling. On the contrary, functions attributed to BRCA2 have mainly been restricted to DNA recombination and repair processes. BRCA2 has a role in the regulation of RAD51 activity. RAD51 is a highly conserved DNA recombinase, involved in the repair of double-strand breaks and arrested replication forks.[30]

Loss of BRCA1 or BRCA2 leads to a deficiency in the repair of DNA double-strand breaks by the conservative mechanism of homologous recombination (HR). This mechanism is an error-free mechanism when the exchange is between identical sister chromatids (or homologous chromosomes). Such a deficiency leads to non-conservative/error-prone, and potentially mutagenic, repair of DNA lesions by mechanisms such as non-homologous end joining and single-strand annealing. The ultimate genomic instability developed is then likely to contribute to the cancer predisposition generated by loss-of-function mutations in BRCA1 or BRCA2.[30]

Histological types

Histopathological analysis of hereditary breast cancers shows that the majority are ductal carcinomas of no-special type (IDC-NST); however, medullary carcinomas are overrepresented in patients with germline mutations in BRCA1.[22,23] Not surprisingly, these tumours have also been reported to have pushing margins and lymphocytic infiltrates more often than sporadic breast cancers (Figure 1a). Two papers with small number of cases suggested that lobular carcinoma, tubular carcinoma, tubulolobular carcinomas (collectively called the tubulolobular group)[27] and pleomorphic lobular carcinomas[13] were associated with BRCA2. This was not substantiated in a much larger study carried out on behalf of the Breast Cancer Linkage Consortium[23] that found an association of lobular carcinoma with non-BRCA1/2 (that is, BRCAX).[22] Bane *et al*[16] also did not find this association. However, there is some evidence showing that BRCA2 may have a role in the aetiology of some sporadic lobular carcinomas, as LOH at the BRCA2 locus has been identified by our group in a subset of pleomorphic lobular carcinomas.[31] Hence, unlike BRCA1, currently no specific morphological type has been associated with either BRCA2 or BRCAX, which seem to be morphologically heterogeneous.

Histological grade

Both BRCA1 and BRCA2 tumours are overall higher grade than sporadic breast cancers. BRCA1 tumours tend to have a higher score for all parameters of grade (tubule formation, pleomorphism, mitotic counts) (Figure 1b), whereas BRCA2 tumours appear to lack tubules and are not more pleomorphic or have high mitotic counts. BRCAX tumours are very similar to sporadic breast cancers.

Multivariate analysis of morphological features

Many of the morphological features described are associated with each other. Multivariate analysis has shown that features predictive of BRCA1 phenotype include pushing margins, lymphocytic infiltrate and high mitotic counts, but not the medullary phenotype *per se*; hence BRCA1 tumours are high-grade, medullary-like cancers. Note that not all BRCA1 tumours have this morphology, just that there is a higher frequency of such cancers in this group. In contrast, the only features found to be significant for BRCA2 were pushing margins and lack of tubule formation;[23] hence, BRCA2 does not have a specific phenotype.

Steroid receptors

BRCA1-associated breast cancers are more likely to be oestrogen (ER) and progesterone receptor (PgR) negative ($\sim$90%) compared with sporadic breast cancers ($\sim$30%) (Figure 1c and d). In contrast, the frequency of ER and PgR for BRCA2- and BRCAX-associated cancers is not significantly different to sporadic cancers.[14,19,24,25,28]

HER2 and TP53

Both BRCA1 and BRCA2 cancers rarely overexpress or show amplification of HER2 (Figure 1e). In contrast, BRCA1 tumours often express/have mutations in TP53, whereas this is not a feature of BRCA2 cancers.[25,28,29]

Other biological markers

BRCA1-associated cancers are often ER negative, and as would be expected, they are also usually negative for bcl2[32] and cyclinD1 (ER-associated genes), and do not show amplification of CCND1.[33] They do, however, overexpress p27 and cyclinE1[34] as other high-grade and 'basal'-like cancers. In a series of patients, amplification of the c-myc gene has been shown to be present in 18.2% of 20 tumours from BRCA1 mutation carriers and in 62.5% of 18 tumours from BRCA2 mutation carriers.[28] BRCA1-associated carcinomas have also been associated with the cell cycle proteins, namely, E2F6, cyclins A and B1, SKP2 and Topo-II-α. BRCA2 tumours were shown to have higher expression of the cell cycle proteins cyclin D1, cyclin D3, p27, p16, p21, CDK4, CDK2 and CDK1.[35]

