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The significance of argyrophilia in human breast carcinomas

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Summary. The significance of demonstrating argyrophilia in human breast carcinomas is a complex issue, although there is general agreement that "true" carcinoid tumours of the breast are rare.

A predominantly unselected series of breast carcinomas has been investigated for evidence of argyrophilia using the Churukian Schenk method (Churukian and Schenk 1979), alpha lactalbumin and prealbumin, a marker of neuroendocrine cells.

Argyrophilia has been detected in 25% of carcinomas, including all of mucinous types. However, only 4 of the 68 tumours had a diffuse cytoplasmic reaction typical of that seen in neuroendocrine cells. The others showed a focal or subluminal/peripheral reaction. Those argyrophilic carcinomas with demonstrable alpha lactalbumin had this latter pattern of reactivity, although the milk protein was always detected in lesser amounts by comparison. Prealbumin was only found to varying degrees in eight tumours and the majority of these had a diffuse or focal cytoplasmic argyrophilic reaction.

It would appear that in only a small number of breast carcinomas, approximately six percent, does the presence of argyrophilia probably represent neuroendocrine differentiation, whilst in others it is related to the secretory nature of the tumour cells.

Key words: Breast carcinoma – Argyrophilia – Milk proteins

Introduction

The significance of demonstrating argyrophilia in human breast carcinomas is a topic that causes confusion and controversy, the major problems relating to whether it represents evidence of neuroendocrine differentiation and is related to a specific type of carcinoma. Until these are resolved it will be difficult to assess the role of argyrophilic cells in the development of breast neoplasms and the bearing that argyrophilia may have on their clinical behaviour.

Cubilla and Woodruff (1977) described a small number of breast carcinomas which exhibited argyrophilia and had an organoid growth pattern typical of carcinoid tumours. Subsequent reports have included carcinomas which merit the term carcinoid on morphological as well as argyrophilic terms (Fisher et al. 1979; Gould and Cheifoc 1980; Taxy et al. 1981; Azzopardi et al. 1982). However, several of these studies (Fisher et al. 1979; Taxy et al. 1981; Azzopardi et al. 1982) as well as others (Fetissof et al. 1983; Bussaloti et al. 1985) have demonstrated argyrophilia in breast carcinomas which have the typical appearances of infiltrating ductal, lobular or mucinous carcinomas. The latter tumour is one in which argyrophilia is frequently demonstrated (Fisher et al. 1979; Capella et al. 1980; Fetissof et al. 1983). The other type of tumour in which argyrophilia can be found more readily is intraductal carcinoma (Azzopardi et al. 1982; Cross et al. 1985). The overall incidence of argyrophilia in a non-selected series of breast carcinomas has ranged from 5% (Azzopardi et al. 1982) to 20% (Fetissof et al. 1983).

The endocrine nature of such carcinomas has been investigated by immunohistochemistry. Fisher et al. (1979) failed to identify any of a range of hormones, whilst Cross et al. (1985) demonstrated ACTH in three of seven argyrophilic intraductal carcinomas and Nesland et al. (1986) detected a range of peptide hormones in 12 argyrophilic tumours. There are single case reports of breast carcinomas secreting ACTH (Cohle et al.

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1979; Woodward et al. 1981). The main approach for demonstrating the neuroendocrine nature of certain breast carcinomas has been the identification of neurone specific enolase (Cross et al. 1985; Nesland et al. 1986) or other neuroendocrine markers (Bussolati et al. 1985; Monaghan and Roberts 1985). However, Nesland et al. (1986) questioned the significance of demonstrating neurone specific enolase, and Bussolati et al. (1985) only detected chromagranin in one third of argyrophilic carcinomas.

A further complication has been the report of Clayton et al. (1982) that argyrophilia is a consequence of lactational differentiation, with the granules containing alpha lactalbumin.

We have considered the problems by examining a group of carcinomas for argyrophilia, for alpha lactalbumin and for prealbumin. The distribution of the latter protein has been shown to parallel that of neuroendocrine cells (Liddle et al. 1985) and it represents an alternative marker to those already used.

Materials and methods

Tissues. Samples were available from 68 breast carcinomas which had been excised at Leicester Royal Infirmary between October 1982 and October 1984. The cases were selected at random, apart from the specific inclusion of four mucinous carcinomas.

