Scanning Electron Microscopy / 1976

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SCANNING ELECTRON MICROSCOPY / 1976 / II

Proceedings of the

WORKSHOPS ON BIOLOGICAL APPLICATIONS OF THE SCANNING & SCANNING TRANSMISSION ELECTRON MICROSCOPE

Part V

ADVANCES IN BIOMEDICAL APPLICATIONS OF THE SCANNING ELECTRON MICROSCOPE

Part VI

SCANNING ELECTRON MICROSCOPY IN REPRODUCTIVE BIOLOGY

Part VII

PLANT SCIENCES APPLICATIONS OF THE SCANNING ELECTRON MICROSCOPE

Part VIII

ZOOLOGICAL APPLICATIONS OF THE SCANNING ELECTRON MICROSCOPE

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SCANNING ELECTRON MICROSCOPY/1976

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FOREWORD

These proceedings contain papers accepted for the four workshops organized for Biological Applications of the SEM and STEM. Although we did not so intend, we must publish these proceedings as a separate volume because Volume I itself contains 800 pages. For all purposes, the two volumes should be considered together since several papers in Volume I (see p.x) will be of direct interest to biologists using this volume. We apologize to the readers and authors for any inconvenience. A full preface describing how these proceedings were prepared, acknowledging the help of various persons, as well as a list of reviewers for both volumes are included in the beginning of Volume I.

Although edited, the authors' replies to reviewers' questions are largely printed as received (see DISCUSSION WITH REVIEWERS appended to each paper). In the reviewing system followed here, the author has the last word and the intelligent reader must make his own critical evaluation of the authors' replies.

April 5, 1976

Om Johari
Robert P. Becker

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LATE PAPERS - PART V

SCANNING ELECTRON MICROSCOPY/1976

PAPERS OF INTEREST TO BIOLOGISTS IN VOL. I

Volume I contains papers presented at the 9th Annual SEM Symposium, three other workshops (including one on Techniques for Particulate Matter Studies), and tutorial papers. Several papers in Vol. I should be of direct interest to those using the SEM for biological studies. A partial list of such papers is given below.

SCANNING ELECTRON MICROSCOPY/1976 (Part V)
Proceedings of the Workshop on
Advances in Biomedical Applications of the SEM
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CELL SURFACE CHANGES ASSOCIATED WITH HUMAN BREAST CANCER

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ABSTRACT

The scanning electron microscope (SEM) reveals differences of potential diagnostic or prognostic importance between the surface morphologies of duct cells from cancerous and dysplastic human breasts. Through examination of tissue samples processed both for SEM and light microscope histology, it has been found that, when a primary carcinoma is present, there are substantial changes in the morphology of the mammary ducts within histologically "normal" areas away from invasive nodules, as well as in hyperplastic regions. These differences, in particular the alterations in surface microvilli, appear to extend throughout the affected breast. The observations call attention to pitfalls associated with using so-called "normal" areas from cancerous breasts for establishing structural or functional characteristics of normal breast tissue. In addition, the observations show that infiltrating duct carcinoma cells which have fewer surface microvilli than duct cells from dysplastic breasts, can be readily identified on the basis of their shape and surface topology.

Whether the observed alterations prove to be an accurate indication of malignant change or an essential part of the neoplastic mechanism remains to be established. Nevertheless, it is possible that recognition of such morphological differences may facilitate the differentiation of benign conditions from infiltrating duct carcinoma and the diagnosis of noninvasive duct carcinoma.

KEY WORDS: Human Breast Cancer, Human Breast Dysplasia, Cell Surface, Microvilli, Scanning Electron Microscopy

Introduction

For several years we have been studying the cell surfaces of human breast lesions and neoplasms with the scanning electron microscope (SEM). Our interest in this problem stems from the observations of others which suggest that the structure and composition of the plasma membrane probably vary among different cell types, as well as the widespread suspicion that cancer cells may have modified plasma membranes.

It is generally assumed that most human breast carcinomas arise from duct epithelium and pass through a period of intraductal noninvasive growth prior to invasion, local extension and metastasis. The segment of the neoplastic process between initiation and invasion has not been studied extensively because there is little agreement concerning the criteria for diagnosing noninvasive duct carcinoma. In addition, morphologic criteria by which the transition from normal to neoplastic can be accurately recognized in the human breast have not been found and may be indefinable. Nevertheless, recent SEM observations from our laboratories revealing structural differences between the microvilli of duct cells from cancerous and dysplastic human breasts[1] raise the possibility that such morphologic evidence may be found in the surface topography of human breast cells. Therefore, the purpose of this paper is to review some of our SEM observations of human breast cells and to indicate where further progress may be made.

