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Ultrastructural and immunohistochemical characteristics of lipid-rich carcinoma of the breast

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Summary. Five cases of lipid-rich carcinomas of the breast were investigated ultrastructurally and immunohistochemically for alpha-lactalbumin (ALA), lactoferrin (Lfr) and human milk fat globule membrane antigen (HMFG-2). Staining for ALA and Lfr showed intensive reaction on nearly all of the tumour cells whereas immunoreaction for HMFG-2 revealed positivity in single cells. All tumours were negative for steroid receptor content. Ultrastructurally the tumour cells showed numerous intracytoplasmic non-membrane bound lipid droplets which were often found within autophagocytic vacuoles. Neither rough endoplasmic reticulum nor Golgi complexes showed any sign of lipid synthesis. Extrusion of lipid droplets and extracellular lipid deposition was not observed. In conclusion, our findings do not justify the consideration of lipid-rich carcinoma of the breast as a clearly defined group of tumours with specific secretory activity. Therefore, the term lipid-rich carcinoma should be used in preference to lipid-secreting, unless there is evidence of active lipid secretion.

Key words: Lipid rich carcinoma – Breast – Immunohistochemistry – Ultrastructure

Introduction

Lipid-rich carcinoma is a rare neoplasm of the breast. The morphology of this tumour was first described by Aboumrad et al. (1963). In a subsequent publication three histological variations were observed, histocytoid type, sebaceous type and carcinoma with apocrine extrusion of nuclei (van Bogaert and Maldague 1977). Lipid-rich carcinomas were reported to comprise about 1%–2% of primary breast carcinomas (Aboumrad et al. 1963; Ramos and Taylor 1974; van Bogaert and Maldague 1977; Wrba and Holzner 1985). The term lipid-rich carcinoma introduced by Ramos and Taylor (1974) is in contrast with the description of lipid-secreting carcinoma (Aboumrad et al. 1963; van Bogaert and Maldague 1977; Vera-Sempere and Llombardt-Bosch 1985) suggesting a distinct entity of breast carcinoma characterized by secretory differentiation of the tumour cells. Ultrastructural studies on two patients performed by different investigators seemed to support these suggestions (Ramos and Taylor 1974, Vera-Sempere and Llombardt-Bosch 1985).

The purpose of this study was to investigate the postulated secretory differentiation of five cases of lipid-rich carcinoma of the breast ultrastructurally. Furthermore, immunohistochemistry for alpha-lactalbumin (ALA), lactoferrin (Lfr) and human milk fat globule membrane antigen (HMFG-2) was carried out on this putative entity.

Materials and methods

We have collected five tumours of this type during the last three years, representing a percentage of 0.8 in the material of breast carcinomas in this period.

Formalin-fixed paraffin-embedded tissue sections were stained with haematoxilin-eosin, PAS, PAS after diastase digestion (D-PAS) and Alcian blue. Additional frozen sections were stained with Sudan III. The tumours were classified according to Aboumrad et al. (1963) The histological grade was determined as described by Bloom and Richardson (1957).

For immunohistochemistry four µm sections were deparaffinized in xylene, placed in absolute ethanol and hydrated in graded alcohols and phosphate buffered saline (PBS, pH 7.4). In addition, indirect immunoperoxidase (three step technique, Ancelin et al.1984) or peroxidase-antiperoxidase (PAP) method (Sternberger 1979) were carried out. The antibodies used are outlined in Table 1. Peroxidase activity was visualized using 3,3 diaminobenzidine-tetrahydrochloride (DAB, 0,05%, Sigma

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Table 1.

Antibody Species	Working dilution[a] (method)	t[b]	Source
ALA r	1:250 (PAP)	30′	Nordic/Netherlands
Lfr r	1:250 (PAP)	30′	Nordic/Netherlands
HMFG-2 m	1:100 (indirect)	30′	Seward (UK)

[a] All antibodies used were diluted in PBS and 5% human AB serum

[b] Duration of incubation in minutes at room temperature

Abbreviations: r, polyclonal rabbit antiserum; m, monoclonal mouse antibody; PAP, peroxidase-antiperoxidase method; indirect, three step technique

Chemical Co, USA) and H_2O_2 (0,01%) in PBS as chromogen. Sections were counterstained with Mayer's haematoxylin and mounted with glycerine-gelatine. Control studies performed by omitting the first antibody yielded negative results.

