Review

The pathology of familial breast cancer
Immunohistochemistry and molecular analysis

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Abstract

Extensive studies of *BRCA1*- and *BRCA2*-associated breast tumours have been carried out in the few years since the identification of these familial breast cancer predisposing genes. The morphological studies suggest that *BRCA1* tumours differ from *BRCA2* tumours and from sporadic breast cancers. Recent progress in immunohistochemistry and molecular biology techniques has enabled in-depth investigation of molecular pathology of these tumours. Studies to date have investigated issues such as steroid hormone receptor expression, mutation status of tumour suppressor genes *TP53* and *c-erbB2*, and expression profiles of cell cycle proteins p21, p27 and cyclin D_1. Despite relative paucity of data, strong evidence of unique biological characteristics of BRCA1-associated breast cancer is accumulating. *BRCA1*-associated tumours appear to show an increased frequency of *TP53* mutations, frequent p53 protein stabilization and absence of imunoreactivity for steroid hormone receptors. Further studies of larger number of samples of both *BRCA1*- and *BRCA2*-associated tumours are necessary to clarify and confirm these observations.

Keywords: *BRCA1*, *BRCA2*, immunohistochemistry

Introduction

In recent years, studies of familial breast cancers associated with mutations in *BRCA1* and *BRCA2* genes have suggested that these tumours have a characteristic histopathological phenotype [1••,2•]. Although tumours associated with *BRCA1* and *BRCA2* are on average of higher grade than sporadic breast cancer, there are also differences between tumours associated with the two genes. These data suggest distinct pathways for tumourigenesis.

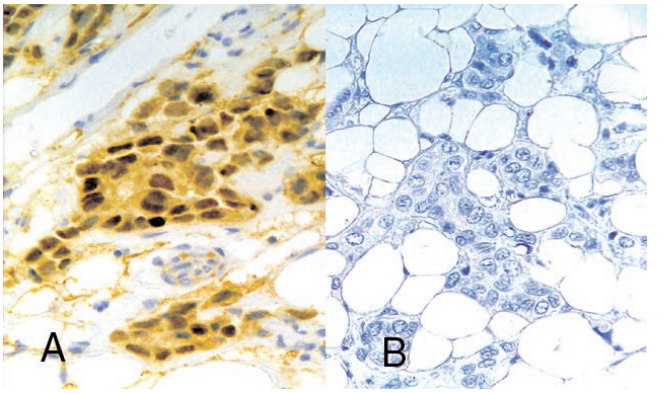
Significant improvement in our understanding of tumour biology in recent years has been achieved by immunohistochemical and molecular analyses of cancer-associated genes and their encoded proteins. Detection of protein expression in sporadic cancers by immunohistochemistry has enabled the identification of new markers that have diagnostic, therapeutic and prognostic value. Examples include hormone [eg oestrogen receptor (ER)] and growth factors receptors (eg epidermal growth factor receptor), tumour-specific oncogene products (eg CerbB2) and cell-cycle proteins (eg cyclin D_1). Molecular analysis of the genetic alterations in tumours has also been correlated with tumour morphology and behaviour. A good example is the identification of mutations in the *E-cadherin* gene in invasive lobular carcinoma of breast and diffuse gastric carcinomas that have specific morphology and metastatic patterns [3–5].

ER = oestrogen receptor; PgR = progesterone receptor.

This article reviews the immunohistochemical and molecular data in tumours associated with mutations in *BRCA1* and *BRCA2* genes. Clearly, this issue is important for more than just reasons of scientific curiosity, because the phenotype of the tumour may provide vital diagnostic and prognostic information for the patient. In addition, with the availability of genetic testing for *BRCA1* and *BRCA2* mutations within high-risk families, the morphological and molecular phenotype may help to identify patients who are suitable for mutation testing of specific genes.

