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CASE REPORT

Immunohistochemical and Ultrastructural Analysis of a Mammary Cystic Hypersecretory Carcinoma

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Cystic hypersecretory carcinoma (CHC) is a rare variant of intraductal carcinoma. A CHC in a 50-year-old woman was excised and processed for light and electron microscopy and immunohistochemistry. The tumor had a marked cystic appearance. The walls of the cysts consisted of epithelial and myoepithelial cells and a well-developed basement membrane. The epithelial cells contained well-developed rough-surfaced endoplasmatic reticulum and Golgi apparatus. Secretory granules were not detected, with the exception of a few mucus-producing cells. The secretion was predominantly homogenous, reminiscent of thyroid colloid, and demonstrated distinct PAS positivity. The cells displayed a strong labeling with epithelial membrane antigen (EMA) and EMA-positive structures were observed within the intraluminal secretion, too. Some of these were stained by alcian blue. In addition, the colloid-like material was admixed with mucus showing a filamentous internal structure and lipid droplets resulting in some heterogenity of the secretion. Intraductal micropapillary proliferation in some of the cysts and adjacent nondistended ducts was a further defining feature of the tumor. Steroid hormone receptor and Ki-67 proliferation marker immuno his Tochemistry showed scattered positivity among the tumor cells. These results are in agreement with previous observations and further clarify the nature of this low-grade in situ cancer. (Pathology Oncology Research Vol 3, No 4, 287–292, 1997)

Key words: breast, cystic hypersecretory carcinoma, electronmicroscopy, immunohistochemistry

Introduction

Cystic hypersecretory carcinoma (CHC) is a rare variant of intraductal carcinoma, characterized by the formation of cysts that contain an eosinophilic, PAS-positive secretory product resembling thyroid colloid. Intraductal epithelial proliferation corresponding to micropapillary carcinoma in some of the cysts and adjacent nondistended ducts is also typical of the lesion.[1] Here we report immunohistochemical and ultrastructural data on a large CHC.

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Abbreviations: AB: alcian blue; BM: basement membrane; CHH: cystic hypersecretory hyperplasia; CHC: cystic hypersecretory carcinoma; DCIS: ductal carcinoma in situ; EM: electron microscopy; EMA: epithelial membrane antigen; FNAC: fine-needle aspiration cytology; HE: hematoxylin and eosin; PAS: periodic acid Schiff; RER: rough surfaced endoplasmic reticulum; SMA: smooth muscle actin

Materials and Methods

The specimen for histology and immunohistochemistry was fixed in 7% buffered formaldehyde and embedded in paraffin. HE staining was supplemented with the PAS reaction, both with and without diastase digestion, and PAS-AB staining at pH, 2.5.

For standard immunohistochemistry the following primary antibodies were used: Epithelial membrane antigen (EMA) (1:50; Biogenex, San Ramon, CA; Cat.# MU182UC); Smooth muscle actin (SMA) (Prediluted; DAKO, Copenhagen; Cat.# U7033); Thyroglobulin (1:100; Novocastra, Newcastle, UK; Cat.# NCL-THY 1D4); Estrogen receptor (1:60; Novocastra, Newcastle, UK; Cat.# NCL-ER-6F11); Progesterone receptor (1:30; Novocastra, Newcastle, UK; Cat.# NCL-PGR 1A6); Ki-67 (1:150; polyclonal, DAKO, Copenhagen, Cat.# A0047)

Microwave antigen retrieval was used for the last 4 of these antibodies. After inhibition of endogenous peroxidases with 3% H_2O_2 solution and reduction of the background staining with blocking serum (Vectastain-Universal Quick Kit, Vector, Burlingame, CA, Cat. #

