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Original Paper

Predicted Anti-oestrogen Resistance in *BRCA*-associated Familial Breast Cancers

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There is controversy concerning the prognosis of breast cancers arising in women carrying loss of function mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2*. This study was carried out to assess the likely hormone dependence of this group of tumours in comparison with an age and grade matched group of control sporadic tumours. We used quantitative immunohistochemical analysis for the oestrogen receptor (ER), progesterone receptor (PgR), cyclin D1 and pS2 on sections of primary tumours and ductal carcinoma *in situ* (DCIS). Expression of PgR ($P < 0.05$) and cyclin D1 ($P < 0.01$) was low in the *BRCA1*- and *BRCA2*-associated cancers compared with sporadic cases. The low frequency of expression of ER (9/40), PgR (2/40) cyclin D1 (5/36) and pS2 (5/36) in the familial tumours indicates that the majority of such tumours will be oestrogen insensitive and unlikely to respond to hormonal manipulation even at the *in situ* stage in their evolution. The low level of PgR (2/40 cases) suggests that there may be some abnormality of transactivating function of the ER in these tumours.

Key words: breast, cancer, familial, anti-oestrogen
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INTRODUCTION

INHERITANCE OF mutant alleles of the recently cloned breast cancer susceptibility genes *BRCA1* [1] and *BRCA2* [2] confers a markedly increased risk of early onset breast, ovarian and prostate cancer [3, 4]. Breast tumours arising in individuals carrying mutant alleles of *BRCA1* or *BRCA2* have a high mitotic fraction and are more frequently of Bloom and Richardson grade 3 than sporadic tumours [5]. It has been demonstrated recently that *BRCA1* and *BRCA2* tumours have a high frequency of *p53* mutations [6]. These features of *BRCA1*- and *BRCA2*-associated tumours would predict that they are likely to behave aggressively and to be associated with a poor prognosis. Some reports, however, suggest a better prognosis in familial breast cancer [7, 8]. This dichotomy between histopathology and apparent tumour behaviour raises the question of other influences on prognosis and tumour behaviour. It has been noted that there is a dense lymphocytic infiltrate associated with *BRCA1* tumours, which, along with a well-circumscribed margin, is a feature of the medullary-like phenotype [5]. It is, therefore, possible that host responses play an important role in the prognosis of these tumours. Another factor that needs to be considered is hormone responsiveness. A recent paper [9] suggested that *BRCA1* positive tumours are more likely to be negative for the oestrogen receptor (ER) and the progesterone receptor (PgR). This raises the question of whether *BRCA1* and *BRCA2* tumours have the biochemical phenotype of tumours that are *de novo* resistant to anti-oestrogens, being either ER negative, or ER positive, but unable to transactivate the PgR [10, 11]. In addition, it could be predicted that such tumours will have low levels of expression of the oestrogen regulated protein pS2 [12]. If these tumours are not hormone responsive it would be important to identify the point in tumour progression at which this phenotype evolves. Therefore, we carried out a detailed study of the expression of ER and the oestrogen responsive genes PgR and pS2 in tumours (with *in situ* and invasive components) of known *BRCA1* and *BRCA2* status. In addition, we assessed cyclin D1 expression which is known to be upregulated by oestrogen in the presence of insulin and by progestins [13], and is downregulated by the

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anti-oestrogen ICI182780 [14]. Cyclin D1 is also known to potentiate transcription of oestrogen receptor-regulated genes [15].

PATIENTS AND METHODS

The familial breast tumours analysed were taken from a database compiled by one of the authors. Paraffin blocks were retrieved from the archives and the diagnosis and grading of each tumour verified by two independent pathologists by examination of haematoxylin and eosin stained tissue sections. Grading was by the Nottingham-modified Bloom and Richardson method that is now accepted as the standard in the U.K. [5]. A series of 40 tumours arising in confirmed carriers of germline mutations in *BRCA1* (25 tumours) and *BRCA2* (15 tumours) were available as paraffin sections. Case selection was based on availability of material. Because *BRCA*-associated tumours are predominantly of high histological grade, 69 sporadic cases of breast cancer of similar grade to the test samples were taken randomly from a group of tumours that had been used to compare the histopathology of familial and sporadic breast cancer by the Breast Cancer Linkage Consortium [5]. The controls were age matched for the Consortium study and the cases used in this study were representative of the group. The overall mean age of the *BRCA*-associated cases was 41.85 years (range 28–58 years) and for the controls was 37.84 years (range 24–85 years). The mean age of the *BRCA1* cases was 40.65 years (range 28–56 years) and the mean age of the *BRCA2* cases was 44.36 years (range 32–58 years). Five (13%) of the familial tumours and six (9%) of the controls were grade 2, the remainder being grade 3. Where available, ductal carcinoma *in situ* (DCIS) associated with the invasive tumour was also studied. No data on hormonal treatment, chemotherapy or clinical follow-up were available. All significance levels were calculated by Fisher's exact test and are two-sided.

