Histol Histopath (1987) 2: 73-77

Histology and Histopathology

Electron microscopy and immunostaining of the normal breast and its benign lesions. A search for neuroendocrine cells

Jahn M. Nesland[1,3], Sigurd Lunde[1], Ruth Holm[1,3] and Jan Vincents Johannessen[1,2,3]

[1]Department of Pathology, The Norwegian Radium Hospital and Institute for Cancer Research, Oslo, Norway;
[2]Department of Pathology, Columbia University College of Physicians and Surgeons, New York, USA;
[3]The Norwegian Cancer Society, Oslo, Norway

Summary. Specimens from 7 patients with normal breast tissue 26 patients with benign breast lesions (6 fibroadenomas, and 4 intraductal papillomas, 2 mammae lactantes, 10 cases of cystic disease and 4 fibrotic lesions) were studied by immunocytochemistry and electron microscopy. Excretory epithelial cells in 2 of the 4 papillomas were immunostained for NSE. Myoepithelial cells were frequently stained as well. All the breast specimens were nonreactive to the antichromogranin antibody we used.

The 2 NSE positive intraductal papillomas were tested for presence of hormone immunoreactivity, but no positively stained cells were observed.

No cells with neuroendocrine features were observed by electron microscopy.

The present study did not reveal neuroendocrine cells in the normal breast specimens and undisputed proof of neuroendocrine differentiation in benign breast lesions was not established. We conclude that if neuroendocrine cells are present in the normal breast, they are very rare, and probably not the cellular origin of all breast carcinomas with neuroendocrine features.

Key words: Breast - Neuroendocrine cells

Introduction

The presence of neuroendocrine cells in the normal breast is a matter of debate (Vogler, 1947; Nesland et al., 1983; Bussolati et al., 1985; Nesland et al., 1986b). Vogler (1947) observed argyrophilic cells scattered in between the excretory epithelium and the layer of myoepithelial cells of normal ducts. He suggested that these cells belonged to the diffusely distributed system of argyrophilic cells, "Das Helle-Zellen Organ", described by Feyrter in 1938.

Bussolati and co-workers (1985) studied a series of benign and malignant argyrophilic breast lesions and observed chromogranin-reactive cells in histologically normal breast tissue from 2 cases obtained by mastectomy and from 1 case obtained by cosmetic surgery. Their conclusion was that besides secretory epithelial and myoepithelial cells, an endocrine-like argyrophilic chromogranin-reactive cell type is normally present.

In earlier studies of neuroendocrine differentiation in breast carcinomas (Nesland et al., 1985, 1986, a,c) we were unable to recognize neuroendocrine cells in the ducts of normal appearance that surrounded the tumour areas, and immunostaining with anti-NSE antibodies was only positive in the myoepithelial cells surrounding these ducts.

In the present study we searched normal breast tissue and benign breast lesions for neuroendocrine cells, using electron microscopy and immunostaining with anti-NSE, anti-chromogranin and antibodies against a series of different hormones.

Materials and methods

Morphologically normal breast tissue from 7 patients who had undergone surgery for cosmetic reasons, and breast tissue from 26 patients treated for benign breast lesions (6 fibroadenomas, 4 intraductal papillomas, 2 mammae lactantes, 10 cases of cystic disease and 4 cases of fibrotic breast tissue) were studied.

Formalin-fixed, paraffin-embedded material was used for immunocytochemical studies with the avidin-biotin peroxidase complex (ABC) method (Hsu et al., 1981), using the antisera and conditions shown in Table 1. After removal of paraffin, sections were treated with 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous

Offprint requests to: Dr. J.M. Nesland, Department of Pathology, The Norwegian Radium Hospital, Montebello, 0310 Oslo 3, Norway.

peroxidase, followed by incubation for 20 minutes with normal goat serum diluted 1:75 in 0.01 M phosphate buffered 0.15 saline (PBS), pH 7.4, containing 25% bovine serum albumin (BSA) to eliminate non-specific staining. The sections were then incubated with the specific antisera, followed by incubation with 1:200 dilution of biotin-labelled secondary antibody for 30 minutes and ABC (10 µg/ml avidin and 2.5 µg/ml biotin-labelled peroxidase) for 60 minutes (Vector, Burlingame, CA). Tissues were stained for 5 minutes with 0.05% 3'3 diamino-benzidine tetrahydrochloride, freshly prepared in 0.05 M Tris buffer pH 7.6, containing 0.01% hydrogen peroxide and then counterstained with haematoxylin before dehydration and mounting. Control studies included 1) relevant positive controls, 2) the use of nonimmune serum as the first layer, 3) incubation with primary antibody preabsorbed with the homologous antigen.

