ORIGINAL ARTICLE

Androgen receptor expression in ductal carcinoma in situ of the breast: relation to oestrogen and progesterone receptors

A-G A Selim, G El-Ayat, C A Wells

J Clin Pathol 2002;**55**:14–16

See end of article for authors' affiliations

Correspondence to: Dr A A Selim, Department of Histopathology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK; aaselim@doctors.net.uk

Aims: Ductal carcinoma in situ (DCIS) of the breast has been diagnosed increasingly since the advent of mammographic screening. In contrast to the situation in invasive breast carcinoma, there are no reports on androgen receptor (AR) status in DCIS and few reports on oestrogen (ER) and progesterone (PR) receptors.

Methods: AR expression was examined in 57 cases of DCIS of the breast and correlated to the degree of differentiation and ER/PR status using immunohistochemical methods.

Results: AR positivity was noted in 19 of the cases, whereas the other 38 cases were negative. There was no significant association between AR expression and the degree of differentiation of DCIS; three of the 13 well differentiated DCIS cases, 10 of the 19 intermediately differentiated cases, and six of the 25 poorly differentiated cases were positive ($p = 0.093$). However, a strong association was shown between the expression of ER ($p < 0.0001$) and PR ($p = 0.002$) and the degree of differentiation of DCIS. In addition, no significant association was found between the expression of AR and the expression of ER ($p = 0.26$) or PR ($p = 0.57$) in DCIS of the breast.

Conclusions: A large number of cases of DCIS of the breast express AR and this may be associated with apocrine differentiation, which may impact on accurate typing of DCIS. Moreover, the expression of AR (but not ER or PR) in DCIS does not appear to be associated with the degree of differentiation.

Ductal carcinoma in situ (DCIS) of the breast without invasion has been reported increasingly since the advent of mammographic screening, but the natural history of this lesion remains unclear. DCIS of the breast does not represent A single entity but is a heterogeneous group of lesions with histological and clinical differences.[1–5] The histological subtype of DCIS influences its biological behaviour, but there are only a few studies correlating the classification with biological markers.[4–7]

The fact that sex steroid hormones and their receptors act in concert has led some investigators to study the role of the androgen receptor (AR) in patients with breast cancer. AR is expressed in approximately 35–75% of breast cancers.[8–10] Variations may be attributable to different methodologies and different fixatives, but a different case mix may also affect these studies. It has been shown that AR values correlate reasonably well with oestrogen receptor (ER) values, but more so with those for the progesterone receptor (PR).[8–11] AR positive breast cancer patients have prolonged survival and a better response to hormonal treatment than AR negative patients. Thus, some workers believe that knowledge of the receptor status of all three receptors may identify more accurately those patients with breast cancer who are most likely to respond to endocrine treatment.[9–13] In addition, androgen stimulation has both stimulatory and inhibitory growth effects on some breast cancer cell lines, depending on the status of receptors and other growth factor effects.[14–16]

The AR is also a marker of apocrine differentiation in normal apocrine epithelium,[17] and this may indicate an association with apocrine differentiation in these tumours. This is supported by the findings of Gatalica in apocrine carcinomas.[18]

In contrast to the situation in invasive breast carcinoma, there are no reports on AR status in DCIS and only occasional reports on ER and PR expression in DCIS.[6 7 19–21] Hence, this study was undertaken to investigate AR expression in DCIS and to correlate it with the expression of ER and PR, in addition to the degree of differentiation of cases of DCIS of the breast.

MATERIALS AND METHODS

Case selection

Fifty seven cases of DCIS were collected from the files of the histopathology department of St Bartholomew's Hospital, London. The age of the patients ranged from 40 to 86 years (mean, 55.0). The cases were classified according to Holland *et al*,[22] based mainly on cytonuclear and architectural differentiation into three categories, namely: well (13 cases), intermediate (19 cases), and poorly (25 cases) differentiated DCIS.