For electron microscopy 5–8 tumour pieces of each case were fixed in phosphate buffered glutaraldehyde (2,5%, pH 7,5) and postfixed in cacodylate buffered osmium tetroxide. Subsequently, the tissue blocks were dehydrated in graded series of alcohols and embedded in Epon. Ultrathin sections were stained with uranyl acetat and lead citrate and examined with a Zeiss EM 9 S electron microscope.

Cytoplasmic estrogen and progesterone receptors (ER and PgR) were quantified by the dextran-coated charcoal (DCC) method (Reiner et al. 1981). Tumours were considered ER and/or PgR positive if the respective receptor concentration was greater than 9 fmol/mg cytosol protein.

Results

The major clinical and pathological findings are summarized in Table 2. All patients were female. The ages at the time of surgery were 62, 64, 80 and 81 years. Two of the tumours were situated in the right breast (case 1: in the upper outer and case 2: in the lower inner quadrant) and three were situated in the left breast (case 4, 5 in the upper outer quadrant, case 3: extended to the whole breast).

The tumours were circumscribed, soft and grey-white in colour. One lesion (case 3) was described as infiltrating and ulcerating the skin.

Light microscopy reveals a similar appearance in all five cases (Fig. 1). With Sudan III staining a positive reaction for neutral fat is observed in nearly all of the tumour cells. PAS, D-PAS and Alcian blue staining are consistently negative.

All tumours are positive for ALA, Lfr, and HMFG-2. Staining for ALA and Lfr show marked reactivity on nearly all of the tumour cells. HMFG-2 reveals strong staining reaction of single tumour cells (Figs. 2, 3, 4).

On electron microscopy the cells possess numerous intracytoplasmic spherical, non-membrane bound lipid droplets of low and high density surrounded by a dense osmiophilic rim (Figs. 5, 6). Numerous autophagocytic vacuoles containing lipid droplets are been detected (Fig. 7). Mitochondria as well as endoplasmic reticulum and Golgi complexes are inconspicuous. Crystals are not obvious. No evidence of glycogen is seen. Small intracytoplasmic lumina with associated microvilli are sporadically detected (not shown). No evidence of extracellular lipid deposits can be seen.

All the tumours are negative for ER and PgR.

Discussion

Ultrastrucutral findings of lipid-rich carcinoma of the breast have been described in two cases (Ramos and Taylor 1974; Vera-Sempere and Llombardt-Bosch 1985). These publications agree on the presence of a prominent rough endoplasmatic reticu-

Table 2. Clinical data and pathological findings

No	Age in years (at surgery)	Size (cm)	Axillary lymphnodes[a]	Treatment	Follow up
1	80	2.5	1/ 6	MRM	died 6 week after surgery of cardiac insufficiency
2	62	2.0	1/ 9	MRM + CT	2.5 years after surgery alive with pulmonary metastasis
3	62	15.0	ND	Qu	died 3 weeks after surgery of generalized metastases
4	64	2.5	3/ 6	RT prior to MRM	2.5 years after surgery free of metastases
5	81	1.9	0/10	MRM	too recent

[a] Number of lymphnodes involved/number of lymphnodes examined

Abbreviations: ND, not determined; MRM, modified radical mastectomy; Qu, Quadrantectomy; CT, Chemotherapy; RT, Radiotherapy

