Reviews

Molecular genetics of solid tumours: translating research into clinical practice. What we could do now: breast cancer

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Abstract
Breast cancer is a common solid malignancy in women. Over the past decade, much progress has been made in understanding the biology of breast cancer. The use of molecular and immunohistochemical techniques is providing insights that will allow us to tailor the management of patients with breast cancer. In this review, progress in the understanding of lobular carcinoma in situ and atypical ductal hyperplasia, the use of the molecular marker CerbB2, and information gained from the morphological analysis of tumours arising in patients with BRCA1 and BRCA2 mutations is discussed.

(Keywords: breast cancer; lobular carcinoma in situ; atypical ductal hyperplasia; CerbB2; BRCA1 mutation; BRCA2 mutation; molecular markers)

There is compelling evidence that breast cancers arise in a multistep fashion through a series of intermediate “hyperplastic” and neoplastic lesions, each of which has a greater chance of becoming malignant than the one that preceded it.1 The reliable recognition of these stages is of great value in learning more about the pathogenesis and possible aetiology of human breast cancer, and in identifying women who are at increased risk of developing the disease.

The introduction of mammographic screening has led to increased detection of premalignant lesions, in particular ductal carcinoma in situ (DCIS).2 3 The classification of intraductal proliferations is controversial and pathologists encounter difficulties in subclassifying DCIS, differentiating it from atypical ductal hyperplasia (ADH), and distinguishing lobular carcinoma in situ (LCIS) from the solid variant of low nuclear grade DCIS. However, in situ carcinomas and atypical hyperplasias are not the only abnormalities thought to be precancerous, and some commonly encountered benign lesions also appear to be associated with increased cancer risk. There can be considerable difficulty in determining whether a particular lesion is premalignant and the degree of risk with which it is associated. Furthermore, it may not be possible to distinguish those that are genuine precursors from those that are merely associated with cancer and consequently markers or indicators of risk.

Lobular carcinoma in situ (LCIS)
LCIS was described in 1941 by Foote and Stewart,4 although it had been recognised as a precancerous lesion some years before.5 Most cases of LCIS are diagnosed between the ages of 40 and 50 years, a decade earlier than DCIS. LCIS is not generally palpable and there are usually no mammographic abnormalities.6 Hence, it is usually an incidental finding in biopsies done for an unrelated benign or malignant condition. It is often multifocal and bilateral. More than 50% of the patients with LCIS have further disease in the ipsilateral breast and approximately a third of patients will have LCIS in the contralateral breast.7 8 9

Although Page et al reported that approximately two thirds of women will develop invasive carcinoma within 15 years of follow up,10 other studies suggest a lower risk (20%), and most cancers do not appear until 15–20 years later.11 12 Interestingly, only half the invasive cancers are lobular in type, the rest being invasive ductal carcinoma. Unlike DCIS, the risk of invasive carcinoma after LCIS is bilateral.13 14

These features have raised questions about the biological nature of LCIS. Although originally described as an “in situ carcinoma”, with the implication that it was an invasive cancer in the making, the view has now changed to a “risk indicator”. Since the original description by Foote and Stewart,4 the trend has swung from mastectomy to follow up, follow up with regular mammography, and even “no action”.15

Although it is recognised that a proportion of women with LCIS will go on to develop invasive carcinoma, at present, there are no clinical or morphological features that allow identification of the women at risk. This has created a problem for surgeons and oncologists managing patients who have a diagnosis of carcinoma in situ but in whom the lesion has uncertain significance.

Although LCIS has been documented as an entity for nearly 50 years, molecular data are
limited. E-cadherin, an epithelial cell–cell adhesion molecule, is often found in DCIS and invasive ductal carcinoma, but is rarely seen in LCIS or invasive lobular carcinoma.\textsuperscript{14–17} Lakhani et al \textsuperscript{25} reported that loss of heterozygosity (LOH) involving chromosomal loci at high frequency in invasive carcinoma can also be detected in LCIS.\textsuperscript{18} The frequency in their study ranged from 8% on chromosome 17p to 50% on 17q. LOH on chromosome 16q, the site of the E-cadherin gene was approximately 30%. LOH was identified in LCIS with and without invasive carcinoma. This confirmed the neoplastic nature of LCIS and suggested that LCIS was probably a direct precursor of invasive cancer. Further support for this hypothesis has come from Nayyar et al,\textsuperscript{19} who showed LOH in 50% of LCIS associated with invasive carcinoma at markers on chromosome 11q13.

The most direct evidence for a precursor role of LCIS comes from mutational analysis of the E-cadherin gene. Bers et al found that 27 of 48 invasive lobular carcinomas had mutations in the E-cadherin gene, whereas none of 50 breast cancers of other types had alterations.\textsuperscript{20–22} The same group subsequently demonstrated that truncating mutations identified in invasive lobular carcinoma were also present in the adjacent LCIS, providing strong evidence that LCIS is a precursor lesion.\textsuperscript{22} There can be little doubt now that LCIS is a precursor of invasive cancer. Unfortunately, the data still do not allow us to stratify patients into meaningful groups for management. We still have no way of identifying the one in five women who need regular follow up or treatment for LCIS. The hope is that newer technologies, such as transcription profiling,\textsuperscript{23} will help us to achieve that aim.

**Atypical ductal hyperplasia (ADH)**

ADH is a proliferation that exhibits some but not all the morphological features of DCIS and hence, by definition, shares histological features with carcinoma. Follow up studies have confirmed the precancerous nature of ADH. Page and his colleagues conducted a series of important prospective studies in the 1980s. In one of these,\textsuperscript{24} they indicated that the relative risk of developing carcinoma in a woman with proliferative disease was 1.9 and this rose to 5.3 if the proliferation showed evidence of atypia. This risk was doubled in the presence of a positive family history of breast cancer. Subsequently, Tavassoli and Norris,\textsuperscript{25} McDivitt et al,\textsuperscript{26} and London and colleagues\textsuperscript{27} have confirmed the increased risk associated with atypical hyperplasia, although the size of the risk has varied between studies.

