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Report

Sex steroid receptor expression in 'carcinoid' tumours of the breast

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Summary

Nine 'carcinoids' of the breast (argyrophilic carcinomas) were examined for the presence of estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR), using immunohistochemistry. The tumours were selected on the basis of their histo-morphological appearance and positive Grimelius stain. All cases were immunoreactive for neuron-specific enolase (NSE). In one case the tumour cells were intensely chromogranin A positive. All cases were ER positive, while 5 cases expressed AR and 5 cases PR. Immunostaining for ER and simultaneous demonstration of argyrophilia or chromogranin A expression in chromogranin A positive argyrophilic carcinoid tumour of the breast provided further evidence that neuroendocrine cells in breast tumours express sex steroid receptors. The similarity in sex steroid receptor expression pattern in 'carcinoids' of the breast and the more common categories of breast cancer suggests an identical responsiveness to endocrine therapy.

Introduction

The term 'carcinoid tumour of the breast' is applied to tumours with the classical organoid histomorphological appearance of a 'carcinoid' [1]. These tumours almost entirely consist of argyrophilic cells. However, there is a lot of confusion surrounding these so-called carcinoid tumours of the breast. Features of neuroendocrine differentiation like argyrophilia may also be present in a variable proportion of tumour cells in conventional infiltrating ductal and lobular carcinomas [2–7]. Besides this, only few argyrophilic breast cancers express chromogranin A [8] and there is little evidence of ectopic hormone production [1, 9]. Now, the term 'carcinoma of the breast with neuroendocrine differentiation' is preferred for mammary tumours expressing neuron-specific enolase (NSE) and/or chromogranin irrespective of the histopathological growth pattern.

The responsiveness of the majority of ductal and lobular carcinomas to endocrine therapy is reflected by their frequent expression of ER and PR. Similarly, a large proportion of breast carcinomas contains AR [10, 11]. Except for a few biochemical studies on ER protein [4, 12, 13], no data are available on sex steroid receptor expression in (argyrophilic) tumours of the breast with neuroendocrine differentiation and the histological growth pattern of 'carcinoid'. The aim of this study was to investigate the presence of ER, PR, and AR in the argyrophilic cells of these 'carcinoids' of the breast with a set of

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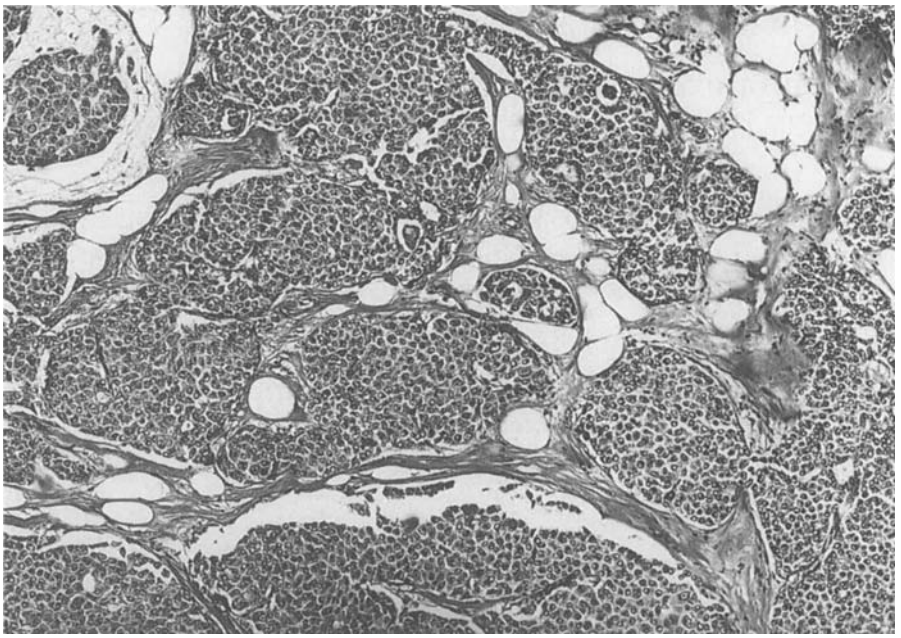

Figure 1. Case 7. Low power micrograph showing the typical histomorphological pattern of a carcinoid tumour. Tumour cells are arranged in larger solid fields. Hematoxylin-eosin. Objective 5 ×.

specific monoclonal antibodies using immunohistochemistry. If the distribution of these sex steroid receptors among 'carcinoids' of the breast is similar to that found in conventional breast carcinomas, they could be regarded as well-differentiated breast cancers with a similar responsiveness to endocrine therapy.

