

Immunohistochemical localisation of androgen receptor in apocrine metaplasia and apocrine adenosis of the breast: relation to oestrogen and progesterone receptors

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Abstract

Aim—To investigate the receptor status of the sex steroid hormones in apocrine metaplasia of the breast.

Methods—82 cases of apocrine metaplasia, including 18 of the rare lesion apocrine adenosis, were studied immunohistochemically for the expression of androgen receptor, oestrogen receptor, and progesterone receptor proteins on formalin fixed, paraffin embedded tissue sections. The standard avidin biotin complex (ABC) technique was followed and appropriate positive and negative controls were used.

Results—All the studied cases (82/82) were positive for androgen receptor, but were negative for oestrogen receptor and progesterone receptor.

Conclusions—Apocrine metaplastic epithelium, unlike the normal breast epithelium, is responsive to androgens, through androgen receptors, rather than to the female sex hormones. This may have clinical implications.

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Keywords: breast; apocrine metaplasia; hormone receptor; antigen retrieval

Apocrine metaplasia is a very common change which is most often seen in dilated cystic structures within fibrocystic change of the breast, but may appear in normal sized tubules as well.¹ The terms "papillary apocrine change"² and "apocrine hyperplasia"³ have been applied to papillary changes in apocrine lesions. Haagensen⁴ showed a relation between large cysts of breast, which are often lined by apocrine metaplastic epithelium, and an increased risk of subsequent development of breast cancer. The evidence for this association, however, is conflicting. While an unexpectedly large number of cancers have been reported after a short follow up of women with cystic change,^{5,6} Dupont and Page,⁷ in their long term follow up study of women with benign breast disease, found an increased relative risk of only 1.7 for those with uncomplicated cystic change. A slightly increased relative risk of 2.4 for subsequent development of breast carcinoma has, however, been noticed for those with complex patterns of papillary apocrine change.⁸

Apocrine adenosis is a rare breast lesion, defined as the presence of apocrine cytology in

a recognisable lobular unit associated with sclerosing adenosis.^{9,10} Wells *et al* have suggested that apocrine adenosis may be a precancerous lesion, mainly on the basis of the expression of the protein product of c-erbB2 oncogene.¹⁰ Apocrine adenosis has been misdiagnosed in the past as carcinoma because of cytological atypia of the apocrine cells. Subsequently, Seidman *et al* reported on the follow up of 37 patients with "atypical apocrine changes superimposed on sclerosing adenosis".¹¹ Four of these, all aged over 60 years, developed carcinoma after a median of 5.6 years, a relative risk of 5.5. To be classified as atypical, the apocrine cells had to have enlarged nucleoli and a threefold variation in nuclear size.

The secretion of gross cystic disease fluid proteins is found in association with apocrine metaplasia, and is thought to be under androgen regulation.^{12,13} Type 1 cysts, which are lined almost always by apocrine epithelium, contain fluid richer in androgens than type 2 cysts, which are lined by flattened epithelium.¹⁴⁻¹⁶ The relative risk of breast carcinoma in patients with type 1 cysts is higher than in those with type 2 cysts,^{17,18} although a recent study disputes this.¹⁹ Androgens in type 1 cysts are thought to be secreted by the lining cells, being present at a higher concentration than in the serum of the patients.^{13,20} This suggests a role for androgen stimulation of the lining cells harbouring the receptors.

This study was undertaken to characterise androgen receptor status in apocrine metaplastic epithelium of the breast and to correlate androgen receptor expression with oestrogen receptor and progesterone receptor status.

Methods

CASE SELECTION

Sixty four cases of apocrine metaplasia and 18 cases of apocrine adenosis were collected from the files of the histopathology department of St Bartholomew's Hospital, London. Some of the apocrine adenosis cases had been sent to CAW as referral cases. The age of the apocrine metaplasia cases ranged from 21 to 81 years (mean 47.3), and that of apocrine adenosis cases from 32 to 63 years (mean 50.3).

