A Histochemical and Ultrastructural Study of Human Breast Carcinomas With a View to Their Classification by Cell of Origin

S. A. BENCOSME, M. J. RAYMOND, R. C. ROSS, B. MOBBS, V. TSUTSUMI, H. ORTIZ, R. GONZALEZ, AND E. SEGURA

1 Department of Pathology, Queen's University and Kingston General Hospital, Kingston, Ontario, Canada K7L 3N6; 2 Department of Pathology, St. Michael's Hospital, Toronto, Canada; 3 Department of Surgery, University of Toronto, Toronto, Canada; 4 Dirección General de Investigación Médica de la S. S. A. Mexico, Mexico; 5 Hospital de Oncología del Centro Médico Nacional, IMSS, Mexico; 6 Instituto de Estudios Biomédicos Universidad Nacional Pedro Henríquez Urena, Santo Domingo, Dominican Republic

We have studied 450 cases of human breast carcinoma at the histochemical and ultrastructural level. The breast biopsies were obtained from Canada, Mexico and Dominican Republic.

This study was undertaken to evaluate the prognostic significance of classification of breast tumors, proposed in 1971 by Murad, based on their cell of origin. Under this classification, ATPase positive tumors represent those of myoepithelial origin, while the remainder are designated ductal or ductular based upon defined ultrastructural characteristics.

We observed a distribution of tumor types essentially similar to that reported by Murad, and no significant differences were observed between the contributing centers. No correlation was observed between this classification and the histological classification described by Stewart. We found no evidence that ATPase positive tumors were myoepithelial in origin, while the remainder are designated ductal or ductular based upon defined ultrastructural characteristics.

An interesting feature requiring further investigation was the high incidence of endocrine-like secretory granules in all histological types of tumors studied.

INTRODUCTION

The pathological classification of disease has long been considered essential to its better understanding.

In 1971, Murad proposed a pathological classification of breast cancer which theoretically seemed to satisfy a basic tenet of tumor pathology which states that once the cell of origin of a tumor is established the foundations are laid for meaningful research into prognosis and therapy. In Murad's view, all breast cancers are derived from the myoepithelial, ductal or ductular cells. These tumor types were differentiated on the basis of their histochemical and ultrastructural characteristics (Murad, 1971).

Using this classification it was found that the ductular type of carcinoma
exhibited a higher frequency of metastases than did the other two types. The author further hypothesized that of the three types of tumor, the ductular carcinoma would be most likely to be responsive to hormonal therapy, a proposal which, if substantiated, would be of obvious value in the management of individual patients. Since Murad's series was very small it was decided to collect a large series where all cases regardless of the pathological diagnosis, would be studied for the purpose of confirming the validity of this classification. Because it is known that geographic or racial differences sometimes play a significant role in the natural history of disease our series included specimens from patients with three different ethnic backgrounds.

In a number of cases the estrogen binding capacity of the tumor was also studied. In all cases a follow-up of the progression of the disease is being carried out.

**MATERIALS AND METHODS**

We have studied a total of 450 consecutive cases of breast cancer with the following distribution: Canada 230; Dominican Republic 121; and Mexico 99. Biopsy specimens diagnosed by frozen section as infiltrating carcinoma of the female breast were obtained at the time of surgery.

**Enzyme Histochemistry**

At the time of frozen section diagnosis, a portion of the tumor tissue measuring approximately 10 × 10 × 2 mm was fixed in 0.1 M cacodylate buffer (pH 7.2) containing 2% glutaraldehyde for 2 to 4 hr at room temperature. The tissue was transferred to the same buffer and stored at 4°C until the enzyme reaction study was performed. The number of cases and their routine pathological diagnosis according to Stewart's classification (McDivitt et al., 1968) is indicated in Table I; the incidence of histologic type diagnosed in our series closely resembled those reported by these authors.

**Magnesium-Dependent Adenosine Triphosphatase (ATPase)**

A modification of the method of Tice and Engel (1964) was used. The substrate, ATP (1 × 10⁻⁴ M), was dissolved in Tris-maleate buffer (pH 7.2) containing 1.1 × 10⁻⁴ M magnesium sulphate and 1.5 × 10⁻⁵ M lead nitrate. Frozen sections 40 μm-thick were incubated in the medium for 15 min at room temperature, washed in 0.3 M sucrose and incubated in 1% ammonium sulphide for 1 to 2 min, washed in 0.3 M sucrose, and a few sections examined by light microscopy. The remaining sections of ATPase positive cases were processed for electron microscopy. In all instances, control sections were incubated in an ATP-free medium. In selected cases, control sections were incubated in a magnesium free medium.