To date, most, if not all studies that investigated the molecular profile of *BRCA*-associated tumours have been performed on relatively small numbers of patients, making statistical analysis of individual studies difficult. Combined data from the literature, however, are beginning to provide a glimpse of the unique immunohistochemical and molecular profile of *BRCA1*- and *BRCA2*-associated tumours.

Figure 1



Immunohistochemical staining of BRCA1 tumour for **(a)** oestrogen receptor and **(b)** progesterone receptor.

Steroid hormone receptors

Since the discovery of the ER in 1960, it has become one of the most important prognostic and predictive markers for breast cancer [6]. It is well known that ER expression is inversely correlated with tumour grade [7]. *BRCA*-associated tumours, which are more often of higher grade than that of sporadic breast cancer, would therefore be predicted to be more often ER negative. Indeed, numerous studies [8•,9,10••,11••,12•] have shown low levels of ER expression in familial breast cancers. Osin *et al* [8•,9] have shown that when ER was assessed in *BRCA*-associated tumours in comparison with a grade-matched control group, the expression of ER in *BRCA1*-associated tumours was still significantly lower (8 versus 26%). In contrast, the expression of ER in *BRCA2*-associated tumours appears to be similar to that in sporadic breast cancers.

The detection of ER immunohistochemically does not necessarily reflect its functional competence, and a certain proportion of tumours that express ER are known to be resistant to anti-oestrogen therapy. The function of ER is dependent on the ability to transactivate so-called ER-dependent genes. Expression of progesterone receptor (PgR) and PS2 protein is indirect evidence of retained transcriptional activation activity of ER, and it has been shown that PgR and PS2 expression have stronger correlation with prognosis in breast cancer than ER expression alone [13]. Osin *et al* [8•] showed that, although nine out of 40 familial breast cancer patients were ER positive, only two of these were also PgR positive (Fig. 1). This suggests that even in cases where ERs could be identified immunohistochemically, their functional ability may be compromised.

Another intriguing question is at what stage of progression do tumours become hormone independent? It has been demonstrated that both the invasive and in-situ component in *BRCA*-associated tumours have a similar status of steroid hormone receptor expression, suggesting that loss of hormonal response is a relatively early event in progression of these tumours [8•,9]. These data strongly suggest resistance to anti-oestrogen therapy of *BRCA*-associated tumours, and undoubtedly could have very serious practical implications in view of proposed anti-oestrogen prophylaxis for patients from high-risk families.

c-erbB2

A large number of studies have been performed on the functional role of *c-erbB2* oncogene (HER-2/neu) in breast cancer. HER-2/neu product is a tyrosine kinase receptor that belongs to the same family as epidermal growth factor receptor. It is overexpressed in approximately 20–30% of high-grade invasive breast cancers and has been shown to be a valuable prognostic indicator. HER2/neu status also predicts response to anti-oestrogen and cytotoxic chemotherapy. Antibodies directed against the HER2/neu protein have attracted a lot of attention recently because of the availability of the monoclonal antibody herceptin for treatment of breast cancer [14]. Clearly, the role of HER2/neu in familial breast cancer is of interest.

Data on HER2/neu are limited and conflicting. Armes *et al* [11••] and Robson *et al* [12•] have not shown a difference in HER2/neu expression between sporadic and familial cancers. The study by Johannsson *et al* [10••], however, demonstrated that *c-erbB2* expression in *BRCA1*-associated cancers is lower than would be predicted on the basis of their histological grade. Data from a large Breast Cancer Linkage Consortium study are awaited to clarify this issue.

TP53

P53 protein is one of most important guardians of stability and integrity of the genome, and acts to prevent cell proliferation after DNA damage and activates apoptosis in

case of unrepairable damage. Mutations in the *TP53* gene are the most common genetic alterations in human cancers and are encountered in 20–40% of sporadic breast cancers. The frequency of these mutations correlates with tumour grade. Detection of p53 protein by immunohistochemistry has become a routine method in pathology practice, and the presence of detectable p53 protein is an important prognostic marker that correlates with higher histopathological grade, increased mitotic activity, aggressive tumour behaviour and therefore a worse prognosis [15,16]. Using immunohistochemistry, Crook *et al* [17••] reported that *BRCA*-associated tumours were more often p53 positive than were grade-matched sporadic breast cancers (77% *BRCA1*, 45% *BRCA2*, 35% sporadic) (Fig. 2).