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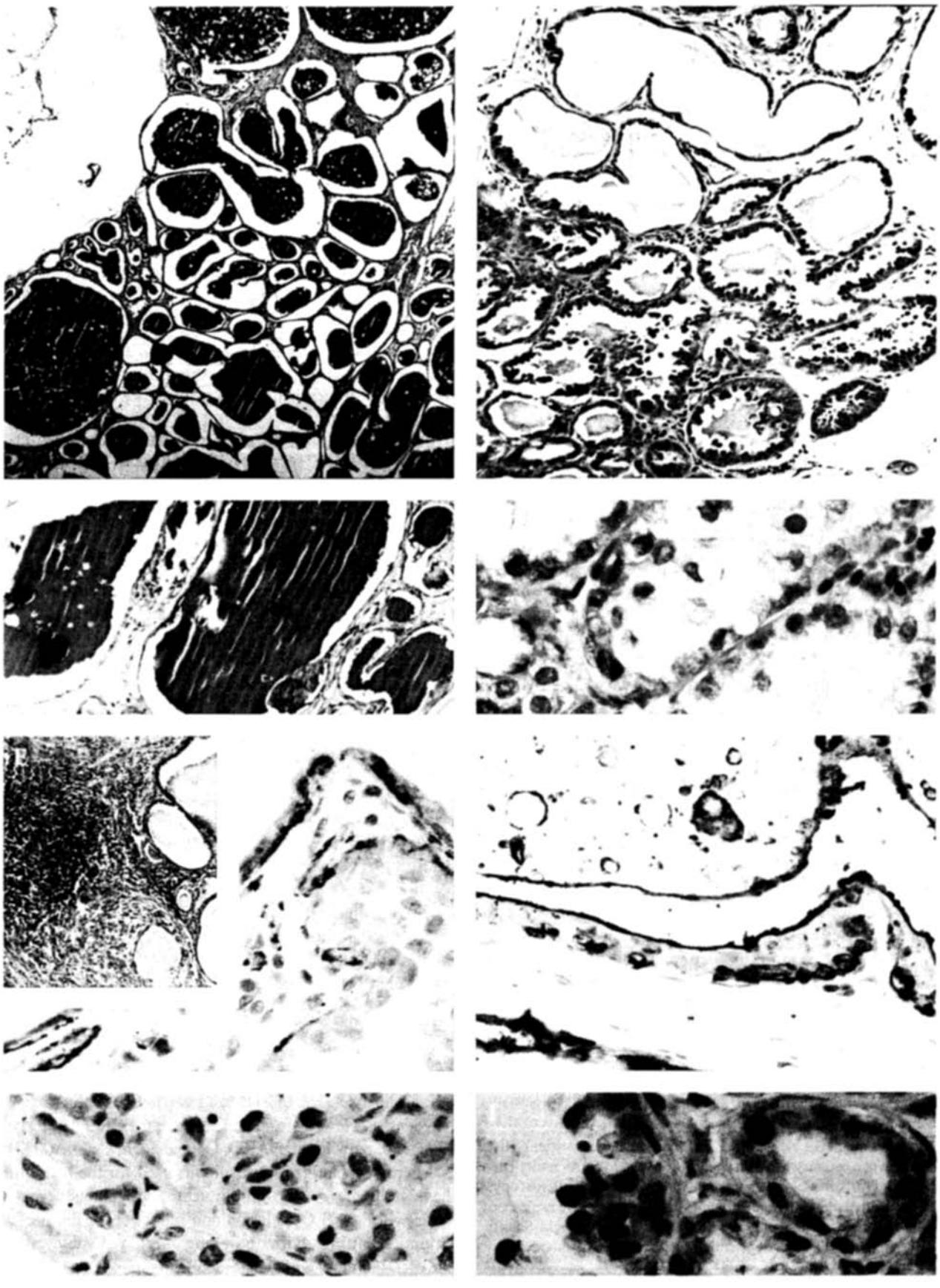


PK8800), all primary antibodies were incubated for 1 hour at room temperature, except for Ki-67, which was incubated overnight. Reactions were then incubated with the secondary antibody and the chromogen of the Vectastain-Universal Quick Kit, following the instructions supplied with the kit. Only the SMA immunostaining deviated from this methodology because of its EPOS detection system. In this case, only the last step (application of the chromogen – aminoethylcarbazole) was performed.

Small pieces of the specimen fixed in 7% buffered formaldehyde were processed for electron microscopy (EM). 1 mm^3 blocks were postfixed in buffered 4% glutaraldehyde and 1% OsO_4 thereafter. The tissue was dehydrated in a series of graded ethanol and embedded in Araldite. Semithin sections were cut and stained with toluidine blue. The ultrathin sections were contrasted with uranyl acetate and lead citrate.