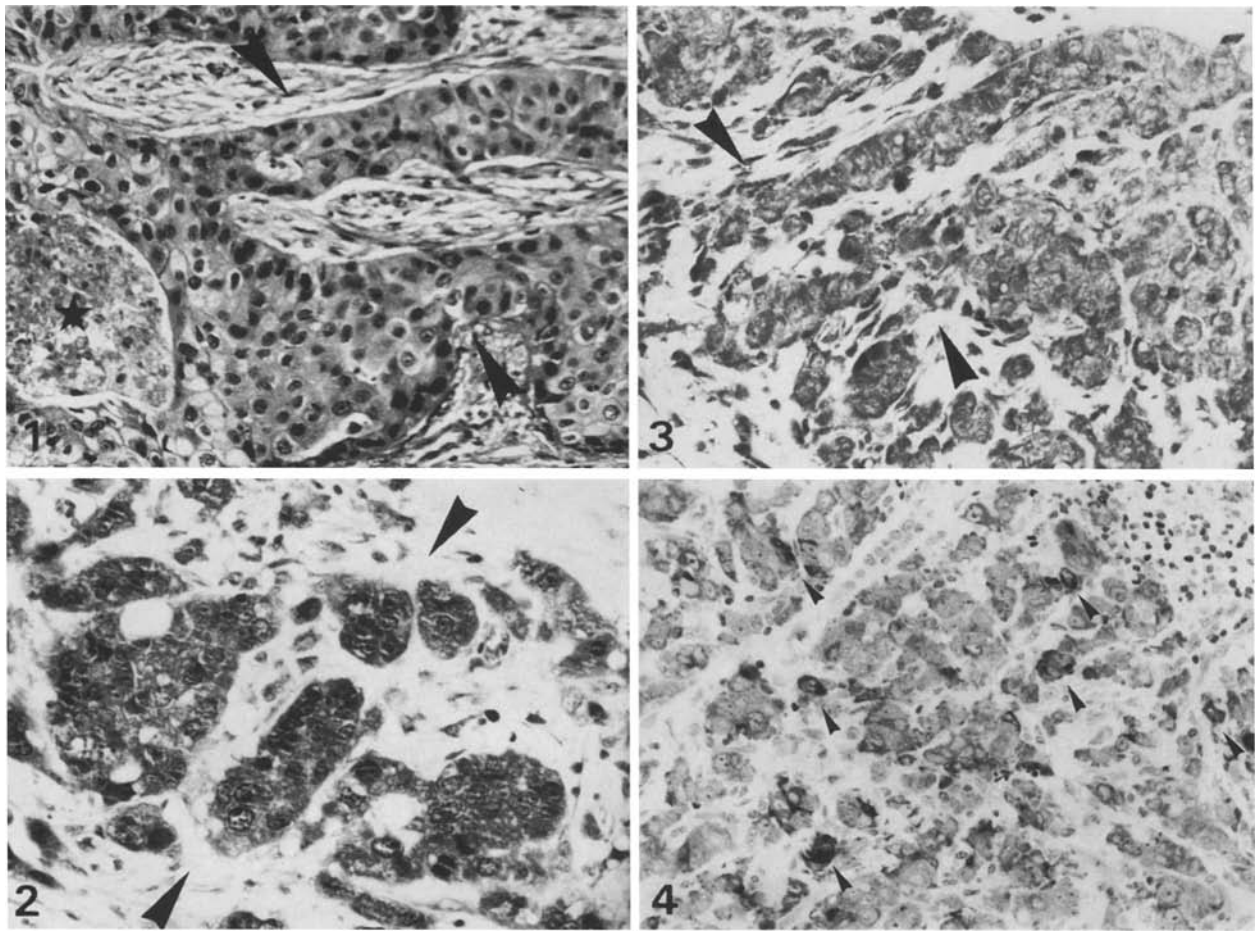


Fig. 1. Ductal carcinoma, G 3. Well defined cells with slight foamy cytoplasm and polymorphous nuclei forming solid sheets (*arrowheads*). Area of tumour necrosis (*asterisk*; case 1, H & E, ×140)