Further evidence for an important role for p53 in familial breast cancer comes from the detection of mutations at a higher frequency than in sporadic cancers. The mutations in *BRCA*-associated cancers were often multiple and their locations unusual, which is in marked contrast to sporadic cancer [17••,18].

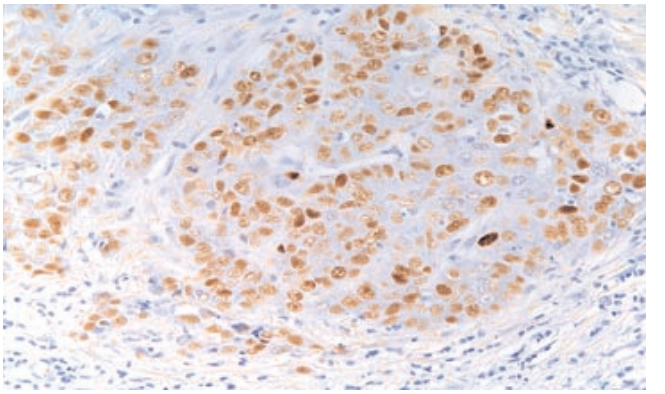
Studies of *TP53* gene function in BRCA tumours have been performed using *in vitro* models. These show that the identified mutants are unique not only in their number and location, but also in their function. The mutants retain some of the wild p53-dependent activities, such as transactivation, suppression of proliferation and apoptosis induction (in particular via *PIG3* transactivation). At the same time, these mutants fail to suppress transformation and they exhibit gain of function [19••]. The retained ability of some of these novel mutants to transactivate *MDM2* may explain the absence of immunodetectable p53 in some *BRCA*-associated tumours with p53 mutations. This can occur because of degradation of the p53 protein by the *MDM2*-regulated, ubiquitin-depended pathway.

In sporadic breast cancer an inverse correlation between loss of p53 expression and high proliferation index on one side, and low expression of the antiapoptotic gene *BCL2* on the other has been demonstrated. Surprisingly two studies [11••,12•] have shown that *BRCA1*/*BRCA2* tumours have the same level of *BCL2* expression as the control group, despite being highly proliferative and with frequent p53 mutations. Clearly the regulation of both cell cycle and apoptosis is multifactorial and relatively high expression of antiapoptotic *BCL2* is probably one of the mechanisms of tumour survival in conditions where apoptosis-inducing genes are still transactivated by mutant p53.

Cell cycle proteins

The cyclin-dependent kinase inhibitor p21 blocks transition from G_1 to S phase and suppresses cell proliferation. The p21 is thought to be a major downstream effector of the wild-type p53-mediated growth arrest pathway that is induced by DNA damage. In sporadic breast tumours the expression of p21 is inversely correlated with p53 expression and high tumour grade [16,20]. The proposed explanation is that mutated p53 is unable to activate p21 transcription. Immunohistochemical studies [17••] have failed to demonstrate a relationship between p21 and p53 in *BRCA1*/*BRCA2* tumours, suggesting that p21 transactivation in this group could be mediated by a p53-independent mechanism. This finding could be of practical significance, because there is an increase in p21 expression and apoptosis in cells with wild-type p53 exposed to chemotherapy [21].

Figure 2



Strong immunopositivity of *BRCA1* tumour for p53 protein.