**Acid Phosphatase**

The method of Hugon and Borgers (1966) was used. After the histochemical reaction, the sections were processed for light and electron microscopy. In all cases, control sections were incubated in a medium containing no β-glycerophosphate.
TABLE I
Distribution of Infiltrating Breast Cancer According to Histological Type (McDivitt et al., 1968) and Histochemical and Ultrastructural Classification (Murad, 1971)

<table>
<thead>
<tr>
<th></th>
<th>Myoepithelial</th>
<th>Ductal</th>
<th>Ductular</th>
<th>Undiagnosed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Carcinoma of the mammary ducts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma with fibrosis</td>
<td>163</td>
<td>36.2</td>
<td>64</td>
<td>14.2</td>
<td>101</td>
</tr>
<tr>
<td>(Scirrhous carcinoma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comedocarcinoma</td>
<td>18</td>
<td>4.0</td>
<td>11</td>
<td>2.4</td>
<td>8</td>
</tr>
<tr>
<td>Medullary carcinoma</td>
<td>5</td>
<td>1.1</td>
<td>2</td>
<td>0.4</td>
<td>13</td>
</tr>
<tr>
<td>Colloid carcinoma</td>
<td>2</td>
<td>0.4</td>
<td>2</td>
<td>0.4</td>
<td>4</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>2</td>
<td>0.4</td>
<td>1</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>Carcinoma of lobules</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobular</td>
<td>2</td>
<td>0.4</td>
<td>---</td>
<td>---</td>
<td>6</td>
</tr>
<tr>
<td>Metaplastic carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma with osseous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metaplasia</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Totals</td>
<td>192</td>
<td>42.7</td>
<td>80</td>
<td>17.7</td>
<td>134</td>
</tr>
</tbody>
</table>

* This undiagnosed column represents those ATPase negative tumors which we were unable to classify (see text).

Alkaline Phosphatase

The method described by Muller et al. (1971) was used. The sections were then processed for light and electron microscopy. Again, control sections were incubated in a substrate-free medium.

Electron Microscopy

For electron histochemistry all sections showing a positive reaction were postfixed in 2% OsO₄ for 1 hr and processed as previously described (Bencosme and Tsutsumi, 1970). In some instances sections were examined unstained or counterstained with uranyl acetate alone.

In most instances the sections were double stained with uranyl acetate and lead citrate. The counterstaining did not interfere with the localization of the reaction product. Representative portions from all biopsies fixed in glutaraldehyde were processed for routine electron microscopy as described above.

ATPase negative tumors were classified as either ductular or ductal carcinoma according to their ultrastructural appearance. The morphological criteria used in the diagnosis of these cases were those described by Murad (1971), and are listed in Table II.

Estrogen Receptor Binding

Measurements of estrogen binding receptors were made in 20 cases using the method previously described (Mobbs and Johnson, 1976). Blind classification of the tumors thus studied was carried out.
TABLE II

Ultrastructural Criteria Used in the Differentiation of Ductal and Ductular Carcinoma (Murad, 1971)

<table>
<thead>
<tr>
<th>Ductular carcinoma</th>
<th>Ductal carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleus</strong></td>
<td><strong>Large; ovoid; membrane showing deep folds</strong></td>
</tr>
<tr>
<td>Spindle; membrane crenated</td>
<td>Few areas of heterochromatin</td>
</tr>
<tr>
<td>Dense heterochromatin</td>
<td>Pars amorpha rare mainly nucleolonema</td>
</tr>
<tr>
<td><strong>Nucleolus</strong></td>
<td><strong>Cell cohesion good microvilli rare ER cisternae less noticeable</strong></td>
</tr>
<tr>
<td>Pars amorpha prominent—up to 50% intertwined with nucleolonema</td>
<td>Cell cohesion poor microvilli frequent ER cisternae prominent</td>
</tr>
<tr>
<td><strong>Cytoplasm</strong></td>
<td><strong>Mitochondria large, ovoid, matrix pale</strong></td>
</tr>
<tr>
<td>Cell cohesion poor microvilli frequent ER cisternae prominent</td>
<td>Mitochondria small, variable shape, dense matrix</td>
</tr>
</tbody>
</table>

RESULTS

MYOEPITHELIAL CELL CARCINOMAS

ATPase Histochemistry

One hundred and ninety-two cases were classified in this category on the basis of their positive ATPase reaction, and their relationship to Stewart's classification is shown in Table I. The enzymatic reaction product was identified by dark precipitate localized on the plasma membrane. In all cases, control slides revealed no specific reaction.