Sections (5 μm) were prepared from archival paraffin blocks. Following passage through graded alcohols to remove the wax and microwaving for antigen retrieval where appropriate, the sections were exposed to the antibodies using standard protocols (see Table 1). Owing to the limited amount of material available a full set of immunohistochemical stains could not be carried out on a few of the cases.

The detection system was a biotinylated rabbit antimouse polyclonal serum (Dako No. E0354, A/S Denmark) used at 1/200 dilution, followed by a horseradish peroxidase labelled streptavidin ABComplex (Dako No. K0377) according to the manufacturer's instructions. Standardisation of staining was carried out against the U.K. National Quality Assurance Standards for ER and PgR staining (NEQUAS Scheme).

Sections were scored for immunocytochemical positivity using the 'quick score' method previously described and validated by Detre and colleagues [17]. This combines both intensity of staining (scored 0–3), multiplied by the percentage of tumour cells positive (on a scale 1–6) giving a range of 0–18. As described by these authors, a Q value of 3 or greater was used to define positive staining. This value correlates well with the biochemical and biological activity for ER and PgR.

RESULTS

The immunohistochemical results summarised in Table 2 indicate that the frequency of expression of ER is similar in *BRCA1*-associated and sporadic breast tumours. To examine the transcriptional activation function of ER, we analysed expression of the oestrogen responsive gene PgR in the same tumours (Figure 1). PgR was expressed in only 2/40 (5%) familial breast cancers compared with 13/69 (19%) sporadic

Table 1. *Antibodies used*

	Antibody (dilution)	Supplier	Antigen retrieval
ER	NCL-ER-6F11 (1/100)	Novacastra	Microwave
PgR	NCL-PgR (1/100)	Novacastra	Microwave
pS2	HIS-pS2-Ab1 (1/50)	CIS U.K. Ltd	None
Cyclin D1	DCS-6 (1/800)	J. Bartek [16]	Microwave

This table shows the antibodies used, their dilution, supplier and whether antigen retrieval is necessary. In all cases microwaving used a 10 min incubation of the sections in citrate buffer using a Proline Micro Chef ST44 on setting 10. ER, oestrogen receptor; PgR, progesterone receptor.

Table 2. *Expression patterns in* BRCA *tumours and sporadic cancers matched for grade*

	ER +ve (%)	PgR +ve (%)	Cyclin D1 +ve (%)	pS2 +ve (%)
BRCA1				
Invasive	3/25 (12)	1/25 (4)	1/21 (5)†	2/23 (9)
DCIS	1/8 (13)	0/7 (0)	0/7 (0)	0/9 (0)
BRCA2				
Invasive	6/15 (40)	1/15 (7)	4/15 (27)	4/14 (29)
DCIS	4/8 (50)	0/8 (0)	2/7 (29)	3/8 (38)
BRCA1 and *BRCA2*				
Invasive	9/40 (23)	2/40 (5)⋆	5/36 (14)⋆	6/37 (16)
DCIS	5/16 (31)	0/15 (0)	2/14 (14)	3/17 (18)
Sporadic				
Invasive	11/69 (16)	13/69 (19)	24/69 (35)	11/67 (16)
DCIS	7/30 (23)	5/31 (16)	10/28 (36)	7/30 (23)

In this table the absolute number of cases examined is shown together with the percentage of cases positive according to reagent and tumour designation. The significance values are based on Fisher's exact test and are two-sided. ER, oestrogen receptor; PgR, progresterone receptor; DCIS, ductal carcinoma *in situ*. ⋆$P < 0.05$ compared with sporadic tumours; †$P < 0.01$ compared with sporadic tumours.