Another cyclin-dependent kinase complex inhibitor that plays an important role in breast cancer pathogenesis is p27. There are reports that patients whose tumours overexpress p27 have significantly higher survival rates. In small breast cancers (stages T1a and b) p27 expression was reported as the only independent prognostic factor [22]. Data regarding p27 expression in familial *BRCA*-associated breast cancer are scarce and contradictory. Robson *et al* [12•] reported that p27 expression does not differ between sporadic and *BRCA*-associated cancers. This is contrary to our own observations, however, where we found p27 to be overexpressed in *BRCA1*/*BRCA2* breast cancers (86% in familial tumours versus 65% in sporadic tumours; Osin PP, unpublished observations). Some studies [23] have demonstrated a better prognosis for familial breast cancer. If this is substantiated (and there is evidence that this may not be correct [24]), the overexpression of p27 could be one possible explanation.

Cyclin D_1 is a regulator of progression from G_1 to S phase in cell cycle. It represents an important part of hormonal regulation of mammary epithelium growth: cyclin D_1 is known to be upregulated by oestrogen and progestins, and to be downregulated by anti-oestrogens [25]. The transcription of ER-regulated genes is also modulated by

cyclin D_1 [26,27]. Overexpression of cyclin D_1 is a common event in breast cancer and is especially frequent in early onset breast cancer, probably because of high levels of oestrogens in this age group [28]. *BRCA1*/*BRCA2*-associated tumours show very low expression of cyclin D_1 in both the invasive and in-situ components, however (14% in both invasive and ductal carcinoma *in situ* components in *BRCA1*/*BRCA2* tumours, versus 35–36% in invasive/ductal carcinoma *in situ* in sporadic tumours) [8•]. Taken together with the absence of ER and PgR in *BRCA1*/*BRCA2* cancers, the absence of cyclin D_1 in these tumours could be additional evidence of hormone independence of *BRCA*-associated familial breast cancers.

Miscellaneous markers

Disruption of normal regulation of the cell cycle leading to increased mitotic activity is a manifestation of tumourigenesis. In addition to traditional counting of mitotic figures, a number of immunohistochemical methods have become a part of routine tumour investigation. A high proliferative index in *BRCA*-associated tumours compared with a grade-matched control group has been demonstrated [1••,2•], and recent studies [11••] have confirmed it by demonstrating high Ki67 labelling index (83 versus 48%).

Cathepsin D belongs to a family of proteases that are involved in the tissue remodelling. Overexpression of cathepsin D in breast cancer has been found to correlate with poor prognosis [29–31] and the expression of cathepsin D in host stromal cells is associated with higher intratumoural microvessel density [32]. Expression of cathepsin D in *BRCA1*/*BRCA2*-associated breast cancers was not found to be different from that in sporadic breast cancers in two studies [11••,12•].

E-cadherin, an epithelial adhesion molecule is mutated at high frequency in invasive lobular carcinoma [3,4]. Epidemiological studies have shown a link between familiality and lobular carcinoma, although this does not appear to be a phenotype of either *BRCA1* or *BRCA2* cancer. Investigation of E-cadherin expression in familial breast cancer is limited, and there is no evidence at present that there are any significant differences between familial and sporadic cancers [11••].

Conclusion

The study of familial breast cancer using immunohistochemistry and molecular techniques is in its infancy. The studies suggest that *BRCA1*-associated tumours are more likely to be steroid receptor negative, and more often express p53 protein. Mutations in *TP53* also appear to be increased in *BRCA1* tumours. Although the data on *BRCA2*-associated tumours are limited at present, they appear to be different from *BRCA1* tumours with respect to ER and PgR. There is no evidence that the expression of ER and PgR is different between sporadic cancers and *BRCA2*-associated cancers. The data on p53 alterations in *BRCA2*-associated tumours requires further clarification.

Combining data regarding the morphological types and immunophenotypes of familial breast cancers indicates that *BRCA1* tumours are different from *BRCA2* tumours and sporadic cancers, and that the biological functions of the proteins these genes encode are likely to be different.