Tissues for electron microscopy were fixed in a cacodylate-buffered mixture of 4% formaldehyde and 1% glutaraldehyde (McDowell and Trump, 1976), postfixed in buffered 1% osmium tetroxide, dehydrated in graded ethanols and embedded in an Epon Araldite mixture (Mollenhauer, 1964). Semithin sections were cut with glass knives, stained with toluidine blue and used for light microscopic orientation. Ultrathin sections were cut with diamond knives and stained with uranyl acetate and lead citrate before examination in the electron microscope.

Results

Immunostaining with anti-NSE

Myoepithelial cells in the 7 cases of normal breast tissue and in the 26 benign breast lesions were frequently stained with rabbit anti-NSE. A diffuse cytoplasmic staining of of varying intensity was present. No other cells in the normal breast tissue specimens revealed immunoreactivity for NSE.

In 2 out of 4 intraductal papillomas NSE immunoreactivity was seen in cells indistinguishable from excretory epithelium. In both cases a diffuse cytoplasmic staining was present in groups of epithelial cells (Fig. 1).

Immunostaining with anti-chromogranin

All breast specimens were unreactive, even the intraductal papillomas where abundant immunoreactivity for NSE was seen in epithelial cells.

Immunostaining for hormones

Serial sections from the 2 NSE positive intraductal papillomas were tested for presence of hormones. However, positively stained cells were not observed in any of the cases.

Control studies

No staining was present in sections incubated with antisera absorbed with their homologous antigens prior to testing, or in sections where nonimmune serum was used instead of specific antiserum. However, tissues used as positive controls all showed specific immunostaining.

Electron microscopy

The ultrastructural features of the cases included in this study were as described in an earlier report (Nesland et al., 1983) and neuroendocrine granules were not seen. Exocrine granules were frequently encountered in the apical parts of cytoplasm and around intracytoplasmic lumina (Fig. 2). Cytoplasmic aggregates of fine filaments, frequently observed in endocrine cells, were not seen.

Fig. 1. Intraductal papilloma with a large group of NSE-positive cells. Other parts of the papilloma (asterisk), as well as the ductal epithelium (arrows) next to the papillomatous area are unstained (anti-NSE X 300).

Table 1.

Antiserum against [1]	Dilution	Incubation time	Source
NSE	1:200	30 min, room temp.	Dako Corp. USA
Chromogranin	1:600	18 - 22 h, 4° C	Immunonuclear Corp. USA
Bombesin	1:200	30 min, room temp.	Immunonuclear Corp. USA
Sub P	1:100	30 min, room temp.	Immunonuclear Corp. USA
Insulin [2]	1:500	30 min, room temp.	Immunonuclear Corp. USA
Leu-enkephalin	1:400	30 min, room temp.	Immunonuclear Corp. USA
Serotonin	1:200	30 min, room temp.	Immunonuclear Corp. USA
VIP	1:300	30 min, room temp.	Immunonuclear Corp. USA
Somatostain	1:200	30 min, room temp.	Dako Corp. USA
Glucagon	1:200	30 min, room temp.	Dako Corp. USA
ACTH	1:200	30 min, room temp.	Dako Corp. USA
Gastrin	1:200	30 min, room temp.	Dako Corp. USA
Calcitonin	1:300	30 min, room temp.	Dako Corp. USA
Neurotensin	1:1000	18 - 22 h, 4° C	Amersham Int. UK
PP	1:1200	18 - 22 h, 4° C	MiLab, Sweden
β-endorphin	1:900	18 - 22 h, 4° C	Amersham int. UK

[1] Unless otherwise specified all primary antisera were produced in rabbits.
[2] Antiserum produced in guinea pig.