In normal breast tissue, myoepithelial cells demonstrated a typical ATPase reaction (Fig. 1). The reactivity of epithelial cells was quite variable. In contrast to the small amount of activity occasionally observed in normal breast epithelial tissue, hyperplastic epithelium was more frequently found to be positive. In the breast carcinomas classified as myoepithelial, the reaction pattern varied considerably. In many cases the reaction was strong and uniformly distributed (Fig. 2), while in the remainder, the proportion of reactive cells was smaller.

Alkaline Phosphatase Histochemistry

Alkaline phosphatase, which is normally present on the plasma membrane of myoepithelial cells of normal breast and in benign lesions was never observed in myoepithelial carcinoma. This enzyme, however, retained its activity in the block vessels of the tumors and in myoepithelial cells of normal ducts in areas adjacent to the tumor.

Electron Microscopy

One hundred and fifty of the ATPase positive cases were studied at the electron microscopic level. In addition, the localization of the ATPase reaction was studied in a small series, and this confirmed the observations made by light microscopy. The traditional cytologic criteria associated with myoepithelial cells, including intracellular myofilaments, were not observed in this study.

An unexpected finding in these ATPase positive tumors was the frequent observation of acid phosphatase negative granules spread throughout the cyto-
FIG. 1. Slightly dilated acinus of a human mammary gland showing a positive ATPase reaction on the plasma membrane of myoepithelial cells (M) but not on the apposing cell membranes of epithelial cells (uranyl acetate-lead citrate), ×4275.

FIG. 2. Infiltrating duct carcinoma showing most tumor cells with a positive ATPase reaction on the plasma membrane, ×380.

FIG. 3. Myoepithelial cell carcinoma showing numerous small granules negative for acid phosphatase reaction. Most of these granules are located close to the cell membrane but a few are in the region of the Golgi complex. There are variations in the appearance of these granules (G). Note also the positive reaction of lysosomes (uranyl acetate-lead citrate), ×4600.
plasm of the tumor cells (Fig. 3). Commonly, there was variation in the distribution of the granules within a particular tumor, with some cells containing more numerous granules.

In most cases these granules were of a similar size and structure within a given neoplasm, although in a few tumors more variability was observed in the size, texture and electron density of the granules (Fig. 3). In many cases the granules were few in number and restricted to an area close to the plasma membrane, requiring careful examination of the section for their identification (Fig. 4). In general, their distribution resembled that found in endocrine cells.

These ATPase positive tumors contained in addition another distinct population of acid phosphatase negative granules which were easier to relate to the exocrine granules observed in the breast epithelium. This population, found in the immediate vicinity of intracellular or extracellular ductules, was composed of either small electron-dense granules (Figs. 5 and 6), or larger granules containing flocculent material (Fig. 7). More irregular granules surrounded by a clear space, and resembling those described as casein granule (Hollman, 1974), were also observed in some cases. Less frequently granules of the type associated with mucin secretion (Fig. 5) were present in some cells which were found either singly or arranged in small groups.

In general, lysosomes could easily be distinguished from the secretory granules on the basis of their size. Electron microscopic study or sections prepared for acid phosphatase histochemically confirmed our impressions, although we did occasionally observe small acid phosphatase positive granules. As expected, changes in the Golgi and in the endoplasmic reticulum were consistent with a secretory function of the tumor cells. In searching for secretory granules care had to be taken to distinguish tumor cells from the granulated inflammatory cells since they are in intimate contact with each other.

**Ductal and Ductular Carcinoma**

By definition these tumor types are ATPase, alkaline phosphatase negative and can be distinguished only on the basis of the ultrastructural criteria outlined in Table II. A summary of the total numbers of ductal and ductular carcinoma diagnosed as well as their relationship to Stewart's classification is presented in Table I.

Characteristically, the cells of ductal carcinoma showed large indented nuclei, with a low electron density nucleoplasm, nucleoli with prominent nucleolonema and a small pars amorpha. ATPase and alkaline phosphatase reactions were always negative, and acid phosphatase limited to the lysosomes. A typical example is shown in Fig. 8.