However, ADH is a controversial entity, which poses considerable difficulties in diagnostic pathology. To solve this problem, Page and Rogers\textsuperscript{28} laid down clear guidelines for diagnosis and a subsequent study by Schnitt et al,\textsuperscript{29} in which the Page and Rogers criteria were used, showed an improvement, with complete agreement in 58% of cases. Other studies, including those associated with the UK National and European Commission Quality Assurance Schemes (EQA), have revealed lower levels of agreement even among experienced breast pathologists.\textsuperscript{30–32} Lakhani et al demonstrated that LOH identified at loci on 16q and 17p in situ and invasive cancer is also present in ADH with a similar frequency.\textsuperscript{33} This indicates that ADH is a neoplastic proliferation and is likely to be part of the spectrum of in situ ductal neoplasia. There is support for this view in the literature from several other studies.\textsuperscript{34–36} O’Connell et al studied 51 cases of ADH at 15 polymorphic microsatellite loci and found LOH for at least one marker in 42% of the cases.\textsuperscript{37} The studies suggest that there is little difference between ADH and DCIS within the limits of current molecular investigations. The failure of EQA schemes to demonstrate reasonable agreement for this category, together with molecular data, which show pronounced overlap between ADH, and DCIS raise serious doubts about the validity of this diagnostic category. If future experiments do show that ADH is distinct from DCIS, more robust diagnostic criteria will have to be developed for use in clinical practice.

**CerbB2 oncogene and Herceptin**

The protooncogene CerbB2 (Her2/Neu) encodes a transmembrane protein, which has homology with epidermal growth factor receptor. CerbB2 is amplified in approximately 20% of invasive cancers and has received interest because of its association with lymph node metastases, short relapse time, poor survival, and decreased response to endocrine and chemotherapy.\textsuperscript{37–39} CerbB2 amplification is almost always associated with an increase in mRNA and protein expression. In contrast to invasive cancer, the CerbB2 protein has been identified in a high proportion (60–80%) of DCIS of high nuclear grade, comedo-type, but is not common in the low nuclear grade forms. Allred et al found that the expression of this protein is higher in invasive carcinomas associated with DCIS than in those without DCIS.\textsuperscript{40} It is very rarely expressed in LCIS.\textsuperscript{41–43} This gene product has not been identified in benign proliferative disease or ADH.\textsuperscript{44} This oncogene represents an excellent example of the translation of basic science to clinical practice. CerbB2 status predicts response to antioestrogen and cytotoxic chemotherapy. CerbB2 has attracted attention because of the availability of the humanised monoclonal antibody Herceptin for the treatment of breast cancer. Initial clinical trials suggest that it will have a useful role in the management of a proportion of breast cancers.\textsuperscript{45}

**Familial breast cancer**

A small proportion of breast cancers result from a heritable predisposition. Two predisposition genes, BRCA1 and BRCA2 have been cloned. The morphological features of tumours from patients with BRCA1 and BRCA2 mutations differ from each other and from sporadic breast cancers.\textsuperscript{46–48} Both are higher grade compared with sporadic cases. An excess of medullary/atypical medullary carcinoma has
been reported in patients with BRCA1 mutations. 40 41 BRCA1 associated tumours are more likely than sporadic cancers to be steroid hormone receptor (oestrogen receptor (ER) and progesterone receptor (PR)) negative, CerbB2 negative, and to have mutations in the p53 gene. 42 In contrast, BRCA2 tumours are not different from sporadic cancers in their ER, PR, or p53 status. The data derived from these studies, combining morphology and clinical data, have implications for clinical practice.

Grade is an independent prognostic indicator and is inversely related to outcome. 43 The higher grade of BRCA1 associated tumours should have a worse prognosis than non–BRCA1 mutation carriers. Paradoxically, there are data within the literature suggesting that familial cancer in general, and medullary carcinomas in particular, have a better prognosis than ordinary ductal carcinomas, no special type. 44–46 A recent study suggests that the disease free interval and survival is not different from patients with sporadic breast cancers. 46 In contrast, Foulkes et al have shown that the worse prognosis predicted by the higher grade is indeed correct. 47 Data on prognosis in patients with BRCA2 mutations are scanty but preliminary reports suggest a similar prognosis to sporadic cancers. The use of breast cancer screening mammography in familial cancer is a controversial issue. It is likely to be replaced by ultrasound or magnetic resonance imaging. Whichever method is used, the pathology data (high grade, with a very high mitotic count and high proliferative index) suggests that the screening interval will have to be smaller than the present three years if interval cancers are to be avoided. The role of BRCA1 as part of a DNA repair complex also raises questions about radiation exposure, although at present there is no convincing evidence that the amount of radiation received by the patient during the mammographic screening is important.

Finally, morphology might have a role in genetic counselling of patients. It has been estimated that patients who develop breast cancers between the age of 25 and 29 years, and who do not have an obvious history of breast cancer in the family, have a risk of approximately 6–7% for carrying mutations in the BRCA1 gene. 48 On the assumption that the odds ratio from the analysis of the morphological features is independent of age, a patient under 30 years who has a high grade tumour (grade II or III) and who is also ER negative (D Easton 2000, personal communication) would have a risk of approximately 40–45% of harbouring a mutation in BRCA1. In the absence of these features the risk would be 3–4%. Hence, the use of morphological and molecular features in addition to the clinical data may enhance the counselling of patients who are likely to harbour mutations in this gene.