Materials and methods

Paraffin-embedded specimens of 'carcinoid' tumours of the adult female breast fixed in phosphate buffered formaldehyde 4% (pH 7.0) were cut in 5 μm thick sections. Eight cases were selected from the archives of the Dept. of Pathology of the Netherlands Cancer Institute in Amsterdam, and one additional case from the Dept. of Pathology of the University Hospital Dijkzigt in Rotterdam. All patients are still alive, two to five years after diagnosis. Expression of chromogranin A was detected with monoclonal antibody LK2H10 (Euro-diagnostica, Apeldoorn, The Netherlands), diluted 1:30 in phosphate buffered saline (PBS) solution (pH 7.4) followed by a routine streptavidin-biotin-peroxidase complex (ABC) method [14]. An additional case of a typical infiltrating ductal carcinoma with focal expression of chromogranin A was included in the study. Immunoreactivity with a rabbit antibody (diluted 1:800 in PBS) to neuron-specific enolase (Dakopatts, Copenhagen, Denmark) was also demonstrated with a routine ABC method.

Immunostaining for ER, PR, and AR

For detection of ER, PR, and AR, an antigen retrieval method was applied as described previously [15]. Briefly, the sections were dried at 60° C for 20 minutes, deparaffinized in xylene for 7 minutes, and immersed in 100% alcohol. Subsequently, endogenous peroxidase activity was blocked by treatment with 0.3% hydrogen peroxide in methanol for 20 minutes, and the sections were rinsed in distilled water. The slides were placed in a plastic box filled with 0.01 M citrate buffer pH 6.0, and processed in the microwave oven 3 times for 5 minutes at 700 W. After cooling down to room-temperature, the slides were rinsed in PBS and pre-incubated for 15 minutes with normal goat serum (DAKO Glostrup,

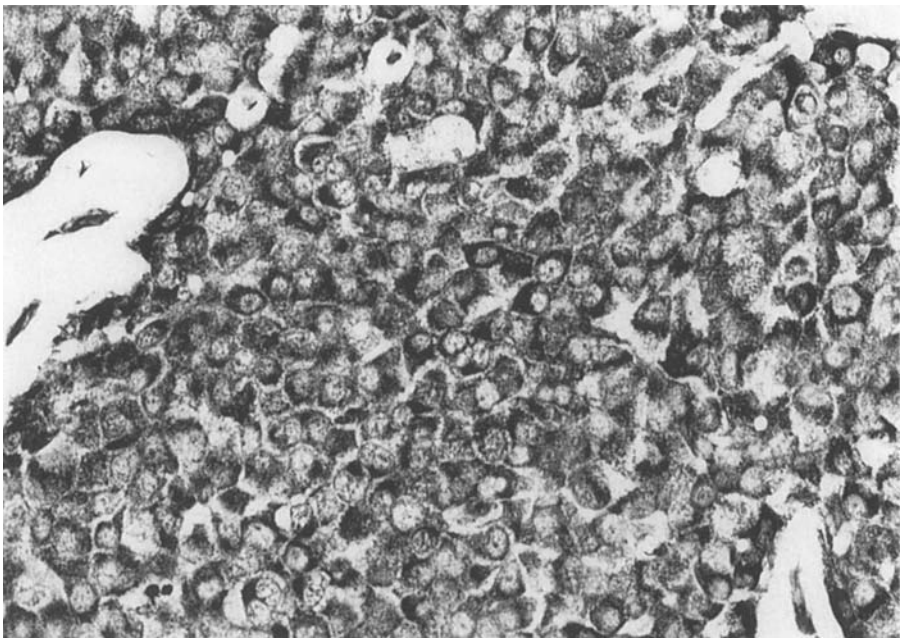

Figure 2. Argyrophilic granules in the cytoplasm (case 7). Most cells are positive. Grimelius silver stain. Objective 40 ×.