References

Articles of particular interest have been highlighted as:
- • of special interest
- •• of outstanding interest

1. •• Breast Cancer Linkage Consortium: **Pathology of familial breast cancer: differences between breast cancers in carriers of *BRCA1* or *BRCA2* mutations and sporadic cases.** *Lancet* 1997, **349**: 1505–1510.
Comprehensive study describing specific histopathological features of *BRCA1*- and *BRCA2*-associated breast cancer.

2. • Lakhani SR, Jacquemier J, Sloane JP, *et al*: **Multifactorial analysis of differences between sporadic breast cancers and cancers involving *BRCA1* and *BRCA2* mutations.** *J Natl Cancer Inst* 1998, **90**: 1138–1145.
Detailed descriptive study of the characteristic cytological and architectural features of *BRCA1*- and *BRCA2*-associated breast cancer.

3. De Leeuw WJ, Berx G, Vos CB, *et al*: **Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma *in situ*.** *J Pathol* 1997, **183**:404–411.

4. Berx G, Cleton-Jansen AM, Strumane K, *et al*: **E-cadherin is inactivated in a majority of invasive human lobular breast cancers by truncation mutations throughout its extracellular domain.** *Oncogene* 1996, **13**:1919–1925.

5. Guilford P, Hopkins J, Harraway J, *et al*: **E-cadherin germline mutations in familial gastric cancer.** *Nature* 1998, **392**:402–405.

6. Osborne CK: **Steroid hormone receptors in breast cancer management.** *Breast Cancer Res Treat* 1998, **51**:227–238.

7. Henderson IC, Patek AJ: **The relationship between prognostic and predictive factors in the management of breast cancer.** *Breast Cancer Res Treat* 1998, **52**:261–288.

8. • Osin P, Gusterson BA, Philp E, *et al*: **Predicted anti-oestrogen resistance in BRCA-associated familial breast cancers.** *Eur J Cancer* 1998, **34**:1683–1686.
Immunohistochemical study of steroid hormone receptors and oestrogen responsive genes in both invasive and in-situ stages of *BRCA1*/*BRCA2*-associated breast cancer compared with sporadic breast cancer.

9. Osin P, Crook T, Powles T, Peto J, Gusterson B: **Hormone status of in-situ cancer in *BRCA1* and *BRCA2* mutation carriers [letter].** *Lancet* 1998, **351**:1487.

10. •• Johannsson OT, Idvall I, Anderson C, *et al*: **Tumour biological features of BRCA1-induced breast and ovarian cancer.** *Eur J Cancer* 1997, **33**:362–371.
Descriptive study of characteristic histopathological and immunohistochemical phenotype of *BRCA1*-associated breast and ovarian cancer.

11. •• Armes JE, Trute L, White D, *et al*: **Distinct molecular pathogeneses of early-onset breast cancers in BRCA1 and BRCA2 mutation carriers: a population-based study.** *Cancer Res* 1999, **59**:2011–2017.
Immunohistochemical analysis of breast cancers arising in young patients and comparison between *BRCA1*/*BRCA2* mutation carriers and a sporadic group.

12. • Robson M, Rajan P, Rosen PP, *et al*: **BRCA-associated breast cancer: absence of a characteristic immunophenotype.** *Cancer Res* 1998, **58**:1839–1842.
Comparative study of histopathological and immunohistochemical profile of early onset breast cancer within sporadic and *BRCA*-associated groups.

13. Ioakim-Liossi A, Karakitsos P, Markopoulos C, *et al*: **Expression of pS2 protein and estrogen and progesterone receptor status in breast cancer.** *Acta Cytol* 1997, **41**:713–716.

14. Ross JS, Fletcher JA: **HER-2/neu (c-erb-B2) gene and protein in breast cancer.** *Am J Clin Pathol* 1999, **112(suppl 1)**:S53–S67.