Tissue from three cases of benign hyperplastic breast disease, one example each of lactating breast, ileum and pancreas and four carcinoid tumours of the appendix were also studied.

All specimens were fixed in 4% formaldehyde in 0.15 M sodium chloride solution, and processed through to paraffin wax.

Reagents. Both primary and secondary antisera were obtained from Dako Ltd. Antiserum to human alpha lactalbumin had been raised in rabbits using purified alpha lactalbumin isolated from human whey and had been shown to react specifically with that and not with serum proteins. Immunoabsorption studies were undertaken using purified alpha lactalbumin from a different source (Sigma). Antiserum to prealbumin was also raised in rabbit. Immunoabsorption was not undertaken but the antibody has been characterised by others (Liddle et al. 1985).

Methods. All tissues were examined for evidence of argyrophilia using the Churukian Schenk modification of the Pascual method (1979).

On the basis of these results all carcinomas with any evidence of staining and ten tumours with no evidence of a reaction were examined for the presence of alpha lactalbumin and prealbumin, as were sections of lactating and benign breast tissue.

The effect of trypsinisation was assessed with parallel sections being treated either with Tris saline buffer pH 7.4 or 0.1% trypsin (Difco 1:250) with 0.12% calcium chloride solution at pH 7.8 for 30 min at 37° C. Endogenous peroxidase was blocked with methanol/HCl for 30 min. Both antisera were applied diluted in Tris-buffer to 1:200 for 18 h at 4° C. After rinsing and washing in buffer, the sections were incubated with antiserum to rabbit immunoglobulin, followed by peroxidase-antiperoxidase complex. The peroxidase was localised by the diaminobenzidine-hydrogen peroxide reaction, and nuclei counterstained with Mayer's haematoxylin.

Controls were: the application of antiserum to alpha lactalbumin absorbed with alpha lactalbumin, 1 mg of antigen being required for 1 ml of antiserum diluted to 1:200 to abolish staining of lactating breast; the substitution of primary antisera by normal rabbit serum; the use of lactating breast as a positive control for alpha lactalbumin; and ileum, pancreas and carcinoid tumours as positive control for prealbumin.

Histology. Haematoxylin and eosin stained sections of all carcinomas were examined and classified according to WHO Criteria (1982).

Results

Argyrophilia

For a carcinoma to be considered to demonstrate argyrophilia there had to be small groups of positive cells present in five or more separate areas throughout the section, as examined with a X10 objective. Seventeen carcinomas were placed in this category (+). However, the proportions of argyrophilic cells and the cellular localisation of the granules varied and three sub-groups could be identified: 1) Carcinomas in which more than 50% of the cells were argyrophilic and the granules were dispersed throughout the cell cytoplasm (Fig. 1), there being four such tumours. Two of these were mucinous carcinomas the other two being infiltrating duct carcinomas, but with one having mucinous areas. 2) Tumours with lesser numbers of argyrophilic cells, never more that 20% in which the granules were present only focally within the cell cytoplasm, this group numbering seven carcinomas and including two infiltrating lobular carcinomas. 3) Carcinomas in which the argyrophilic granules were present just under or at the periphery of cells either in relation to lumens (Fig. 2) or extracellular mucin (Fig. 3). The proportion of reactive cells varied throughout the group which totalled six. Two of the tumours were mucinous, two were tubular and the others infiltrating duct carcinomas. A further 13 carcinomas contained a very occasional argyrophilic cell and were categorised as (±). Thirty eight of the 68 carcinomas were negative. The relationship between the category of argyrophilic reaction and type of tumour is shown in Table 1.

There was no evidence of argyrophilia in the hyperplastic breast tissue. Subluminal argyrophilic granules were present in many of the cells of the pregnant breast.