Immunohistochemistry

Tissue

Formalin fixed, paraffin wax embedded blocks of DCIS tissue were selected from the files and sectioned at a nominal 4 µm. The standard avidin biotin peroxidase complex method[23] was used. Heat mediated antigen retrieval using the pressure cooker method[24] was used for all staining. Appropriate positive and negative controls omitting the primary antibodies were included with each slide run. In addition, the normal breast tissue in the sample served as an internal control.

Antibodies

Table 1 summarises the monoclonal antibodies used against the AR, ER, and PR proteins.

Abbreviations: AR, androgen receptor; DCIS, ductal carcinoma in situ; ER, oestrogen receptor; PR, progesterone receptor

Table 1 Details of primary monoclonal antibodies used

Antibody against	Source	Clone	Dilution	Positive control
AR	Novocastra	2F12	1/50	Prostate
ER	Dako	ID-5	1/300	Breast carcinoma
PR	Novocastra	IA-6	1/200	Breast carcinoma

AR, androgen receptor; ER, oestrogen receptor; PR, progesterone receptor.

Table 2 Expression of AR, ER, and PR in the three categories of DCIS

	AR		ER		PR	
Differentiation	+	–	+	–	+	–
Well (n = 13)	3	10	13	0	12	1
Intermediate (n = 19)	10	9	10	9	8	11
Poor (n = 25)	6	19	8	17	8	17
Total (n = 57)	19	38	31	26	28	29
p Value	0.093		<0.0001		0.002	

AR, androgen receptor; DCIS, ductal carcinoma in situ; ER, oestrogen receptor; PR, progesterone receptor.

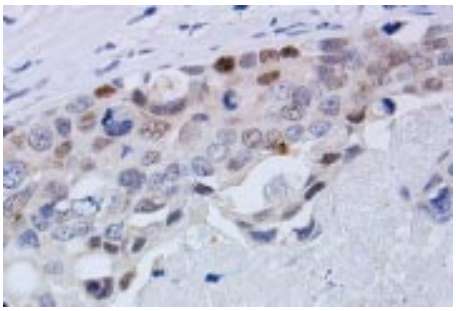

Figure 1 Androgen receptor nuclear staining of poorly differentiated ductal carcinoma in situ of the breast (immunoperoxidase).

Assessment

Nuclear staining was taken as positive, with cytoplasmic staining being ignored. The Quick Score method[25] was used for semiquantitation of AR, ER, and PR status as follows.

(1) Intensity of staining. Slides were assessed for the average degree of staining on low power (×10) and the following scores allocated: weak (1), moderate (2), or strong (3).

(2) The percentage of cells with positive nuclei was counted on high power (×40) and the following scores were allocated: < 25% (1), 25–< 50% (2), 50–< 75% (3), > 75% (4).
The scores from (1) and (2) were added together to give a final score ranging from 0 to 7, designated as negative or positive as follows: score of 0–3, negative; score of 4–7, positive.

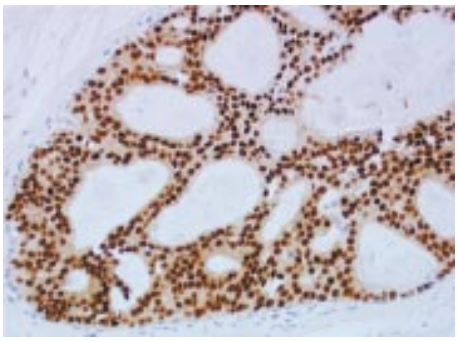

Figure 2 Strong nuclear staining for the oestrogen receptor in well differentiated ductal carcinoma in situ of the breast (immunoperoxidase).

Statistical analysis

To evaluate significance the χ^2 and Fisher exact tests were applied as appropriate. A p value of < 0.05 was considered to be significant.