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Tissue

Formalin fixed, paraffin embedded blocks of apocrine metaplasia and apocrine adenosis were selected from the files and sectioned at a nominal 4 µm. The standard avidin biotin per-

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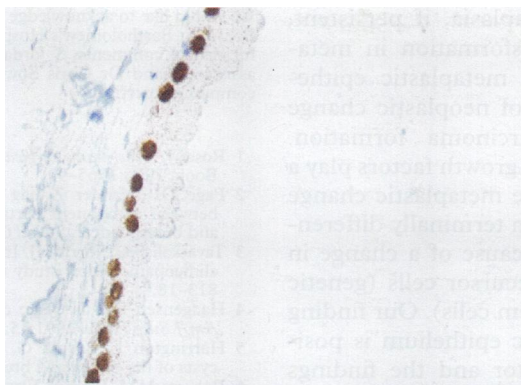


Figure 1 Strong nuclear staining for androgen receptor in apocrine metaplasia of the breast (immunoperoxidase).

oxidase complex (ABC) method²¹ was used. Heat mediated antigen retrieval using the pressure cooking method²² was employed for all staining. Positive controls were prostate for androgen receptor and a known positive breast carcinoma for oestrogen receptor and progesterone receptor. Negative controls omitting the primary antibodies were included with

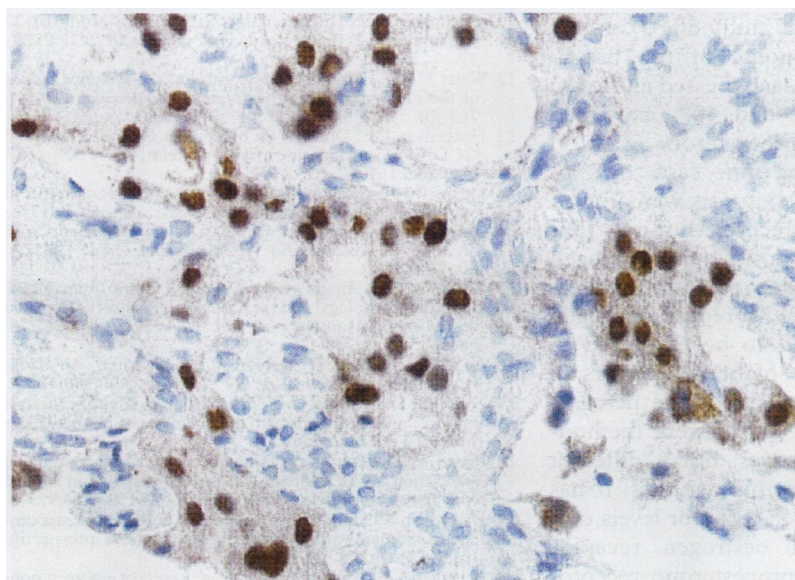


Figure 2 Strong nuclear staining for androgen receptor in apocrine adenosis of the breast (immunoperoxidase).

