Virchows Arch (1998) 433:311–314

ORIGINAL ARTICLE

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Immunohistochemical study of hormone receptor and hormone-regulated protein expression in phyllodes tumour: comparison with fibroadenoma

Received: 26 January 1998 / Accepted: 5 April 1998

Abstract The histogenesis of phyllodes tumour (PT) and that of fibroadenoma (FA) of the breast appear to be closely related. FA is thought to be hormonally responsive, while the hormone-responsiveness of PT is uncertain. To gain insight into hormone-responsiveness of PT, we performed immunohistochemical analysis of oestrogen-regulated pS2 and androgen-regulated prostate-specific antigen (PSA) protein expression and also of oestrogen receptor (ER), progesterone receptor (PgR) and androgen receptor (AR) expression in paraffin sections obtained from 50 female PT patients. Paraffin sections taken from 50 female fibroadenoma (FA) patients were analysed for comparison. ER, PgR, pS2, AR and PSA expression were detected in 32%, 96%, 20% 98% and 4.0% of PT sections and in 28%, 96%, 42% 80% and 10% of FA sections, respectively. No correlations were detected among ER, PgR and pS2 expression or between AR and PSA expression in PT or FA sections. PgR expression was significantly associated with AR expression in PT ($P<0.0001$). The present investigations indicate that PT and FA have almost similar hormone receptor status. However, different positivities of pS2 expression suggest that oestrogen-responsiveness may differ between PT and FA. In addition, a wide-ranging co-expression of AR and PgR in PT sections suggests that these receptors may play an important part in the proliferation, although the functional significance of these receptors should be elucidated.

Key words Phyllodes tumour · Androgen receptor · Immunohistochemistry

Introduction

Phyllodes tumour (PT) is a rare neoplasm that accounts for less than 1% of all breast neoplasms [16]. Fibroadenoma (FA) is the third most frequent tumour of the breast, only gross cystic disease and carcinoma being more frequent [5]. Although these tumours resemble each other histologically, there are distinct differences in their clinical courses [5, 6]. FA is thought to be hormonally responsive, because these tumours occur predominantly in the two decades following menarche and because some FA involute after the menopause [4]. PT has a broader age distribution at the time of clinical presentation than FA, with the median age being around 45 years [6]. Rao et al. [19, 20] demonstrated the presence of progesterone receptors (PgR) in PT and suggested the possibility of effective endocrine therapy. However, the presence of specific steroid hormone receptors and their function in PT have not been fully investigated. The expression of several proteins is known to be oestrogen-regulated, including PgR and the product of the *pS2* gene [10, 15]. The prostate-specific antigen (*PSA*) gene is regulated via the androgen receptor (AR) [18]. To gain further insight into the hormone responsiveness of PT, therefore, we performed immunohistochemical analysis of pS2 and PSA protein expression and also of oestrogen receptor (ER), PgR and androgen receptor (AR) expression in surgically resected paraffin-embedded specimens obtained from female PT patients.

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Materials and methods

Tissue samples of PT were obtained from 50 female patients who underwent local excision, wide local excision or simple mastectomy at the Sagara hospitals (Kagoshima, Japan) between 1990 and 1997. Their ages ranged from 17 to 72 years, with a mean of 45 years. Tumour size ranged from 1.0 to 30 cm, with a mean of 5.7 cm. Tissue samples of FA were also obtained from 50 female patients who underwent excisional biopsy or wide local resection. Their ages ranged from 14 to 53 years, with a mean of 34 years. Their tumour ranged in size from 1.0 to 14 cm, with a mean of 2.9 cm. All tumour tissues were fixed in 10% neutrally buffered formalin for 24–48 h and embedded in paraffin. Sections 3 μm thick were stained with haematoxylin and eosin, and were examined histopathologically. The diagnosis of PT and FA were made according to the World Health Organization's criteria [24]. On the basis of the criteria proposed by Azzopardi et al. [1], PT was di-

vided into benign (34 cases, 68%), borderline (12 cases, 24%) and malignant (4 cases, 8%) cases.

Monoclonal anti-human ER antibody (clone: 1D5) and anti-human pS2 antibody (clone: BC04) were purchased from Immunotech (France). Monoclonal anti-human PgR antibody (clone: 1A6) was purchased from Novocastra (UK). A polyclonal anti-AR antibody (AN-15) directed against amino acids 1 through 15 in the NH_2-terminal region of AR cDNA was used [22]. This antibody was generously provided by Dr. Shutsung Liao (University of Chicago, Chicago, Ill.). Monoclonal anti-human PSA antibody (clone: ER-PR8) was purchased from DAKO (Denmark).