Association with 'basal' phenotype

As BRCA1-associated cancers are often triple negative (ER−, PgR−, HER2−), it is not surprising that a high proportion show a 'basal' phenotype (expression of basal/myoepithelial markers such as CK5/6, CK14, SMA, EGFR, P-cadherin and caveolin 1)[17,22,36,37] (Figure 1f). This is not a significant feature of BRCA2/BRCAX-related breast cancers, in that, the frequency is not higher than in sporadic cancers. Conversely, the BRCA1 pathway seems to be altered

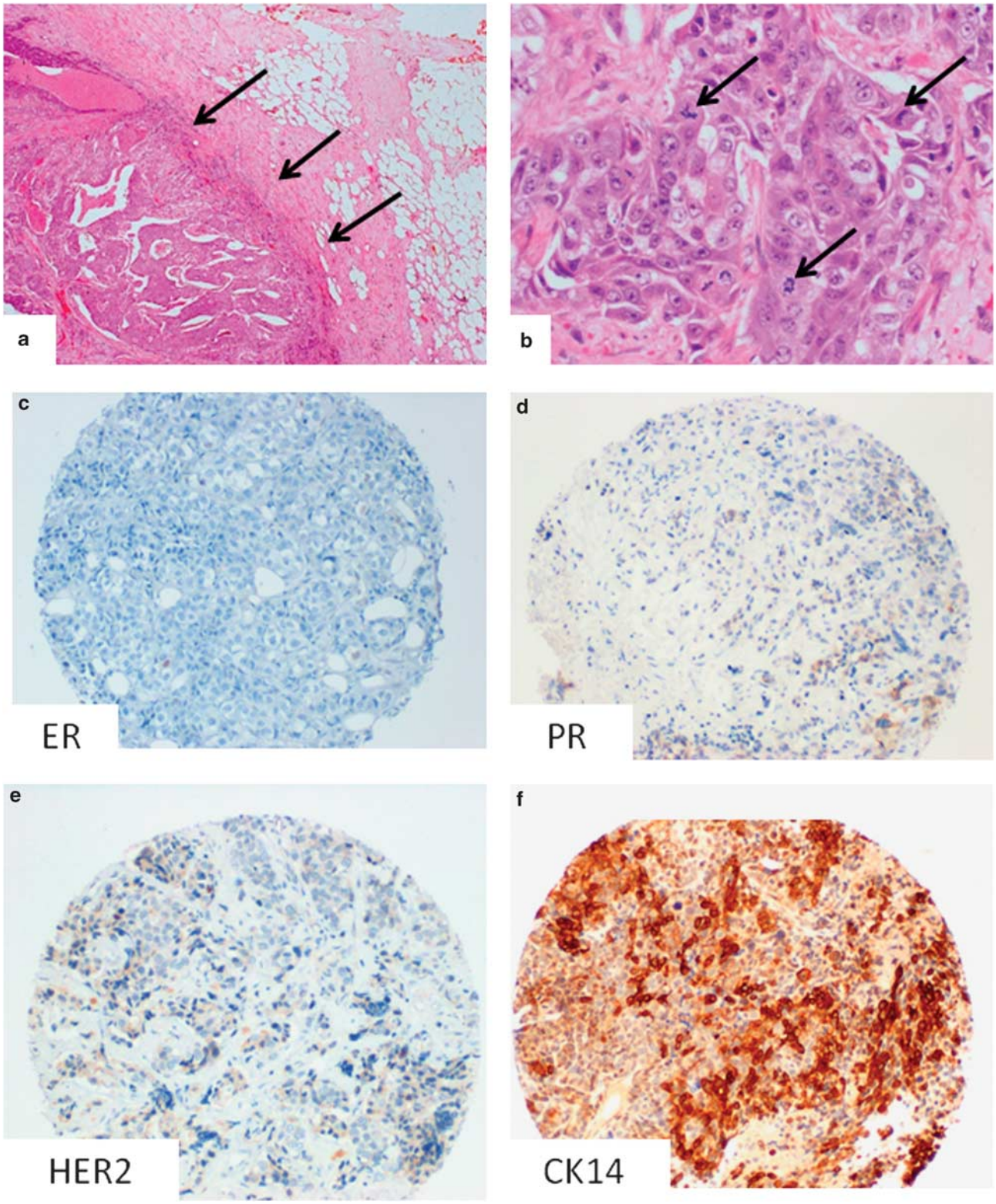

Figure 1 Example of a BRCA1 basal-like cancer: (**a**) Low power (× 40) view depicting the pushing border (arrows). (**b**) High magnification of tumour A (× 400) showing high-grade nuclei and mitotic figures (arrows); (**c–f**) cores of a single tumour from a tissue microarray of basal-like cancers showing negativity for oestrogen receptor (ER, **c**), progesterone receptor (PR, **d**) and HER2 (**e**) and positivity for Cytokeratin 14 (CK14, **f**).

in sporadic breast cancers with a 'basal' phenotype.[38] The trend of basal-like cancers to metastasise to brain,[39,40] which was also shown in patients with BRCA1 mutations, is also noteworthy.[41]

Can pathology aid clinical decision making?

Unlike BRCA2/BRCAX, the morphology of tumours associated with BRCA1 is fairly distinct, and it has been postulated that this may help to identify potential carriers of germline mutation and hence triage patients towards testing. Current risk estimation models are not very specific and accurate, and new parameters that aid this process would be advantageous. Although a recent paper suggested that the use of basal markers was not predictive of BRCA1 status,[42] this study had a very small data set and there are several studies that do show a role of morphology and immunohistochemistry in improving risk estimations for BRCA status, particularly in young women <40 years of age.[24,43,44]

Other predisposition genes

TP53, PTEN, CHEK2 and ATM

Hereditary breast cancers are also a part of a number of cancer syndromes including the Li–Fraumeni syndrome (TP53) and Cowden's syndrome (PTEN), which are caused by high-penetrant genes, but overall make a small contribution to familial breast cancer.[45,46] In contrast, CHEK2 and ATM are low-to-moderate-risk genes. Overall, there is little significant data relating pathology to these predisposition genes.[47,48]

SNP Polymorphisms