Case report

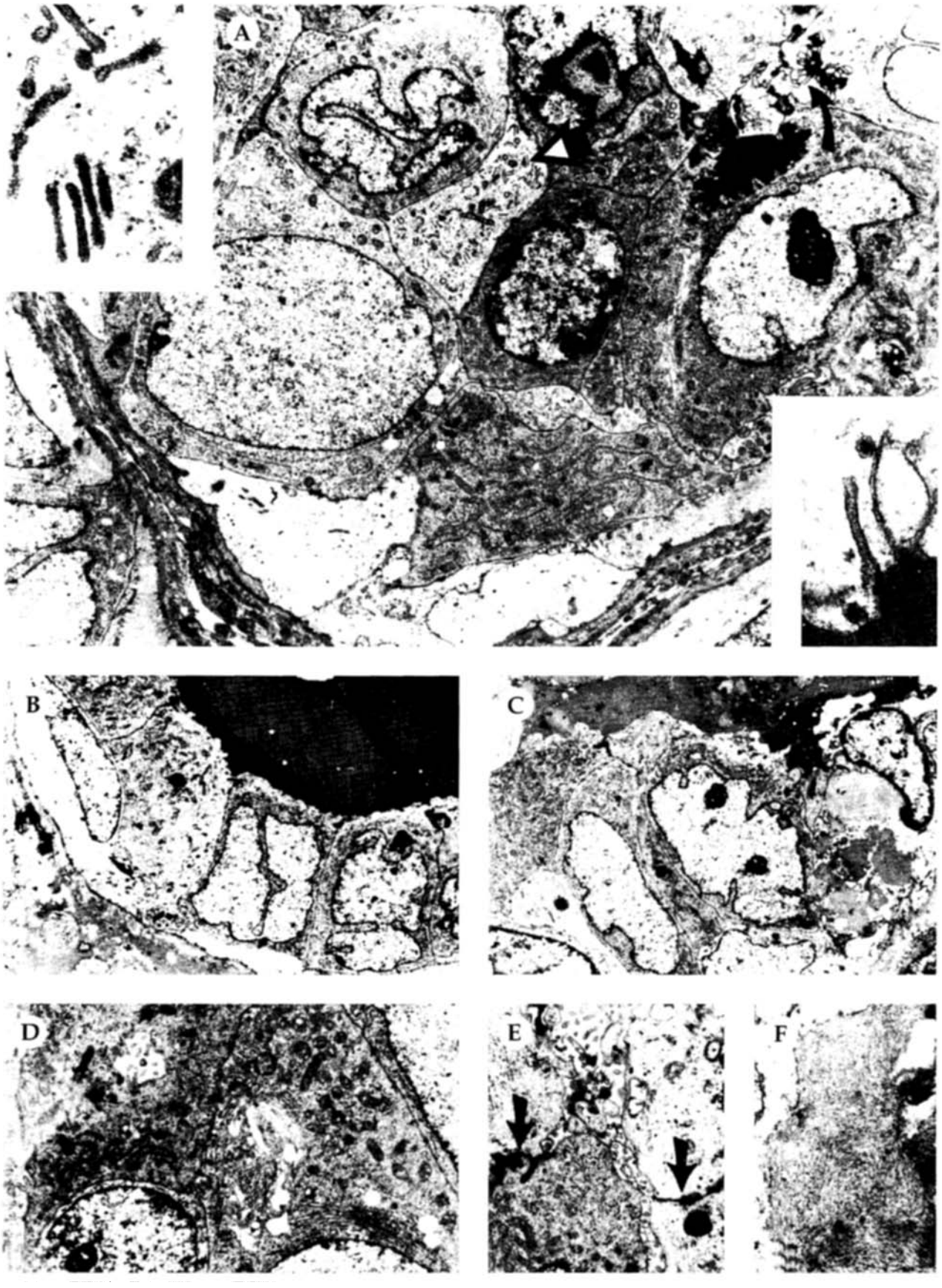
Mammography of a 50-year-old woman demonstrated a large tumor filling the upper outer quadrant of the left breast, which was characterized by massive calcification. Physical examination revealed no other abnormalities. The patient underwent operation. An intraoperative incision biopsy was taken from the central part of the tumor for frozen sections. A large number of cystic ducts were seen. Some cells exhibited larger nuclei and nucleoli, but the overall cytologic features were bland. The secretion was interpreted at this time as necrotic debris, suggestive of comedo necrosis (and carcinoma) with random calcifications, but the cellular features were not consistent with this entity, and the diagnosis was deferred for paraffin embedding. Because of the inconclusive nature of the frozen section. The tumor, measuring 10 x 8.5 x 4 cm, was excised in toto and no further surgery was carried out.