Denmark), diluted 1:10 in PBS. Next, the sections were incubated overnight at 4° C with a monoclonal antibody against ER, PR (Immunotech, S.A.) [16], and monoclonal antibody F39.4 (Biogenex, CA, USA) directed against AR [17]. The specificity of F39.4 on paraffin sections for AR has been described earlier [18–20]. A standard ABC technique (DAKO Glostrup, Denmark) was used for visualization with 3,3′-diaminobenzidintetrahydrochloride (Fluka, Basel, Switzerland) as chromogen and H_2O_2 as substrate. As a positive control, breast carcinoma sections known to be positive for ER and PR were used. Prostatic tissue was used as a positive control for AR. As negative controls the primary antibodies specific for ER, PR, AR, and chromogranin A or neuron-specific enolase were substituted by PBS.

Grimelius silver staining

All cases were selected on the basis of their positive reaction with the Grimelius silver-stain essentially as described by Grimelius [21].

Simultaneous detection of ER and argyrophilic granules

Immunostaining for ER was followed by the Grimelius stain. To intensify the argyrophilic staining reaction, the procedure was repeated by performing the cycle of incubations with Grimelius A and B solution up to 4 times five minutes. For visualization of ER by the bound ABC complex, the sections were immersed in freshly prepared 3-amino-9-ethyl-carbazole (AEC) solution yielding a red precipitate. After the Grimelius staining reaction the silver grain precipitate was amplified with an 0.1% (v/v) sodium acetate solution containing 0.02% $AuCl_3$. This solution was kept on the slides for two minutes. Next, the non-specific metal-complex was removed by rinsing with 5% sodium-thiosulfate. The incubation with $AuCl_3$ is probably based on a redox reaction. By this procedure the precipitated silver grains, linked to protein in neuroendocrine cells, assume a darker brown violet colour. In this way background staining was minimized without influencing the intensity of the AEC precipitate. As positive controls pancreas and lung tissue were used.

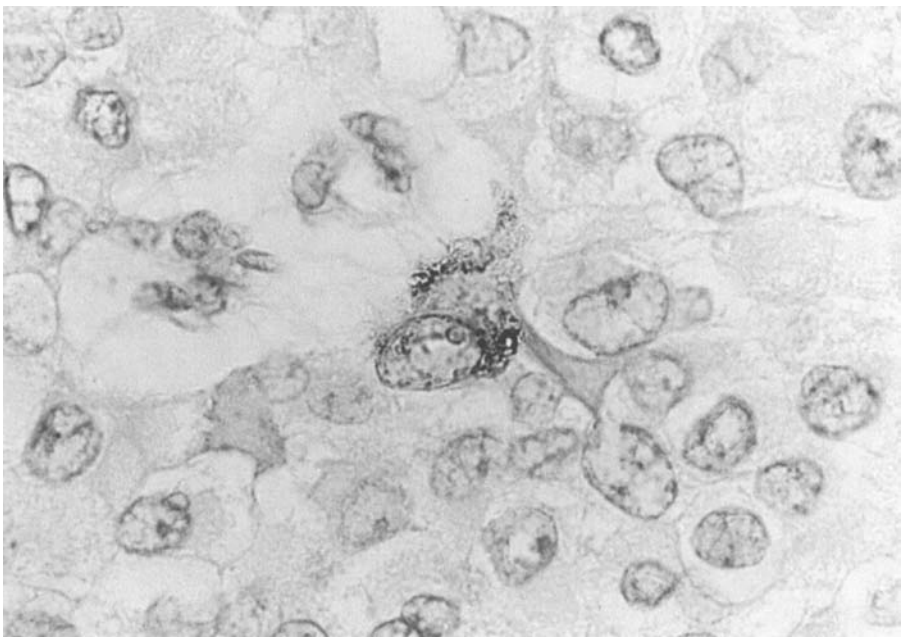

Figure 3. 'Carcinoid' tumour of the breast (case 7) immunostained for chromogranin A. Dispersed cells show immunoreactivity. Objective 100 ×.

Double-staining with chromogranin A and ER

In two cases of 'carcinoid' of the breast, a sequential immunostaining for ER and chromogranin A was performed. We first applied the antibody to chromogranin A in a 1:30 dilution in PBS for 30 minutes, using alkaline phosphatase conjugated rabbit anti-mouse immunoglobulin (DAKO, Denmark) as secondary antibody. The sections were thoroughly washed and subsequently the procedure for ER detection was followed.