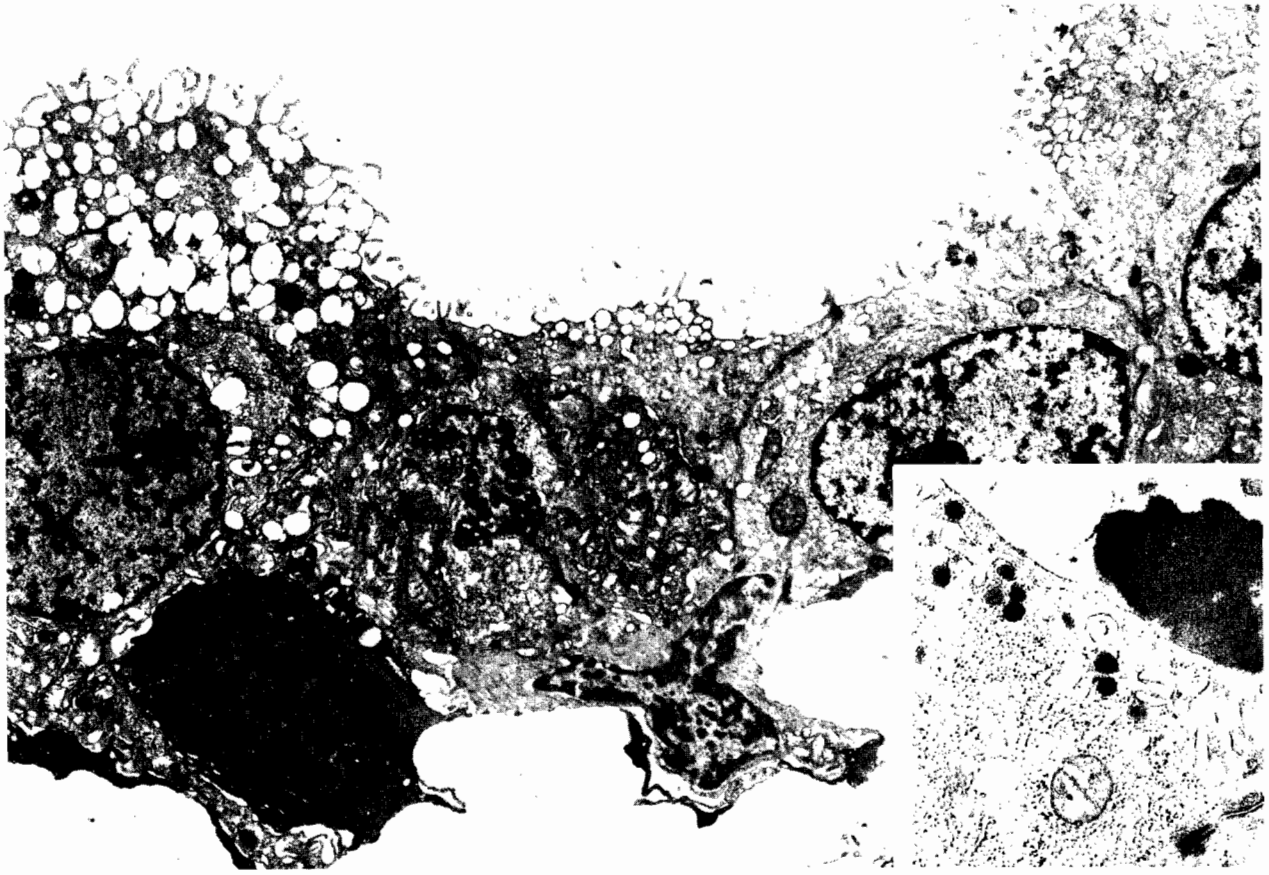

Fig. 2. Fibrocystic disease: An accumulation of light stained vacuoles is seen in the apical part of the cells. A thickened basal lamina is present towards the stroma, in addition to a layer of myoepithelial cells. (UA/LC X 5,600). **Inset:** Area with presence of small electron dense granules of exocrine nature in the vicinity of an intercellular lumen (UA/LC X 32,000).

Discussion

Neuroendocrine differentiation is seen in about 30% of all breast carcinomas (Monaghan and Roberts, 1985; Nesland et al., 1985, 1986a) and several hormones have been identified in the neoplastic cells (for review see Nesland et al., 1986b). The cellular origin of these tumours has not been determined. The cells may derive from specialized cells different from excretory or myoepithelial cells or the neuroendocrine activity may simply represent a differentiation feature of common breast carcinomas.

Vogler (1947) felt that the argyrophilic cells lying between the excretory epithelium and the myoepithelial cell layer in the normal breast were of endocrine nature.

Toyoshima (1983) and Partanen and Syrjänen (1981) carefully studied large series of normal breast tissue and benign lesions but did not identify neuroendocrine cells in a single case and nor did we. Even though Bussolati and co-workers reported chromogranin-reactive cells to be "few in number and focally located in sparse endocrine ductule-lobular units", it seems reasonable to conclude that if neuroendocrine cells are present in the normal breast, they are very rare and probably not the cellular origin of all breast carcinomas with neuroendocrine features.