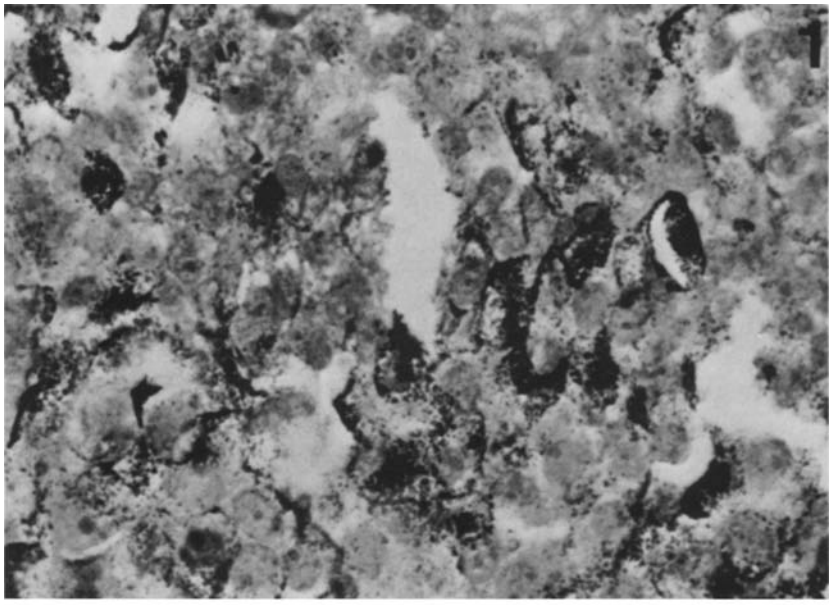

Fig. 1. Infiltrating duct carcinoma in which many of the cells are argyrophilic with a predominance of a diffuse cytoplasmic reaction. Churukian Schenk. (Magnification × 220)

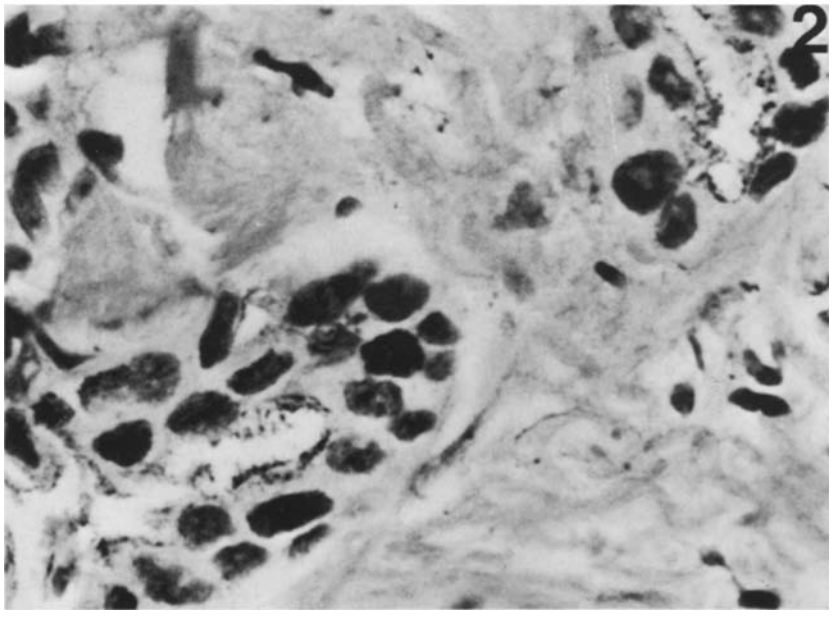

Fig. 2. Tubular carcinoma in which the argyrophilic granules are beneath the luminal membrane of the cells. Churukian Schenk. (Magnification × 220)

Alpha lactalbumin

Trypsinisation of sections always resulted in more clearly defined staining. No reaction was seen in sections treated with antiserum absorbed with 1 mg/ml of alpha lactalbumin in comparison to unabsorbed antiserum.

Forty carcinomas were examined for the presence of alpha lactalbumin (17 argyrophilic positive; 13 (±), 10 negative). In eight there were small groups of cells having a staining reaction present in three or more separate areas of the tumour (Fig. 4). A further eight carcinomas had very occasional reactive cells. The comparison between expression of alpha lactalbumin and argyrophilia is shown in Table 2. Six of the eight carcinomas with detectable alpha lactalbumin were argyrophilic, having either focal cytoplasmic or subluminal granules. Two were mucinous, two tubular and the other two infiltrating duct carcinomas. Within each tumour the number of argyrophilic cells exceeded those in which alpha lactalbumin was detected. In some cases the reactions were possibly in the same cell but in others the staining for alpha lactalbumin was observed in different areas to that of the argyrophilia. The other two tumours with

Table 1. The numbers of carcinomas having a particular category of argyrophilic reaction related to tumour classification

Type of carcinoma	Argyrophilic category		
	Positive	A few positive cells	Negative
Infiltrating duct	9	12	35
Infiltrating lobular	2	1	3
Mucinous	4	0	0
Tubular	2	0	0

detectable alpha lactalbumin had very occasional argyrophilic cells, whilst the majority of those with minimal reactivity for the milk protein were argyrophilic.