Quantification

The percentage of ER, PR, and AR positive tumour nuclei was calculated by counting the number of positive cells on a total of 300 cells in three different tumour areas. Tumours with a staining percentage less than 10% were regarded as negative. We designate our tumours as carcinoids, instead of argyrophilic tumours, to stress that they possess a classical endocrine appearance and are not mucinous tumours (Figure 1).

Results

In the nine 'carcinoids' of the breast we observed three staining patterns after the Grimelius reaction. Five tumours had a faint, rather homogenous granular staining. In three tumours we found scattered intensely positive cells in association with the faint, more diffuse, distribution pattern. One tumour almost entirely consisted of cells with a dense granular staining (Figure 2). In the latter tumour a positive chromogranin A staining was observed (Figure 3), while the remaining cases lacked immunoreactivity for this marker. In all nine cases a homogenous expression of neuron-specific enolase was observed, consistent with their putative neuro-endocrine differentiation.

All nine cases were ER positive (Figure 4), and in 5 cases a large proportion of PR positive tumour cells was present. The percentage of AR positive tumour cells within a tumour was on average lower than that of ER and PR. Three tumours expressed ER as the only receptor and 4 showed ER and PR as well as AR. In some tumours cytoplasmic staining for PR and AR was noted. No stromal sex steroid receptor expression was noted. The distribution of

ER, PR, and AR expression among the 9 'carcinoid' cases is given in Table 1.

Double-staining performed on the 9 'carcinoid' tumours of the breast revealed a coexpression of both argyrophilic granules in the cell's cytoplasm and ER in the cell's nucleus. Similarly, in the one case with a positive chromogranin A staining, tumour cells expressing ER were also chromogranin A positive. The infiltrating ductal carcinoma with scattered neuroendocrine tumour cells showed occasional coexpression of chromogranin A and ER. However, the majority of the chromogranin A positive cells were ER negative.

Discussion

This paper demonstrates that all nine examined 'carcinoid' tumours of the breast express ER. Similarly, Chabon et al. [12] and others [4, 13] found very high levels of ER in breast carcinomas with argyrophilic tumour cells, using a biochemical ligand binding assay. This coincides with the high proportion of ER positive cases in well-differentiated infiltrating ductal carcinomas [22]. The lower number of PR and particularly AR positive tumour cells in argyrophilic carcinomas is also in line with the PR and AR expression in infiltrating ductal carcinomas [10].

A controversial issue is whether (argyrophilic) 'carcinoids' of the breast should indeed be considered true neuroendocrine tumours similar to those encountered in other organs (e.g. those of the gastrointestinal tract). According to several authors, argyrophilia in breast tumours is not necessarily related to the neuroendocrine nature of a breast tumour. These authors related argyrophilia to the presence of milk proteins such as lactalbumin [23–25]. However, these authors used the Sevier-Munger method for demonstration, while Cross [26], Toyoshima [7], and Fetissof et al. [2], did not find any evidence of argyrophilia in lactating breast tissue using the Grimelius procedure. They considered the possibility of lactalbumin as a cause of argyrophilia less likely. In an ultrastructural study Ferguson and Anderson [27], in accordance with Clayton [23], attributed the presence of dense core granules (DCG) to prelactational differentiation rather than neuroendocrine differentiation. The presence of dense core granules in normal non-pathological breast tissue confirms this finding [2, 4–6, 27]. Another argument against the view of 'carcinoids' being a distinct type of breast tumour, is the finding of a high incidence (50%) argyrophilia-positive 'normal breast cancers' by Taxy et al. [4]. Unlike this, Partanen and Azzopardi et al. [5, 6] only found an incidence of resp. 3.3% and 4.5%. The frequently observed absence of chromogranin A (a constituent of neuroendocrine granules) expression in 'carcinoids' of the breast and the lack of con-

Table 1. Sex steroid receptor expression and Grimelius staining pattern in carcinoid tumours of the breast. Tumours with a staining percentage less than 10% were regarded as negative

Cases	Grimelius		ER (%)	AR (%)	PR (%)	Age	Stage
	Diffuse	Focal					
1	+	–	53	21	8	60	T1N0
2	+++	–	11	2	10	68	T1N0
3	++	–	63	33	52	82	T2N0
4	+/–	–	51	0	8	89	T2N1
5	+	++++	54	0	7	78	T2N0
6	++	++++	33	0	6	83	T2N1
7[1]	++++	–	70	14	49	71	T2N1
8	+++	–	63	28	42	75	T1N0
9	+	+++	26	19	13	70	T2N0

[1] On average two chromogranin A positive cells, less than 1%, per tumour field (10 × objective) were seen.