After endogenous peroxidase activity had been blocked, deparaffinized sections (3 μm thick) were pretreated in 10 mM citrate buffer (pH 6.0) by microwaving (500 W, full power) for 15 min. After cooling for 60 min, sections were incubated with primary antibodies diluted as follows: ER;1:1; PgR; 1:40, pS2; 1:1, AR; 1:1000, PSA; 1:20, overnight at 4° C in a moist chamber. The sections were incubated with biotinylated goat anti-mouse or rabbit immunoglobulin (diluted 1:150, Vector Lab., UK) for 10 min and horseradish peroxidase-conjugated streptoavidin complex (diluted 1:100, Zymmed, Calif.) for 10 min. To visualize immunoreactivity, diaminobenzidine tetrachloride (1 mg/ml) containing 0.1% hydrogen peroxide (30% w/v) was used. Previously defined strongly ER+, PgR+ or pS2+ metastatic breast cancer tissues from the regional lymph nodes were used as positive controls [23]. Non-metastatic regional lymph nodes in the same sections served as negative controls. Nude mice tumours originating from LNCaP cells, which contain high levels of the AR and PSA protein, and PC-3 cells, which show no expression of AR and PSA protein, were used as positive and negative controls, respectively [22].

The percentage of positive cells was estimated semiquantitatively, and tumours were assigned to two categories: positive and negative. For ER, PgR and AR, nuclear stained cells were interpreted as positive cells. A section with more than 20% of positive cells was considered ER or PgR positive [8]. For pS2 and PSA, cytoplasmic stained cells were interpreted as positive cells. For pS2, PSA and AR, the presence of any percentage of positive cells was regarded as a positive result. Each slide was assessed independently by two pathologists (Y.U. and H.Y.). The few discordant cases were referred to a third pathologist in our laboratory.

Associations among these five proteins were assessed by Chi-square analysis. The statistical significance level was set at a *P*-value of less than 0.05.

Results

In PT and FA sections, positive rates of ER, PgR, pS2, AR and PSA were 32, 96%, 20%, 98% and 4.0%, and 28%, 96%, 42%, 80% and 10%, respectively (Table 1). Immunohistochemical staining for ER and PgR was localized to the nucleus only in epithelial cells (Figs. 1, 2). The AR immunolocalization was nuclear in epithelial cells in most cases. Both stromal and epithelial cells were positively stained in some cases (PT: 6 cases, FA: 4 cases; Fig. 3). Immunolocalization of pS2 and PSA was restricted to the cytoplasm of epithelial cells and some luminal surfaces (Figs. 4, 5). Staining within specimens was heterogeneous, with both positively and negatively stained cells evident in tumour foci. Of the positive cases of PT and FA, a high percentage (>50%) of immunoreactive cells for ER, PgR, pS2, AR and PSA was observed in 40%, 75%, 16%, 67% and 14%, and in 42%, 66%, 9%, 57% and 20%, respectively. No positive correlations were observed among ER, PgR and pS2 expression, or between AR and PSA expression (Table 2). PgR expression was significantly correlated with AR expression in PT ($P<0.0001$) (Table 3). The different types of PT (benign, borderline and malignant) did not differ significantly in positive rates of ER, PgR, pS2, AR and PSA expression.

Table 2 Androgen receptor (*AR*) and prostate-specific antigen (*PSA*) expression in phyllodes tumour and fibroadenoma

	AR+		AR–	
	PSA+	PSA–	PSA+	PSA–
Phyllodes tumour				
Malignant (*n*=4)	0	3	0	1
Borderline (*n*=12)	0	11	0	1
Benign (*n*=34)	2	31	0	1
Fibroadenoma (*n*=50)	4	36	1	9

Table 3 Correlation between PgR and AR expression in phyllodes tumour and fibroadenoma