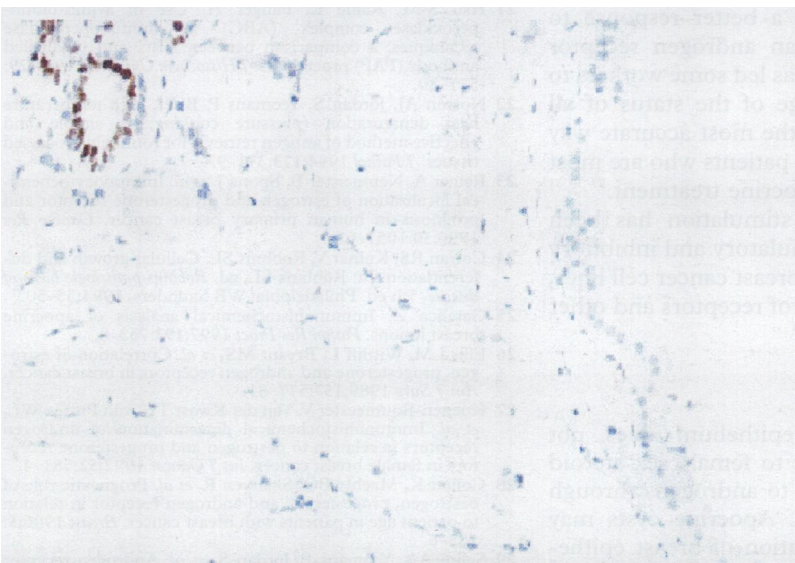


Figure 3 Lack of progesterone receptor nuclear staining in apocrine metaplasia (right). Normal breast epithelium shows progesterone receptor nuclear staining (left) (immunoperoxidase).

each slide run. In addition, the normal breast tissue in the sample served as an internal control.

Antibodies

Monoclonal antibodies against androgen receptor protein (Novocastra, clone 2F12 at 1:50), oestrogen receptor protein (Dako, clone ID-5 at 1:300), and progesterone receptor protein (Novocastra, clone IA-6 at 1:200) were used.

Assessment

Nuclear staining was taken as positive, any cytoplasmic staining being ignored. The quick score method²³ was used for semiquantitation of androgen receptor, oestrogen receptor, and progesterone receptor status.

Results

All cases of apocrine metaplasia (64 cases) (fig 1) and apocrine adenosis (18 cases) (fig 2) studied showed intense nuclear staining with the antibody against androgen receptor in nearly 100% of apocrine cells. The surrounding normal breast epithelium showed weak focal nuclear androgen receptor positivity. Immunostains for oestrogen receptor and progesterone receptor were completely negative in all cases of apocrine metaplasia (fig 3) and apocrine adenosis, but normal breast epithelium showed moderate to strong nuclear staining for oestrogen receptor and progesterone receptor in a proportion of cells. These showed the expected normal distribution pattern.

Discussion

The relation between apocrine cystic changes and breast carcinoma has always been controversial. Clinical follow up studies of women with breast apocrine cystic change have shown an increased risk of subsequent breast carcinoma.^{15 18 20} Wellings and Alpers¹⁵ discovered that cystic apocrine metaplasia is more common in cancer associated breasts than in the normal breast, and foci of apocrine cysts are more numerous in cases of breast carcinoma. The data regarding the relation between apocrine epithelium, cysts and cancer of the breast are conflicting, and the stimuli which lead to these processes are unknown. The identification of androgen receptor immunoreactivity in apocrine metaplastic cells in both apocrine metaplasia and apocrine adenosis is intriguing as the normal breast epithelium shows only focal weak positive cells. These may be a precursor of apocrine metaplasia. In addition, the absence of oestrogen receptor and progesterone receptor in apocrine cells, but not in normal breast epithelium, reflects the fact that these apocrine metaplastic cells differ from normal cells not only morphologically but also biologically.

The stimulus for apocrine metaplasia is unknown, but metaplastic changes are known to relate to cellular stresses or abnormal stimuli in other organs. In the bronchus, for example, columnar epithelium is replaced by squamous epithelium owing to chronic irritation by cigarette smoke. The influences that

predispose to such metaplasia, if persistent, may induce cancer transformation in metaplastic epithelium. This metaplastic epithelium may form the site of neoplastic change and squamous cell carcinoma formation. Chemicals, vitamins, and growth factors play a role in metaplasias.²⁴ The metaplastic change does not take place within terminally differentiated cells but arises because of a change in the differentiation of precursor cells (genetic reprogramming of the stem cells). Our finding that apocrine metaplastic epithelium is positive for androgen receptor and the findings that apocrine cysts contain high concentrations of androgens and other proliferative growth factors^{14 18 20} lead us to postulate a possible role for the continuous stimulation of the normal breast epithelium by androgens and growth factors in the development of metaplastic apocrine cells.