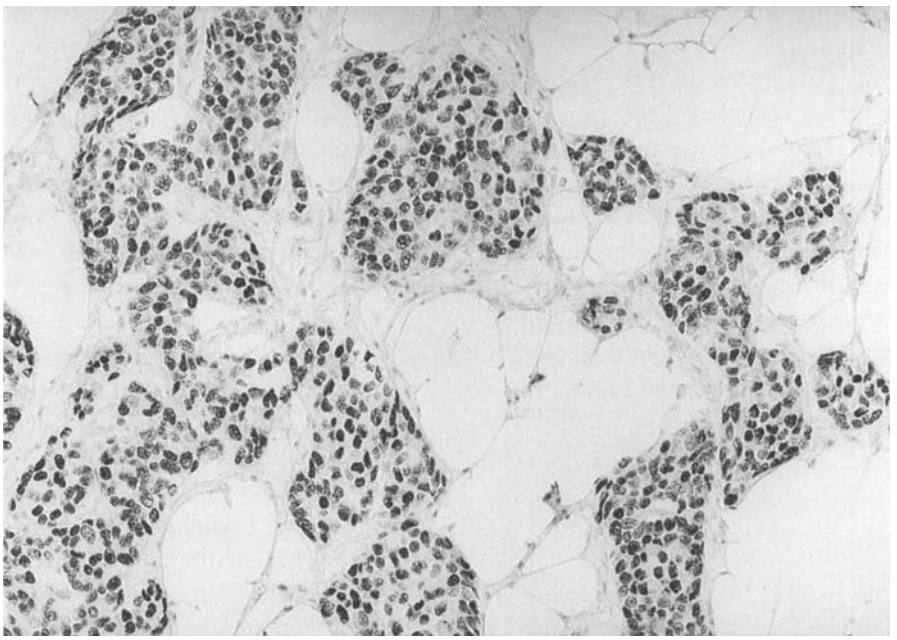

Figure 4. 'Carcinoid' tumour of the breast immunostained for ER. Most nuclei are positive for estrogen receptor (case 7). Objective 20 ×.

sistent evidence that neuroendocrine cells produce ectopic hormones [2, 4, 8], are additional arguments for the view that at least a proportion of the 'carcinoids' of the breast are not true neuroendocrine tumours.

Currently, the presence of scattered (chromogranin A positive) neuroendocrine tumour cells within an infiltrating ductal carcinoma is attributed to a multidirectional differentiation of tumour cells during the neoplastic process [2, 28, 29]. In one case, an ER positive infiltrating ductal carcinoma with focal neuroendocrine differentiation, we showed the expression of ER in only a few chromogranin A positive cells; the bulk of chromogranin A positive cells was ER negative. This observation is comparable to the lack of AR in tumour cells with neuroendocrine differentiation in human prostatic carcinomas [30, 31].

Whatever hypothesis on the nature of carcinoids of the breast holds true, one of our cases represents a true neuroendocrine tumour in that all tumour cells are intensely argyrophylic associated with a strong chromogranin A expression in a small amount of tumour cells (Figures 2 and 4). The high expression of ER (Figure 3) and PR in this case clearly shows that neuroendocrine 'carcinoid' tumour of the breast may express sex steroid receptors like conventional breast carcinomas. Double staining confirmed the ER expression in chromogranin A reactive tumour cells in this case.

Our results with regard to sex steroid receptor expression indicate that 'carcinoids' of the breast have more in common with conventional breast cancer. As to the prognosis, this small series of 'carcinoids' of the breast seems to display a favourable biological behaviour, since all patients are alive two to five years after diagnosis. The low tumour stage similarly reflects a favourable prognosis. This is consistent with the view that 'carcinoids' of the breast should indeed be considered as well-differentiated carcinomas.

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