Alpha lactalbumin was demonstrated in all epithelial cells of the pregnant breast but in none of the cases of benign fibrocystic disease.

Prealbumin

Again, trypsinisation was found to improve the quality of the staining reaction. Prealbumin was detected in cells of the pancreas (Fig. 5), ileum and carcinoid tumours.

In 3 of the 40 carcinomas examined there were small groups of cells with a cytoplasmic reaction in three or more separate areas of the tumour (Fig. 6). A further five had a very occasional reactive cell. Six of these eight had evidence of argyrophilia, with either diffuse or focal cytoplasmic granules (Table 2). One was a mucinous carcinoma, the others infiltrating duct tumours.

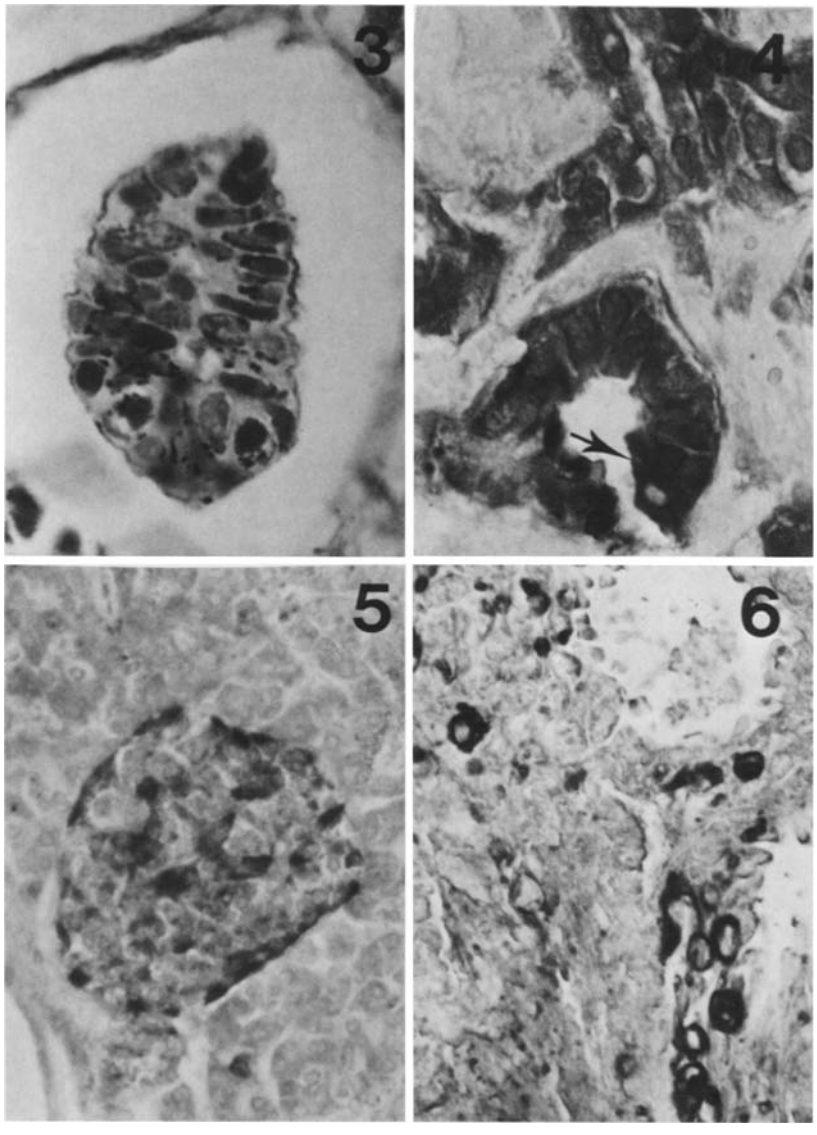

Fig. 3. Mucinous carcinoma with argyrophilic granules at the periphery of some cells, beneath the luminal membrane in others and with some having focal reactivity. Churukian Schenk. (Magnification × 220)