The fact that sex steroid hormones and their receptors act in concert has led some investigators to study the role of the androgen receptor in apocrine and non-apocrine breast cancer patients. Gatalica,²⁵ in his study of 10 cases of invasive apocrine carcinoma of the breast, reported that 80% of these expressed androgen receptor, but only 60% and 40% expressed oestrogen receptor and progesterone receptor, respectively. Also, among nine cases of apocrine ductal carcinoma in situ, six were immunopositive for androgen receptor but only four and three were immunopositive for oestrogen receptor and progesterone receptor, respectively (unpublished data). Moreover, androgen receptor has been found to be expressed in between 35% and 75% of breast cancers in various studies.²⁶⁻²⁹ Variations in the percentages may be attributable to different methodology and different fixatives, but a different case mix may also affect these studies. It has been shown that androgen receptor levels correlate reasonably with oestrogen receptor levels, but better with progesterone receptor levels.^{26-28 30} Androgen receptor positive breast cancer patients have been shown to have prolonged survival and a better response to hormonal treatment than androgen receptor negative patients. This has led some workers to conclude that knowledge of the status of all three receptors may be the most accurate way to identify breast cancer patients who are most likely to respond to endocrine treatment.^{27 30 31} In addition, androgen stimulation has been shown to have both stimulatory and inhibitory growth effects on some breast cancer cell lines, depending on the status of receptors and other growth factor effects.^{32 33}

CONCLUSIONS

Apocrine metaplastic epithelium does not appear to be responsive to female sex steroid hormones but responds to androgens through the androgen receptors. Apocrine cysts may form because of stimulation of breast epithelium by androgens, and women developing such cysts could benefit from androgenic hormone manipulations.