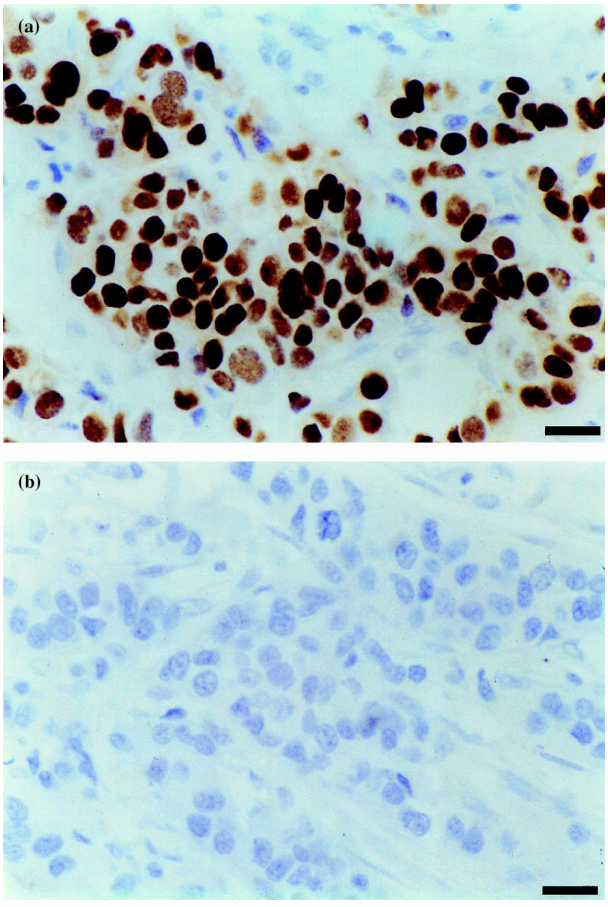

Figure 1. Immunohistochemical staining of the same grade 3 *BRCA1* tumour for oestrogen receptor (a) and progesterone receptor (b). Bar = 50 μm.

cases ($P < 0.05$). Even among ER positive cancers, PgR was expressed in only 2/9 (22%) familial cases compared with 7/11 (64%) sporadic cases ($P = 0.09$). In sporadic cases, there were five tumours that were PgR positive and ER negative.

The low levels of PgR in the *BRCA*-associated tumours raised an important question relating to the possible loss of transactivating activity of ER and led to the further analysis of pS2, another oestrogen regulated protein. In the case of pS2, expression was observed in two *BRCA1*-associated invasive tumours, of which one was positive for ER expression and one negative. In the *BRCA2*-associated tumours, pS2 expression was detected in three tumours which were ER positive and one which was ER negative. The two cases of a PgR positive tumour were both positive for ER, but only one was positive for pS2. pS2 positivity in some ER negative tumours is not unexpected as pS2 has a complex promoter/enhancer region responsive to epidermal growth factor (EGF), c-Ha-ras oncoprotein and c-jun [18]. There was no significant difference between pS2 expression in the *BRCA*-associated tumours and the control sporadic tumours.

Cyclin D1 expression was significantly less common in *BRCA1*-associated invasive tumours than in *BRCA2*-associated ($P < 0.05$) or sporadic tumours ($P < 0.01$). Only one (5%) of the *BRCA1*-associated tumours expressed cyclin D1, compared with 27% of the *BRCA2* tumours and 35% of sporadic tumours.

There were no significant differences between the expression of any of the receptors in primary and the corresponding DCIS, with the latter having a similar pattern to the invasive tumours (Table 2).

DISCUSSION

These results indicate that PgR and cyclin D1 expression are significantly reduced in *BRCA1*- and *BRCA2*-associated breast cancers compared with sporadic tumours. The low frequency of PgR (2/40 cases) expression suggests that there may be some abnormality of transactivating function of the ER in these tumours. The results for cyclin D1 are similar to previously published data. Overexpression of cyclin D1 is associated with a good prognosis [18]. Gillett and colleagues reported that tumours that had high levels of expression of cyclin D1 and ER responded to tamoxifen, whereas tumours lacking both proteins had less than a 10% chance of responding. Neilsen and associates have confirmed these findings [19] and have also shown that low levels of cyclin D1 increased the risk of relapse and death. The high grade (and associated low incidence of ER expression), the loss of PgR and the low levels of expression of pS2 and cyclin D1 all indicate that the *BRCA*-associated tumours in this series have an anti-oestrogen resistance phenotype and a predicted poor prognosis [10, 11]. The immunohistochemical pattern among the DCIS samples was similar to that for the invasive disease (Table 2), suggesting that this is a relatively 'early' phenotype in the evolution of the tumour. The loss of PgR in ER positive tumours as seen in the *BRCA2* tumours may be due to an inability of the ER to bind DNA [11]. Our data indicate that high grade tumours in *BRCA1*/*BRCA2* cancers are less likely to respond to endocrine manipulation than grade matched sporadic tumours. The molecular basis for the apparently good prognosis of *BRCA1*/*BRCA2* tumours does not, therefore, reside in frequent expression of functional ER circuitry. It is also to be noted that when matched for grade, there appear to be significant differences in the phenotype between *BRCA1* and *BRCA2* tumours with respect to cyclin D1 expression.

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