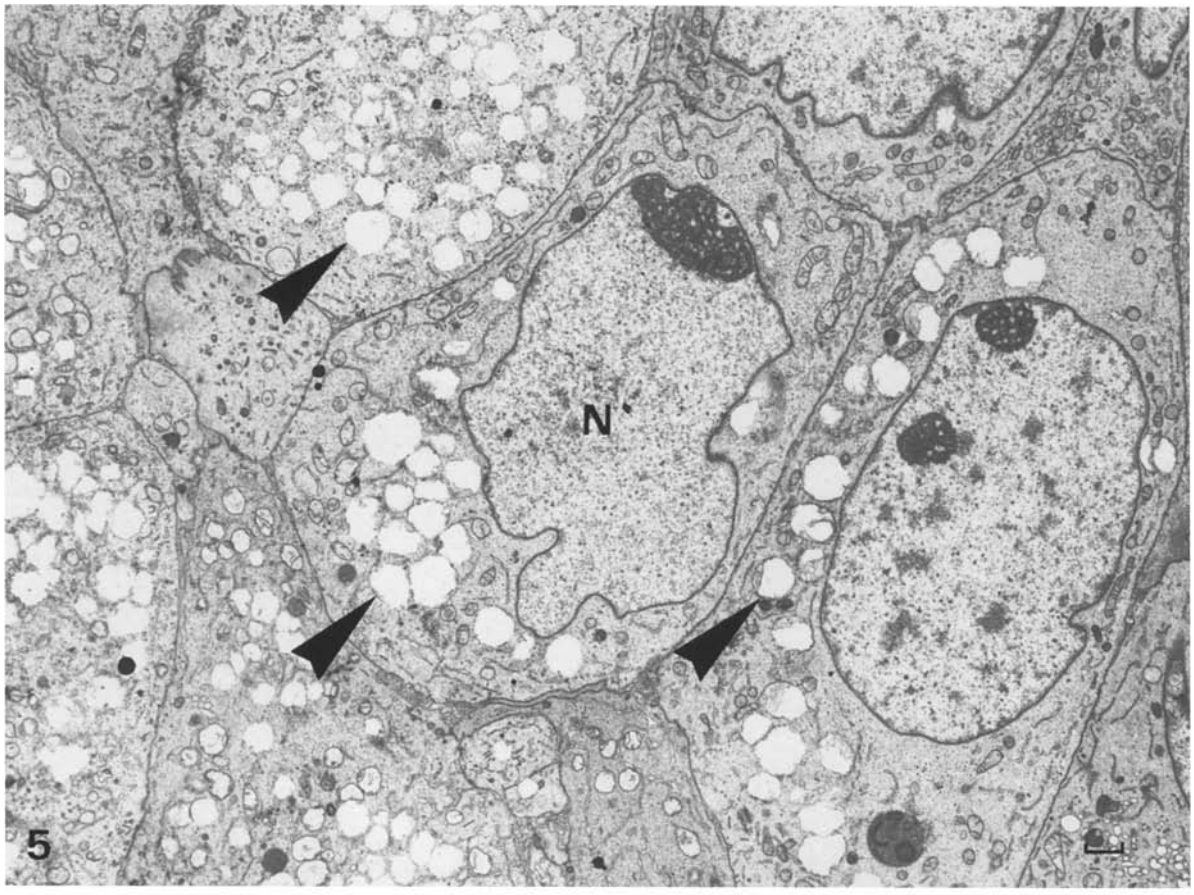
Fig. 2. Paraffin section of case 3, showing intense staining of tumour cells for ALA (*arrowheads*; immunohistochemistry, ×70)

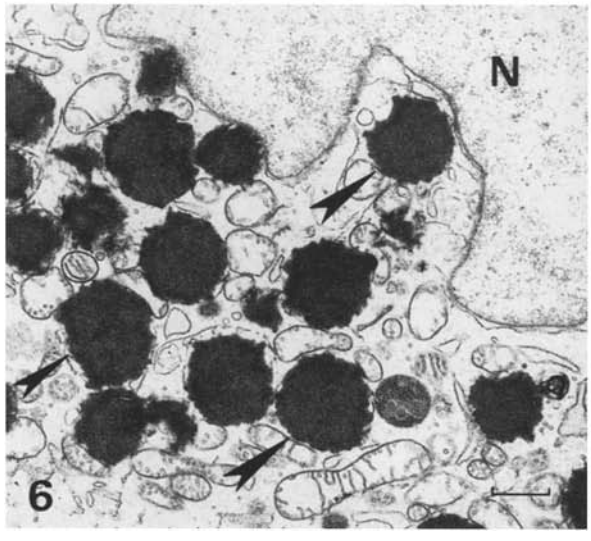
Fig. 3. Paraffin section of case 4, showing intense staining of tumour cells for Lfr (*arrowheads*; immunohistochemistry, ×70)