The cells of ductular carcinoma typically exhibited spindle-shaped nuclei and prominent nucleoli which consisted largely of pars amorpha. The nuclear chromatin often appeared as small clumps of heterochromatin scattered throughout the nucleoplasm (Fig. 9).

Whereas Murad did not emphasize the occurrence of forms of tumor intermediate between the ductal and ductular types, in our series we noted a significant number of cases that were classified according to the criteria laid down in Table II rather subjectively. In most of these cases, the tumor displayed features that were characteristic of both types of tumors, and in others there were addi-
Fig. 4. Myoepithelial cell carcinoma with large lysosomes scattered throughout the cytoplasm of tumor cells. Note the presence of very small dark granules mostly localized close to the cell membrane. ×5940.

Fig. 5. Myoepithelial cell carcinoma showing several intracellular ductules and numerous small dark granules situated close to the cell membrane of these ductules. Large lysosomal granules are also present in these cells. In one area there is a small portion of cytoplasm containing large mucin-like granules. ×4275.

Fig. 6. Another tissue sample from the case illustrated in Fig. 5 showing positive acid phosphatase reaction on a lysosome but not in the granules situated just beneath the plasma membrane of an intracellular ductule. ×26,600.
tional features not attributed to a specific type. In a small proportion of tumors, the features were sufficiently ambiguous that no diagnosis was made.

The appearance of the secretory components observed in ATPase negative tumor types could not be distinguished, either qualitatively or quantitatively, from those seen in the ATPase positive "myoepithelial" carcinomas.

**Geographic Distribution**

No difference in the overall distribution of the three types of tumors classified in this study was related to the location of the collections of the tumors.

**Estrogen Binding Receptors**

The 20 cases studied for estrogen binding receptors were classified as follows: myoepithelial 10; ductular 9; ductal 1. Because of this distribution it was impossible to draw any conclusions concerning the single case of ductal carcinoma. There was no difference in the range of estrogen receptor values in the other two types of tumor. Values for the ductular type ranged from 0 to 22.3 fm/mg tissue and for the myoepithelial from 0 to 14.3 fm/mg tissue.

**DISCUSSION**

In this study we have shown that 40% of infiltrating breast carcinoma are ATPase positive, irrespective of the ethnic origin of the patients. It was also found that the proportions of ductal and ductular carcinoma were similar when the series was considered on a geographical basis. The disparities in the percentage distribution of the three types of tumors in this study and that of Murad, can probably be attributed to the much larger number of cases studied in our series than in that of Murad (1971). We were unable to determine any correlation between tumor type and histological diagnosis. Indeed, it was notable that of 370 scirrhous carcinomas, only 163 were ATPase positive.

From our work it appears that, with some exceptions, ATPase negative tumors can be divided into ductal and ductular carcinomas. However, the theoretical significance of the distinction thus drawn is not entirely clear. Striking similarities between the two types of tumor cells were observed, such as intracellular and extracellular duct formation and the presence of secretory granules. These granules were situated either close to the plasma membrane limiting the ductules, reminiscent of exocrine cells, or they were present at the periphery of the cell, a distribution resembling those of endocrine cells.

Several authors have attempted to establish whether or not myoepithelial cells play a significant role in human breast cancer. Recently, Van Bogaert and Maldaque (1976) have discussed the lack of agreement in the literature regarding this point. Buell et al., (1976), have more specifically investigated the value of the ATPase reaction in the identification of myoepithelial tumors. These authors concluded that the majority of infiltrating ductal carcinomas did not possess a uniform ATPase activity. Because some mucin producing breast tumors

---

**Fig. 7.** Myoepithelial cell carcinoma showing pale granules mostly composed of a flocculent material, and figures suggest emiocytosis of these granules. ×5960.
FIG. 8. A typical breast carcinoma diagnosed as ductal carcinoma, according to the criteria described in Table II. Note the presence of intracellular ductules surrounded by small granules. ×4160.

FIG. 9. A typical breast carcinoma diagnosed as ductular carcinoma, according to the criteria laid down in Table II. ×4160.

and papillary carcinomas with apocrine metaplasia have been shown to be ATPase positive, two groups of investigators have strongly questioned the belief that this reaction on the plasma membrane of the tumor indicates a myoepi-
thelial origin (Buell et al., 1976; Ahmed, 1974). It has been further suggested that the ATPase activity in infiltrating duct carcinoma may be similar to the phenomenon observed in fetal rat skin in which the basal layer is transiently ATPase-positive, with the reaction disappearing after birth (Moynahan et al., 1972).