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- Rosai J. *Ackerman's surgical pathology*, 8th ed., Mosby-Year Book, 1996:1565-9.
- Page DL, Vander Zwaag R, Rogers LW, et al. Relation between component parts of fibrocystic disease complex and breast cancer. *J Natl Cancer Inst* 1978;**61**:1055-63.
- Tavassoli FA, Norris HJ. Intraductal apocrine carcinoma: a clinicopathological study of 37 cases. *Mod Pathol* 1994;**7**: 813-18.
- Haagensen DE. Is cystic disease related to breast cancer? *Am J Surg Pathol* 1991;**15**:687-94.
- Harrington E, Lesnik G. The association between gross cysts of the breast and breast cancer. *Breast* 1980;**7**:13-17.
- Roberts MM, Jones V, Elton RA, et al. Risk of breast cancer in women with history of benign disease of the breast. *BMJ* 1984;**288**:275-8.
- Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative disease. *N Engl J Med* 1985;**312**: 146-51.
- Page DL, Dupont WD, Jensen RA. Papillary apocrine change of the breast: association with atypical hyperplasia and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 1996;**5**:29-32.
- Rosen PP, Oberman HA. Tumours of the mammary gland. In: *Atlas of tumour pathology*, 3rd series, fasc 7, Washington, DC: Armed Forces Institute of Pathology, 1993:49-66.
- Wells CA, McGregor IL, Makunura CN, et al. Apocrine adenosis: a precursor of aggressive breast disease. *J Clin Pathol* 1995;**48**:737-42.
- Seidman JD, Ashton M, Lefkowitz M. Atypical apocrine adenosis of the breast: clinicopathological study of 37 patients with 8.7-year follow up. *Cancer* 1996;**77**:2529-37.
- Wales NAM, Ebling FJ. The control of the apocrine glands of the rabbit by steroid hormones. *J Endocrinol* 1971;**51**: 763-70.
- Haagensen DE, Dilley WG, Mazoujian G, et al. Review of GCDFP-15: an apocrine marker protein. *Ann NY Acad Sci* 1990;**586**:161-73.
- Dixon JM, Lumsden AB, Miller WR. The relationship of cyst type to risk factors for breast cancer and the subsequent development of breast cancer in patients with breast cystic disease. *Eur J Cancer Clin Oncol* 1985;**21**: 1047-50.
- Wellings SR, Alpers CE. Apocrine cystic metaplasia: subgross pathology and prevalence in cancer associated versus random autopsy breasts. *Human Pathol* 1987;**18**: 381-6.
- Angeli A, Bradlow HL, Bodian AC, et al. Criteria for classifying breast cyst fluids. *Ann NY Acad Sci* 1990;**586**:49-52.
- Ness JC, Sedghinasab M, Moe RE, et al. Identification of multiple proliferative growth factors in breast cyst fluid. *Am J Surg* 1993;**166**:237-43.
- Bruzzi P, Dogliotti L, Naldoni C, et al. Cohort study of association of risk of breast cancer with cyst type in women with gross cystic disease of the breast. *BMJ* 1997;**314**:925-8.
- Dixon JM, McDonald C, Elton RA, et al. Risk of breast cancer in women with palpable breast cysts: a prospective study. *Lancet* 1999;**353**:1742-5.
- Belanger A, Caron S, Labrie F, et al. Levels of eighteen non-conjugated and conjugated steroids in human breast cysts fluid; relationship with cyst type. *Eur J Cancer* 1990;**26**: 277-81.
- Hsu S-M, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *J Histochem Cytochem* 1981;**29**: 577-80.
- Norton AJ, Jordan S, Yeomans P. Brief, high temperature heat denaturation (pressure cooking): a simple and effective method of antigen retrieval for routinely processed tissues. *J Pathol* 1994;**173**:371-9.
- Reiner A, Neumeister B, Spona J, et al. Immunocytochemical localization of estrogen and progesterone receptor and prognosis in human primary breast cancer. *Cancer Res* 1990;**50**:1057-61.
- Cotran RS, Kumar V, Robbins SL. Cellular growth and differentiation. In: Robbins SL, ed. *Robbins pathologic basis of disease*, 5th ed. Philadelphia: WB Saunders, 1994:35-50.
- Gatalica Z. Immunohistochemical analysis of apocrine breast lesions. *Pathol Res Pract* 1997;**193**:753-8.
- Ellis LM, Wittliff L, Bryant MS, et al. Correlation of estrogen, progesterone and androgen receptors in breast cancer. *Am J Surg* 1989;**157**:577-81.
- Kuonen-Boumeester V, Van der Kwast TH, van Putten WL, et al. Immunohistochemical determination of androgen receptors in relation to oestrogen and progesterone receptors in female breast cancer. *Int J Cancer* 1992;**52**:581-4.
- Collett K, Maehle BO, Skjarven R, et al. Prognostic role of oestrogen, progesterone and androgen receptor in relation to patient age in patients with breast cancer. *Breast* 1996;**5**: 123-6.
- Selim AA, Yeomans P, Jordan S, et al. Androgen receptor expression in ductal carcinoma in situ of the breast: relationship to Gross cystic disease fluid protein-15, apolipoprotein-D and zinc alpha-2 glycoprotein. *J Pathol* 1998;**186**:4.

- 30 Langer M, Kubista E, Schemper M, *et al.* Androgen receptors, serum androgen levels and survival of breast cancer patients. *Arch Gynecol Obstet* 1990;247:203-9.
- 31 Isola JJ. Immunohistochemical demonstration of androgen receptor in breast cancer and its relationship to other prognostic factors. *J Pathol* 1993;170:31-5.
- 32 Hackenberg R, Hawighorst T, Filmer A, *et al.* Medroxyprogesterone acetate inhibits the proliferation of estrogen- and progesterone-receptor negative MFM-223 human mammary cancer cells via the androgen receptor. *Breast Cancer Res Treat* 1993;25:217-24.
- 33 Liberato MH, Sonohara S, Brentani MM. Effects of androgens on proliferation and progesterone receptor levels in T47D human breast cancer cells. *Tumour Biol* 1993;14:38-45.2-9.

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