Recently, a number of polymorphisms at genetic loci have been identified through large-scale genome-wide association studies.[49] These include FGFR2, TNRC9, MAP3K1 and LSP1. It is not clear what the role of these genes is in breast cancer development, and hence nothing is currently known about the pathology or biology associated with these loci.

Unclassified BRCA1 and BRCA2 mutation variants

The BRCA1 and BRCA2 genes are frequently sequenced in kindred from families presenting with multiple cases of breast cancer, and as a consequence, pathogenic mutations are identified in some families. On the other hand, many rare sequence variants of unknown clinical significance are also reported. These rare missense substitutions and in-frame deletions of BRCA1 and BRCA2 genes pose a challenge for genetic counselling of individuals carrying such unclassified variants. Notwithstanding, variant classification has been shown to be improved by parallel analysis of oestrogen receptor, cytokeratin 5/6 and cytokeratin 14 tumour expression and use of updated methods that are able to estimate the clinical relevance of amino acid evolutionary conservation and position. This combination may assist genetic counselling of individuals with unclassified sequence variants.[50,51]

Therapeutic potential

It has become increasingly clear that BRCA1 and BRCA2, as well as a number of other predisposition genes, such as CHEK2 and ATM, have a role in DNA repair. In particular, BRCA1 and BRCA2 are involved in an error-free type of repair called HR.

Cells with loss of HR have been shown to be sensitive to DNA crosslinking agents such as the mitomycin-C and platinum-based drugs.[52] Further damage caused by these drugs in the absence of error-free repair leads to cell death. Data that these drugs may have an important role in managing patients harbouring these mutations are beginning to accumulate.

When HR is compromised, other error-prone repair mechanisms kick in—these include base excision and single-strand break repair. Counter intuitively, blocking these pathways in cells that have lost the BRCA function seems to be a good thing, as further damage caused by loss of the repair mechanisms leads to cell death. Poly-ADP ribose polymerase (PARP) is an enzyme involved in these pathways and PARP inhibitors look set to provide a novel way of targeting BRCA1 and BRCA2-associated breast cancers.[53,54]

Disclosure/conflict of interest

The authors declare no conflict of interest.

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