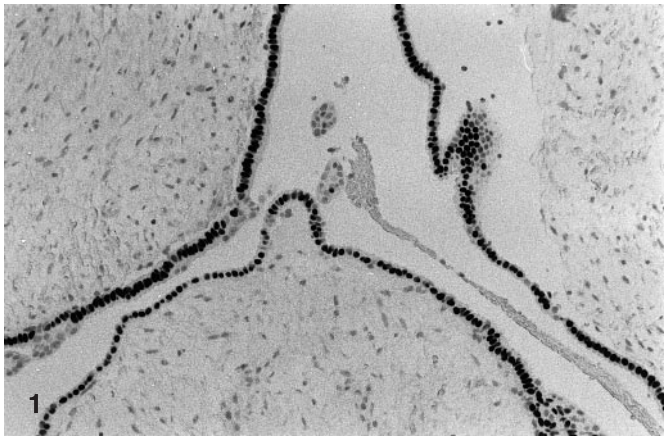
	Phyllodes tumour		Fibroadenoma	
	AR+	AR–	AR+	AR–
PgR positive (*n*=48)	45	3	39	9
PgR negative (*n*=2)	2	0	1	1

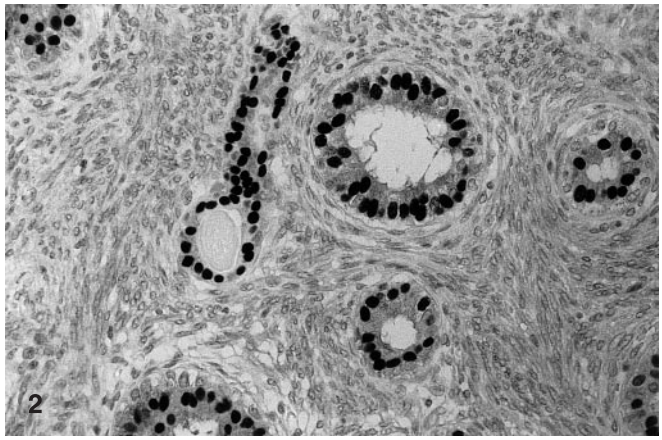
Discussion

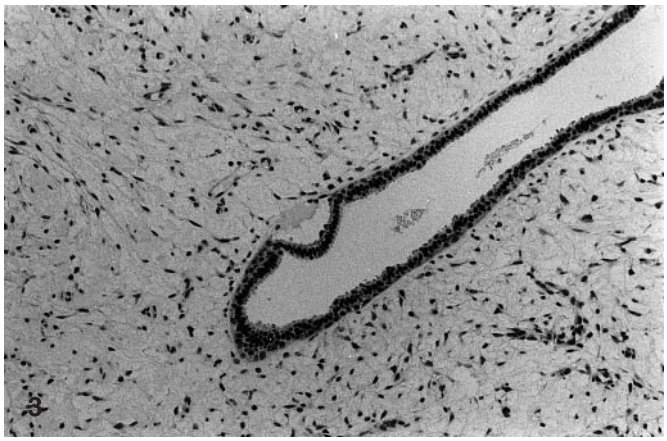
Rao et al. were the first to demonstrate cytosolic PgR in PT [19]. They also suggested that most PT and FA contained PgR but lacked ER, and that PgR were localized in the stromal cells [20]. They proposed that progestational therapy should be tested in the treatment of ad-

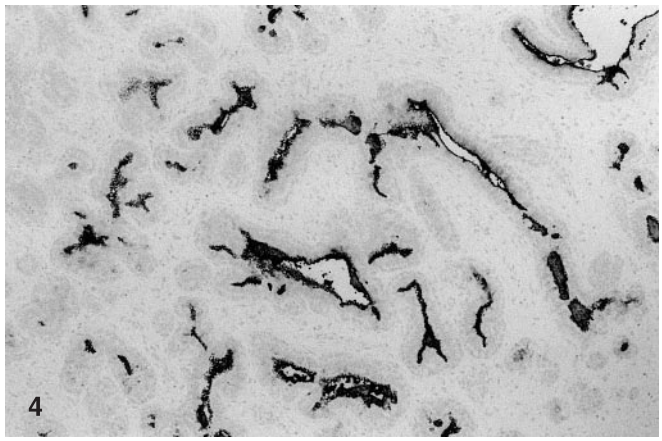
Table 1 Oestrogen receptor, progesterone receptor, and pS2 expression in phyllodes tumour and fibroadenoma

	ER+				ER–			
	PgR+		PgR–		PgR+		PgR–	
	pS2+	pS2–	pS2+	pS2–	pS2+	pS2–	pS2+	pS2–
Phyllodes tumour								
Malignant (*n*=4)	0	0	0	0	1	3	0	0
Borderline (*n*=12)	2	3	0	0	0	6	0	1
Benign (*n*=34)	3	8	0	0	3	19	1	0
Fibroadenoma (*n*=50)	5	9	0	0	16	18	0	2