Fig. 4. Paraffin section of case 2, showing staining reaction of single tumour cells for HMFG-2 (*arrowheads*; immunohistochemistry, ×70)

lum, interpreted as a sign of an active secretory cell. Moreover, Ramos and Taylor (1974) described a well developed Golgi complex as a further hint of the presence of secretory cell type. In our material rough endoplasmatic reticulum and Golgi complexes were inconspicuous. Numerous tumour cells in the cases investigated showed autophagic vacuoles containing lipid droplets which are probably a sign of degenerative processes. Additionally, neither extracellular lipid deposition nor signs of an active secretion could be found. Ramos and Taylor (1974) described the presence of hydroxyapatite crystals in the mitochondria as being typical of lipid-rich carcinomas. In our material deposition of intramitochondrial crystals was not obvious, suggesting considerable ultrastructural heterogeneity of this tumour type.

Almost all tumour cells of the five cases examined reacted positvely for ALA and Lfr. Therefore it is conceivable that the intracytoplasmic lipid droplets may contain ALA and Lfr. However, positivity for ALA and Lfr seems not to be specific for certain histological tumour type. As reported earlier a large number of breast carcinomas demonstrated positivity for ALA and Lfr (Walker 1984; Wrba et al. 1988). Staining for HMFG-2 was weak and only a small number of positive cells were present in the tumours investigated. Since all tumours were poorly differentiated (G 3) this finding is in accordance with the fact that HMFG-2





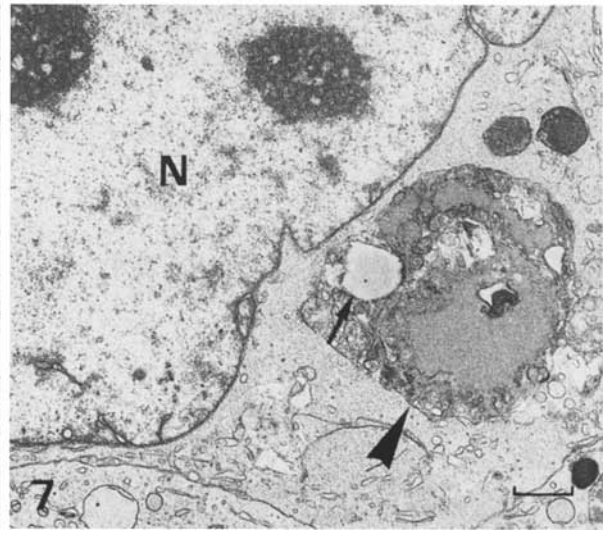


Fig. 5. Electron microscopy: tightly apposed tumour cells containing numerous lipid droplets of low density surrounded by a dense osmiophilic rim (*arrowheads*; *N*, nucleus; case 3, ×4800; space bar 1 μm)

Fig. 6. Electron microscopy: lipid droplets of high density (*arrowheads*; *N*, nucleus; case 1, ×7500; *space bar* 1 μm)

Fig. 7. Electron microscopy: autophagocytic vacuoles (*arrowheads*) containing lipid droplets (*arrow*; *N*, nucleus; case 1, ×7800; *space bar* 1 μm)

expression correlates with histological tumour grade (Wrba et al. 1988). Additionally, all the tumours were negative for ER and PgR content, presumably by virtue of low degree of differentiation.

In conclusion, even if negativity for ER and PgR and low immunoreactivity for HMFG-2 are due to the poor differentiation, our ultrastructural findings do not justify the consideration of lipid-rich carcinoma of the breast as a morphologically well defined group of tumours with specific lipid secretion. Moreover, in our opinion, the term lipid-rich carcinoma should be used in preference to lipid-secreting, unless there is evidence of secretory activity of lipid substances.

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