Permanent sections revealed the same type of cystic lesion as did the frozen ones *(Figure 1A)*, but a micropapillary proliferation of the epithelium was present adjacent to and also in some of the cysts *(Figure 1B)*. The cysts were lined with flattened epithelium and a myoepithelial layer was consistently identified, even on HE sections. Besides the homogenous pale eosinophilic and strongly PAS-positive secretions in the lumina, some cysts contained rounded holes mixed with the colloid-like secretion. Some of the holes stained blue with PAS-AB while others did not *(Figure 1C)*, a phenomenon that might be artifactual. The homogenous-appearing, colloid-like secretion was often separated from the epithelium by an artifactual retraction space, and parallel "cracks" in the secretion were also frequently present. Some noncystic or cystic areas contained epithelial cells with a vacuolated cytoplasm, suggesting an active secretory function *(Figure 1D)*. A fen of these latter cells were reminiscent of signet-ring cells. Tissue reactions were also seen in some areas of the tumor. These included lymphocytic and histiocytic reactions adjacent to a visualized or hypothesized cyst rupture. A few isolated granulomas were also seen *(Figure 1E)*. The diagnosis of CHC was made on the basis of the features reported, especially the presence of micropapillary intraductal carcinoma.

Immunohistochemistry yielded negative result with thyroglobulin. SMA clearly demonstrated a myoepithelial layer around the cysts and micropapillary intraductal carcinoma *(Figure 1F)*. EMA was strongly positive in the epithelium, and both membrane fragments and rounded isolated vacuoles were seen in the lumen of many cysts, suggesting apocrine secretory activity *(Figure 1G)*. Estrogen and progesterone receptors were observed in some scattered cells, but on the whole the tumor was considered negative for these receptors on the basis of the histology (H-) scores[2] *(Figure 1 H)*. The Ki-67 proliferation marker identified only a few positive nuclei, which corresponded to the very rare mitotic figures in the specimen *(Figure 1I)*. Adjacent to the tumor, benign papillary hyperplasia (with epithelial and myoepithelial cells) and cystic dilation of the involved duct were also present.

A small representative area containing various-sized cysts and compact cell groups was chosen for EM. The fine structure of the cuboidal cells lining the cysts and that of the compact cell groups were practically identical. In the compact areas many small *intercellular* lumina were observed, and in places *intracytoplasmic* crypts also occurred *(Figure 2A&D)*. The apical cellular surface was almost smooth in the larger cysts, while small and fine microvilli were seen in the smaller intracytoplasmic crypts *(Figure 2 B&E)*. The cells forming the cystically dilated ducts were surrounded by a well-developed basement membrane (BM), and typical myoepithelial cells were located between the BM and epithelial cells *(Figure*

Figure 1. *(A) Cystic and (B) micropapillary area of the tumor (PAS and HE respectively, objective X10, original magnification X32). (C) AB-stained secretory component in a PAS-positive background. Note the parallel cracks and shrinkage artifact of the colloid-like secretion (PAS-AB, objective X10, original; magnification X32). (D) Marked secretory activity seen in some areas of the tumor. Note signet-ring-type cells (center of the field) (HE, objective X40, original magnification X130). (E) Tissue reaction with granuloma formation to a ruptured cyst (bottom right of the field) (HE, objective X4, original magnification X13). (F) Myoepithelial layer around the epithelium (SMA, objective X40, original magnification X130). (G) EMA-positive round vacuoles in the secretion (objective X40, original magnification X130). (H) Scant estrogen receptor and I/ Ki-67 positivity between the tumor cells (objective X40, original magnification X130).*


A
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2 A&B). These different cells were interconnected with desmosomes. Typical terminal bars consisting of zonula and fascia adherentes as well as desmosomes were present at the luminal poles of the epithelial cells *(Figure 2B&E).* In some places, the cysts contained an electron-dense secretion product reminiscent of thyroid colloid *(Figure 2B).* Detached epithelial cells, loosened amorphous material and lipid droplets also occurred within the lumina. Vermicular cell surface invaginations with a fine periodic inner structure were observed in the luminal surface of some epithelial cells *(Figure 2A,* lower insert). These invaginations detached from the cell surface and accumulated in the apical cytoplasm *(Figure 2A,* upper insert). A calcifying spherule was also visualized in the central part of a cyst.