15. Rudolph P, Olsson H, Bonatz G, *et al*: **Correlation between p53, c-erbB-2, and topoisomerase II alpha expression, DNA ploidy, hormonal receptor status and proliferation in 356 node-negative breast carcinomas: prognostic implications.** *J Pathol* 1999, **187**:207–216.

16. Elledge RM, Allred DC: **Prognostic and predictive value of p53 and p21 in breast cancer.** *Breast Cancer Res Treat* 1998, **52**:79–98.

17. •• Crook T, Brooks LA, Crossland S, *et al*: **p53 mutation with frequent novel condons but not a mutator phenotype in BRCA1- and BRCA2-associated breast tumours.** *Oncogene* 1998, **17**:1681–1689.
Molecular analysis of *TP53* status in *BRCA1*- and *BRCA2*-associated breast cancer. Molecular and functional analysis of specific *TP53* mutants characteristic for *BRCA1*- and *BRCA2*-associated tumours.

18. Crook T, Crossland S, Crompton MR, Osin P, Gusterson BA: **p53 mutations in BRCA1-associated familial breast cancer.** *Lancet* 1997, **350**:638–639.

19. •• Smith PD, Crossland S, Parker G, *et al*: **Novel p53 mutants selected in BRCA-associated tumours which dissociate transformation suppression from other wild-type p53 functions.** *Oncogene* 1999, **18**:2451–2459.
This study identifies a novel class of p53 mutants that dissociate transformation suppression from other wild-type functions such as apoptosis.

20. McClelland RA, Gee JM, O'Sullivan L, *et al*: **p21(WAF1) expression and endocrine response in breast cancer.** *J Pathol* 1999, **188**: 126–132.

21. el-Deiry WS, Harper JW, O'Connor PM, *et al*: **WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis.** *Cancer Res* 1994, **54**: 1169–1174.

22. Tan P, Cady B, Wanner M, *et al*: **The cell cycle inhibitor p27 is an independent prognostic marker in small (T1a,b) invasive breast carcinomas.** *Cancer Res* 1997, **57**:1259–1263.

23. Mohammed SN, Smith P, Hodgson SV, *et al*: **Family history and survival in premenopausal breast cancer.** *Br J Cancer* 1998, **77**: 2252–2256.

24. Robson M, Gilewski T, Haas B, *et al*: **BRCA-associated breast cancer in young women.** *J Clin Oncol* 1998, **16**:1642–1649.

25. Gillett CE, Lee AH, Millis RR, Barnes DM: **Cyclin D1 and associated proteins in mammary ductal carcinoma *in situ* and atypical ductal hyperplasia.** *J Pathol* 1998, **184**:396–400.

26. McMahon C, Suthiphongchai T, DiRenzo J, Ewen ME: **P/CAF associates with cyclin D1 and potentiates its activation of the estrogen receptor.** *Proc Natl Acad Sci USA* 1999, **96**:5382–5387.

27. Neuman E, Ladha MH, Lin N, *et al*: **Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4.** *Mol Cell Biol* 1997, **17**:5338–5347.

28. Barnes DM, Gillett CE: **Cyclin D1 in breast cancer.** *Breast Cancer Res Treat* 1998, **52**:1–15.

29. Duffy MJ: **Proteases as prognostic markers in cancer.** *Clin Cancer Res* 1996, **2**:613–618.

30. Rochefort H, Liaudet-Coopman E: **Cathepsin D in cancer metastasis: a protease and a ligand.** *APMIS* 1999, **107**:86–95.

31. Fulco RA, Petix M, Salimbeni V, Torre EA: **Prognostic significance of the estrogen-regulated proteins, cathepsin-D and pS2, in breast cancer.** *Minerva Med* 1998, **89**:5–10.

32. Gonzalez-Vela MC, Garijo MF, Fernandez F, Buelta L, Val-Bernal JF: **Cathepsin D in host stromal cells is associated with more highly vascular and aggressive invasive breast carcinoma.** *Histopathology* 1999, **34**:35–42.

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