Fig. 4. Alpha lactalbumin demonstrated in a well differentiated carcinoma. Immunoperoxidase. (Magnification × 220)

Fig. 5. Prealbumin detected in the cells of the Islets of Langerhans, confirming its association with neuroendocrine cells. Immunoperoxidase. (Magnification × 220)

Fig. 6. Infiltrating duct carcinoma in which cells containing prealbumin were detected. Immunoperoxidase. (Magnification × 160)

Table 2. The correlation between argyrophilic reaction and staining for alpha lactalbumin and prealbumin within carcinomas

Argyrophilia	Alpha lactalbumin			Prealbumin		
	Present	A few cells	Absent	Present	A few cells	Absent
Present	6	6	5	2	3	12
A few cells	2	1	10	1	0	12
Absent	0	1	9	0	2	8

There was no evidence of prealbumin in pregnant or benign fibrocystic breast.

Discussion

In this study convincing evidence of argyrophilia has been found in 25% of predominantly unselected breast carcinomas with an occasional reactive cell demonstrated in a further 19% of tumours. The incidence is greater than other studies (Azzopardi et al. 1982; Fetissof et al. 1983). This may be related to the argyrophilic procedure employed since the majority of studies have used the Grimelius method, whilst we have employed the Churukian Schenk modification of that method. This technique has been shown in other tissues and particularly in carcinoid tumours to result in a higher incidence of detection of argyrophilia (Smith and Haggit 1983). However, three patterns of staining could be discerned. Only four of the carcinomas had a diffuse cytoplasmic granular reaction, typical of that observed in "true" carcinoid tumours, although none had the morphological features of such a tumour. Subluminal or peripheral granules were a feature of six carcinomas and also pregnant breast. A similar distribution has been observed in a tubular carcinoma and pregnant breast by Anderson et al. (1985), who advocated caution in the interpretation of results from argyrophilic studies.

Several studies have detected argyrophilia in conventional infiltrating carcinomas (Partanen and Syrjanen 1981; Taxy et al. 1981; Fetissof et al. 1983). Capella et al. (1980) found a high incidence of argyrophilia in a subtype of mucinous carcinomas and similar observations have been made by Fisher et al. (1978). All four mucinous carcinomas in the present study had evidence of argyrophilia, two having a diffuse cytoplasmic granular reaction and two subluminal/peripheral granule distribution. Cross et al. (1985) identified an argyrophilic endocrine variant of ductal carcinoma-in-situ but commented that only a minority of argyrophil invasive carcinomas had a comparable endocrine structure.

Neuroendocrine differentiation of breast has been investigated using a variety of antibodies directed against enzymes and proteins present in neural and neuroendocrine cells. Bussolati et al. (1985) suggested that the presence of chromogranin in certain breast carcinomas was evidence of the endocrine nature of these tumours, and Monaghan and Roberts (1985) drew similar conclusions from demonstrating E36 immunoreactivity. We considered another protein, prealbumin, whose tissue distribution parallels that of neuroendocrine cells and which has been reported to be a marker of carcinoid tumours (Miller et al. 1984). In only a small number of carcinomas with argyrophilia could prealbumin be detected giving further support to the view that the demonstration of argyrophilia in carcinomas of the breast does not equate them to carcinoid tumours.

Clayton et al. (1982) suggested that argyrophilia in breast carcinomas was related to milk secretory products rather than the neuroendocrine nature of the cells. In the present study those carcinomas with detectable alpha lactalbumin were more likely to be argyrophilic, and to have either focal or subluminal granule localisation. The degree of argyrophilia was always more than the detectable alpha lactalbumin. The presence of the milk protein probably reflects the general secretory capacity of the carcinomas. We would suggest that in those carcinomas which have focal or subluminal/peripheral granules argyrophilia is related to the secretory nature of the cells, which would include the presence of alpha lactalbumin.

These studies confirm that the presence of argyrophilia in breast carcinomas is complex. In a small group it probably does reflect evidence of neuroendocrine differentiation but in others it is related to the secretory nature of the cells, as suggested by Anderson et al. (1985), hence care is needed in the interpretation of its demonstration.

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