RESULTS

Our study comprised 57 cases of DCIS, which were classified according to Holland and colleagues[22] into three categories, namely: well (13 cases), intermediate (19 cases), and poorly (25 cases) differentiated DCIS. Nine cases were morphologically of the apocrine type. Table 2 summarises the results of the three markers tested in the three categories of DCIS studied. Nuclear staining of the tumour cells was counted as positive. All non-specific cytoplasmic staining was ignored. In cases with normal tissue present, staining of nuclei in normal ducts or lobules was taken as a positive internal control. The intensity of nuclear staining varied between individual tumour cells. Of the 57 DCIS cases studied; 19, 31, and 28 cases were positive for AR (fig 1), ER (fig 2), and PR, respectively. No association between AR expression and the degree of differentiation of DCIS was identified; three of 13 cases of well differentiated DCIS, 10 of 19 cases of intermediately differentiated DCIS, and six of 25 cases of poorly differentiated DCIS were AR positive ($p = 0.093$). Six of the nine morphologically apocrine cases were positive for AR. A strong positive association between ER and PR expression and the degree of differentiation of DCIS was found. All the 13 cases of well differentiated DCIS, 10 of 19 intermediately differentiated DCIS, and eight of 25 poorly differentiated DCIS cases were positive for ER ($p < 0.0001$). Four of the morphologically apocrine cases showed immunopositivity for ER. Twelve of the 13 cases of well differentiated DCIS, eight of the nine intermediately differentiated DCIS, and eight of the 25 poorly differentiated DCIS cases were positive for PR ($p = 0.002$). Three of the morphologically apocrine cases were positive for PR. In the 19 DCIS cases positive for AR there were eight cases also positive for ER and PR, but the other 11 cases were negative for ER and PR. Table 3 shows no significant association between AR expression and the expression of ER ($p = 0.260$) or PR ($p = 0.57$) in the cases of DCIS studied.

DISCUSSION

In our study, using the European classification of Holland and colleagues[22] to categorise cases into well, intermediately, or poorly differentiated DCIS, no association was found between immunoreactivity for AR and the degree of differentiation of DCIS. In addition, no association was found between AR

Table 3 Association between AR expression and ER and PR expression in DCIS

		AR		
		+ (19)	– (38)	p Value
ER	+	8	23	0.26
	–	11	15	
PR	+	8	20	0.57
	–	11	18	

AR, androgen receptor; DCIS, ductal carcinoma in situ; ER, oestrogen receptor; PR, progesterone receptor.

Take home messages

- Many ductal carcinoma in situ (DCIS) cases are positive for the androgen receptor (AR) but negative for oestrogen (ER) and progesterone (PR) receptors
- There was no association between AR expression and the degree of differentiation in DCIS of the breast
- There was no association between AR expression and the expression of ER and PR in DCIS of the breast

expression and the expression of ER or PR. However, Isola[13] found A strong association between AR detected immunohistochemically and histological grade in 76 cases of invasive breast carcinoma using frozen sections. A strong positive association between AR and ER was also found in his study. Ellis *et al* found no significant association between AR and ER expression in invasive breast carcinoma; however, a strong positive association was found in their study between AR and PR expression.[8] The difference in the number and nature of cases studied, in addition to technical differences may explain the disagreement between our study and those of others. A larger series of cases of DCIS would be needed to exclude a weak association of AR with the degree of differentiation.

Our findings agree with those of Bobrow *et al*,[4] Millis *et al*,[7] and Pallis *et al*,[19] in that most poorly differentiated DCIS cases were lacking immunoreactivity for ER and PR, and most well differentiated DCIS cases were immunoreactive with ER and PR.

In conclusion, it seems that a large number of DCIS cases are positive for AR but negative for ER and PR, and this indicates the need for further investigation of AR status, in addition to conventional ER and PR. This could yield potentially useful information for establishing new therapeutic strategies and evaluating the prognostic outcome in patients with DCIS, and may relate partially to apocrine differentiation of these tumours.

ACKNOWLEDGEMENT

The authors thank S Jordan for technical assistance and Dr C Sowter for digital photography and computer expertise.

Authors' affiliations

A A Selim, G El-Ayat, C A Wells, Department of Histopathology, St Bartholomew's Hospital, St Bartholomew's and the Royal London School of Medicine and Dentistry, Queen Mary and Westfield College, University of London, West Smithfield, London EC1A 7BE, UK

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