Vinores et al. (1984) observed immunoreactivity for NSE in 2 out of 11 fibroadenomas, in 1 out of 2 cases of fibrocystic disease and in none of 4 cases of normal breast tissue. They concluded that immunostaining for NSE should be interpreted with caution. We share this view (Nesland et al., 1986a), although immunostaining for NSE seems to be the best screening marker for neuroendocrine cells available (Nesland et al., 1986c). In our series, 2 out of 4 intraductal papillomas contained NSE-positive cells that looked like secretory cells and undoubtedly were different from myoepithelial cells. However, they did not give positive staining for hormones in parallel sections.

Immunostaining with anti-chromogranin is in our experience less reliable than staining with anti-NSE (Nesland et al., 1986c), and we even feel that immunoreactivity for hormones should be required as a final proof of neuroendocrine differentiation. Such cells have not yet been adequately documented in the normal breast and in benign lesions.

Electron microscopy may be useful but should be interpreted with caution. Ferguson and Anderson (1985) frequently observed dense core granules in both benign and malignant breast lesions as well as in the normal breast, and were unable to separate neuroendocrine granules from other types of granules. The frequently encountered electron dense granules in our study did not fulfil the minimum criteria necessary to label them neuroendocrine. They were mainly situated in apical parts of the cells or around intracytoplasmic lumina and their distribution was different from that seen in neuroendocrine cells (Nesland et al., 1985).

The present study did not reveal neuroendocrine cells in the normal breast specimens. Two intraductal papillomas were NSE-immunoreactive, but we were unable to prove the presence of hormone-immunoreactive cells in any of the cases, and electron microscopy did not reveal neuroendocrine granules. Undisputed proof of neuroendocrine differentiation in normal breast specimens and benign breast lesions has thus not been obtained.

Acknowledgements. We thank Inga Finseth, Ellen Hellesylt, Liv Inger Håseth, Mette Myre and Elisabeth Emilsen Mølsted for excellent technical assistance.

References

Bussolati G., Gugliotta P., Sapino A., Eusebi V. and Lloyd R.V. (1985). Chromogranin-reactive endocrine cells in argyrophilic carcinomas ("carcinoids") and normal tissue of the breast. Am. J. Pathol. 120, 186-192.

Ferguson D. J.P. and Anderson T.J. (1985). Distribution of dense core granules in normal benign and malignant breast tissue. J. Pathol. 147, 59-65.

Feyrter F. (1962). Über den derzeitigen Stand der Lehre von den peripheren endokrinen (parakrinen) Drüsen. Acta Neuroveg. (Wien) 25, 63-89.

Hsu S-M., Raine L. and Fanger H. (1981). A comparative study of the peroxidase antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones wisth radioimmunoassay antibodies. Am. J. Clin. Pathol. 75, 734-738.

McDowell E.M. and Trump B.F. (1976). Histological fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100, 405-414.

Mollenhauer H. (1964). Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39, 111-114.

Monaghan P. and Roberts J.D.B. (1985). Immunocytochemical evidence for neuroendocrine differentiation in human breast carcinomas. J. Pathol. 147, 281-289.

Nesland J.M., Høie J. and Johannessen J.V. (1983). The fine structure of the human breast and its benign disorders. Diagn. Histopathol. 6, 51-67.

Nesland J.M., Memoli V.A., Holm R., Gould V.E. and Johannessen J.V. (1985). Breast carcinomas with neuroendocrine differentiation. Ultrastruct. Pathol. I, 225-240.

Nesland J.M., Holm R., Johannessen J.V. and Gould V.E. (1986a). Neurone specific enolase immunostaining in the diagnosis of breast carcinomas with neuroendocrine differentiation. Its usefulness and limitations. J. Pathol 148, 35-43.

Nesland J.M., Holm R. and Johannessen J.V. (1986b). Breast carcinomas with neuroendocrine activity. In: New Frontiers in Mammary Pathology. Hollman K.H. (ed). Martinus Nijhoff. The Netherlands. in press.

Nesland J.M., Holm R. and Johannessen J.V. (1986c). A study of different markers for neuroendocrine differentiation in breast carcinoma. Path. Res. Pract. 181, 524-530.

Partanen S. and Syrjänen K. (1981). Argyrophilic cells in carcinoma of the female breast. Virchows Arch. (Pathol. Anat.) 391, 45-51.

Toyoshima S. (1983). Mammary carcinoma with argyrophil cells. Cancer 2, 2129-2138.

Vinores S.A., Bonnin J.M., Rubinstein L.J. and Marangos P.J. (1984). Immunohistochemical demonstration of neuron-specific enolase in neoplasms of the CNS and other tissues. Arch. Pathol. Lab. Med. 108, 535-540.

Vogler E. (1947). Über das basilare Helle-Zellen-Organ der menschlichen Brustdrüse. Klin. Med. 2, 159-168.

Accepted November 10, 1986