In our work, the only feature differentiating the "myoepithelial" from the other carcinomas was the positive reaction to the ATPase stain; no other clear indicators of the myoepithelial nature of a tumor could be observed. Myofilaments could not be positively identified, and morphological signs of secretory activity were observed as often as in the ductal and ductular tumors. The general consensus in the literature is that no correlation exists between the histologic type in breast cancer and the presence of estrogen receptors (McGuire et al., 1975). In our small series in which estrogen receptors were studied we found no difference between myoepithelial and ductular tumors in this regard. The presence of acid phosphatase negative granules with a cytoplasmic distribution similar to that observed in exocrine and endocrine cells further militated against a myoepithelial cell origin of these tumors. Rather, we feel that the tumors originating from the myoepithelial cell may present an altogether different picture. A revealing case has been reported by Cameron et al. (1974), in which a study of biopsies taken from the same breast tumor, and a recurrent nodule of that tumor, strongly suggested that a leiomyosarcoma developed from the myoepithelium of the breast. It therefore appears possible that at least some leiomyosarcomas of the breast represent tumors of the myoepithelial cells, and in the light of this fact, compelling evidence would be required to demonstrate that tumors from myoepithelial cells can also manifest themselves as carcinomas. On this basis, and on the basis of our own observations, we conclude that myoepitheliomas of the breast must be exceedingly rare in man.

Morphological evidence of the secretory activity of the breast epithelium has been well documented in experimental animals and extensively reviewed by Hollmann (1974), but such data on human breast is still fragmentary. Nevertheless, the information available is sufficient to allow speculation as to the potential value of a more comprehensive understanding of this subject in mammary carcinoma. Recently some authors have reported on the exocrine secretory activity of human mammary cancer cells (Bussolati et al., 1975; Dermer and Sherwin, 1975; Harris et al., 1975), but only a few have indicated that these cells may also have an endocrine function (McCarty et al., 1977).

That breast tumors may be the site of ectopic hormone production has been emphasized only recently. Elevated levels of chorionic gonadotrophin have been found in around 13% of breast cancer patients (Sheth et al., 1974). Cushing's syndrome is known to occur secondary to ACTH production by a breast carcinoma. Ultrastructural examination of one such carcinoma revealed typical endocrine-like secretory granules in the tumor cells (McCarty et al., 1977). According to these authors, the fixation and staining characteristics of these granules were similar to those found in the ACTH producing cells of the adenohypophysis. Ectopic production of parathyroid hormone by a breast carcinoma has also been documented (Mavligit et al., 1971).

The frequent occurrence in our material of granules which did not appear to belong to the lysosomal system nor to that granule population secreted into
the intracellular or extracellular ductules, strongly suggests that breast cancer may be responsible for some ectopic endocrine secretions. If this were to be the case it would add another dimension to our understanding of the biological behaviour of breast cancer. Furthermore it is conceivable that variations in the nature and amount of the exocrine secretions of these tumors may also alter their micromilieu and ultimately their biological behaviour. Qualitative and quantitative differences regarding these secretory products have been reported (Bussolati et al., 1975; Harris et al., 1975). These findings are consistent with our views. Most of the ultrastructural changes discussed by Hollman (1974) in his comprehensive review of the development and structure of the mammary gland as correlated with functional activity, were also observed in our study. Although we did not carry out a systematic investigation of these changes, they appeared with sufficient frequency that study in greater detail is warranted with a view to a better understanding of the functional pathology of these cells. This fact we believe to be sufficient justification for the assessment of the possible role of the secretory potential of breast cancer by routine ultrastructural investigation.

In conclusion, we do not believe that the ATPase positive epithelial tumors are of myoepithelial origin; they are by all other criteria, epithelial tumors as are the ductal and ductular tumors.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Ontario Cancer Treatment and Research Foundation; Medical Research Council of Canada (PG2); Clare Nelson Bequest; Burl Ives Cancer Research Fund; Edith S. Ross Bequest; Non-Governmental Organizations Division; Canadian International Development Agency and Dr. W. F. James. We wish to acknowledge the helpful comments provided by Drs. D. M. Robertson and N. Kaufman; the excellent technical assistance of B. Gubbins and M. Chiong; and the expert secretarial assistance of M. Conley.

REFERENCES