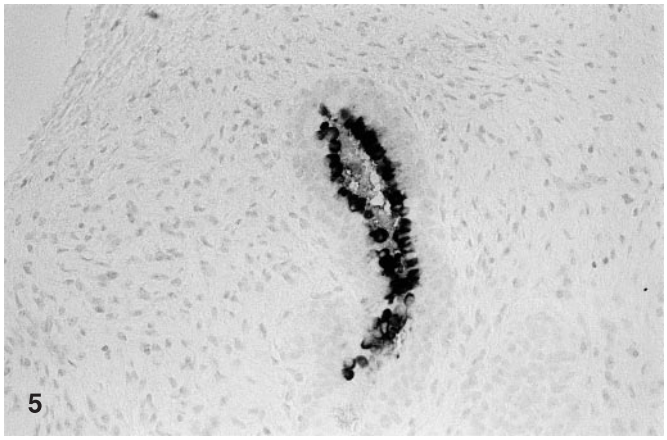


Fig. 1 Immunostaining for oestrogen receptors, showing nuclear localization in the epithelial components but not in the stromal components of phyllodes tumour. ×140

Fig. 2 Immunostaining for progesterone receptors, showing nuclear localization in the epithelial components but not in the stromal components of phyllodes tumor. ×220

Fig. 3 Immunostaining for androgen receptors, showing nuclear localization in both epithelial and stromal cells in phyllodes tumour. ×115

Fig. 4 Immunoreactivity for pS2 was seen in the cytoplasm of the epithelial cells and the luminal surface in fibroadenoma. ×140

Fig. 5 Immunoreactivity for prostate-specific antigen was seen in the cytoplasm of the epithelial cells in phyllodes tumour. ×180

vanced malignant PT. However, no immunohistochemical tests were performed to confirm these observations in these earlier studies. Our study also shows that most PT and FA have PgR and that their expression is localized only in the epithelial cells. These findings are almost consistent with those of Mechtersheimer et al. [17]. Although only 4 cases of malignant PT were investigated in our study, no stromal expression of PgR was observed. Thus, presence of PgR in stromal cells is unlikely to have an important role in proliferation of malignant PT. The *pS2* gene, which was originally isolated by virtue of its oestrogen-inducibility [15], has been shown to be associated predominantly with ER-positive breast cancers [9]. Although significant pS2 positivity has been reported in benign proliferative conditions of the breast [14], its positivity in PT has not been reported. We found more frequent expression of pS2 in FA than in PT regardless of the similarities in positivity for ER and PgR. It has been suggested that pS2 expression indicates a functional oestrogen-regulatory system and may predict clinical responsiveness to hormonal therapy [21]. Therefore, it was speculated that oestrogen-responsiveness might differ between PT and FA. Although PSA gene expression is regulated via the AR [18], 30% of breast cancer cases show PSA-immunoreactivity [3]. We found PSA-immunoreactivity with a monoclonal antibody in a small num-

ber of PT and FA regardless of high positivity for AR expression. Therefore, it was speculated that PSA might not be regulated by AR in PT and FA. We found stromal AR expression in a small number of cases. Although only one study revealed stromal AR expression in nonmalignant breast specimens [12], negative AR expression in breast cancer-associated stromal cells has been reported by several investigators [7, 11, 13]. To date, there is no report concerning AR expression in stroma of PT or FA. Therefore, we should confirm the stromal AR expression by using different AR antibodies or in situ hybridization methods. We found, for the first time, that most PT showed co-expression of PgR and AR. The divergent proliferative response to androgen seen in several breast cancer cell lines in vitro is considered to be due in part to alterations in the *AR* gene or interaction between AR and other steroid hormone receptors, such as ER and PgR [2]. In addition, it has been proposed that endothelin-1 synthesized by the epithelial component of PT may play an important part in stimulating the growth of stromal cells of PT in a paracrine fashion [25]. Therefore, it was hypothesized that AR- or PgR-induced growth factors might have an important role in stimulating the growth of stromal cells of PT in a paracrine fashion. As structural alterations or alternative isoforms of the AR have been suggested in human breast cancer [7], genetic analysis is needed to give us further insight into the function of AR in PT:

Acknowledgements The authors are grateful to Mr. T. Kodama for his assistance in immunohistochemical analysis. This work was supported in part by the Kodama Memorial fund for Medical Research.

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