The shape of the nuclei was roundish on light microscopy, but many irregular nuclei were observed with EM. The cytoplasm was rich in polyribosomes; the rough-surfaced ER was moderately/well developed. The Golgi apparatus was prominent. Mitochondria were elongated and moderate in number. The majority of the secretory cells were devoid of typical secretory granules. A few osmiophilic lysosome-like granules were dispersed in the cytoplasm. In some ductules, a few cells produced mucus droplets which accumulated as a secretion in the lumen *(Figure 2C).* The mucus granules exhibited a fine filamentous internal structure *(Figure 2F).* No dense-core granules were present in the specimen.

The EM findings correlated well with the light microscopy results.

Discussion

CHC is a rare form of low-grade intraductal carcinoma first described in 1984 by Rosen et al.[1] It should be distinguished from benign lesions such as extensive fibrocystic changes (cysts and epithelial proliferation),[1,3] mucocele-like lesions,[3] juvenile papillomatosis[3] and malignant tumors, including secretory carcinoma,[3,4] and mucinous or colloid carcinoma.[1,3,4]

A very important differential diagnostic help is that CHH lacks the micropapillary neoplastic component.[4] It is particularly important to sample this lesion thoroughly to exclude a carcinomatous component. Interestingly, the sample sent for frozen sections was virtually devoid of proliferation and contained only cysts. CHH and CHC seem to be parts of a spectrum of lesions that include CHH with atypia[4,5] between these two entities and invasive carcinoma at the worst end. The size of this neoplasm is comparable with that of the largest tumor from the series of Guerry et al.[4] The prognosis of CHC seems good, and similar to that of other low-grade intraductal cancers,[4] but invasive cancer associated with CHC has been reported so far to be poorly differentiated, losing the hypersecretory nature of the intraductal cancer.[1,4-6] (It is to be noted that rupture of the cysts and spillage of their content into the surrounding tissues does not comprise invasion.)

To the authors' knowledge, only one such tumor has been subjected to EM prior to the present case. This produced a result similar to ours except for a few details, some of which have not been described previously. Notably, we demonstrated the heterogenous nature of the intracystic secretion. This contained a colloid-like homogenous substance and mucin with a filamentous internal structure. Lipid droplets and membrane-bound particles (seen with EMA) were also present in the lumina. No dense-core granules were observed in our specimen. The presence of microvilli seemed to correlate with the size of the extracellular lumen. Small intracellular crypts and intercellular "holes" had easily identifiable microvilli *(Figure 2D & E),* while in the larger cysts the microvilli were reduced both in size and in number. This feature is consistent with the finding that the "apical cytoplasm (is) virtually devoid of surface microvilli".[4]

On the basis of previously reported cases and the present one, the main diagnostic features of CHC are cystic dilatation of ducts or acini filled with a colloid-like, PAS-positive secretion and areas of micropapillary intraductal carcinoma in the lesion. These features may aid the FNAC diagnosis of these tumors.[3,6] This is the first case that highlights the probable apocrine nature of the hypersecretory process by demonstrating membrane-bound secretory particles within the lumen of the cysts by conventional histochemical and immunohistochemical methods. EM also helped to clarify the heterogenous nature of the secretion.

Figure 2. *(A) Part of a compact cell group surrounded by BM. Myoepithelial cells (light in the micrographs) are located between the densely packed epithelial cells and the BM. Osmiophilic homogenous secretion material is seen in small irregular intercellular lumina (upper right corner). The lower insert is an enlargement of the upper lumen (arrow), revealing a vermicular enfolding of the plasmolemma. The upper insert shows a detail of an epithelial cell (double arrow) and demonstrates detached vermicular enfoldings. (B) Part of a cyst lined with cuboidal cells. Note the myoepithelial cell and the terminal bars at the apical poles of the cells. The content of the cyst is homogenous and electron-dense. (C) Mucus-producing cell (right) in the wall of a cyst containing mucus and homogenous material (see also 2F). (D) Densely packed epithelial cells with well-developed RER and Golgi apparatus. A small intracellular crypt containing microvilli is observed (upper left). (E) Microvilli are seen in an irregular lumen. Note also terminal bars at the apical poles of the cells (arrows). (F) The intracellular mucus droplet consists of fine filaments. The mucus of the cysts exhibited an identical filamentous structure.*

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