Materials and Methods

Breast tissue was obtained from 52 women at biopsy and mastectomy. There were 18 dysplasias, 5 fibroadenomas, 2 lobular carcinomas *in situ*, 23 infiltrating duct carcinomas, 1 cytosarcoma phyllodes, 1 colloid carcinoma, 1 papillary carcinoma and 1 medullary carcinoma.

We have tried many different preparative procedures during the course of this study. Routinely, however, the surgical specimens are rinsed briefly in 0.1 M cacodylate buffer, pH 7.4, and fixed four days to two weeks at 4°C in a modified Karnovsky's fixative[2] containing 2% glutaraldehyde, 1% paraformaldehyde and 0.1 mM $CaCl_2$ in 0.067 M sodium cacodylate buffer, pH 7.4. Tissues are dehydrated from 3-5 minutes at 4°C in 20, 30, 50, 70, 80, 95 (2x) and 100 (2x) per cent ethanol. A final change of absolute ethanol is made at room temperature. The specimens are then immersed and frozen in supercooled liquid Freon. The frozen tissue is cracked with a pre-cooled scalpel[3] and placed in absolute ethanol for 10 minutes to defrost. The tissue is critical-point dried in a Bomar SPC-900/EX critical-point dryer using liquid CO_2.[4] The dried tissue is viewed under a dissecting microscope and the upper halves of ducts close to the surface of the specimen are teased away. This allows the observer to view essentially all cell surfaces bordering one half of the duct lumen. The specimens are mounted on stubs using silver conducting paint, sputter coated in a Hummer II apparatus with ca 200 Å AuPd and viewed at 20 kV in a Kent-Cambridge S-4 SEM.

Specimens examined and photographed in the SEM were processed for histology using the first six steps of the method developed by Ayres *et al.*[5] Celloidin sections were cut at 5 μ on a sliding microtome and stained with hematoxylin and eosin.

Observations and Discussion

Dysplasias

The term dysplasia refers to a perturbation in the usual orderly organization of cells and tissues. Originally it was applied only to developmental disorders, but now it is generally used to refer to morphological disturbances in growth processes. Fibrous and fibrocystic mastopathy, mazoplasia, adenosis, sclerosing adenosis and adenofibrosis are terms commonly applied to the chief variants of mammary dysplasia. Dysplasia is characterized by proliferative changes in the epithelium and stroma which may be associated with cyst formation. Mammary dysplasia is of interest because: (1) certain forms are easily confused with carcinoma; (2) chronic fibrocystic disease may be a precancerous lesion; and (3) the incidence of carcinoma in women with gross cystic disease is 3-5 times that of the general female population.

Figure 1 is a low magnification micrograph from a patient with fibrocystic mastopathy. A hyperplastic duct, microscopic, epithelial-lined cysts and a grossly evident blue dome cyst with a bare connective tissue surface are present in this specimen. Cysts are believed to be formed from dilated ducts. When present, cells in a cyst wall often are flattened or cuboidal and usually show moderate variation in size and shape (Fig. 2). Flattened cells usually have a prominent row of microvilli encircling the terminal bar region (Fig. 3).

Some cysts show papillary proliferation of the epithelium while others have a diffuse papillomatosis (i.e., fully developed branching papillae with long stalks) in the dilated ducts - the lesion of fibrocystic disease thought by some to be associated with malignant transformation (Fig. 4). Trabeculae composed of epithelial stalks may bridge the smaller cysts, while in others the lumen may be obliterated by the proliferative epithelium.

The apical surface of the glandular epithelial cells within both normal and hyperplastic ducts from dysplastic breasts is covered with microvilli. The diameter of the microvilli is fairly constant (ca 0.1 μm) and the average microvillus is 1.5 - 2 μm long. Cells undergoing

Fig 1: This specimen, from a patient with fibrocystic mastopathy, contains a hyperplastic duct (upper left), microcysts (upper right) and a large blue dome cyst (lower half).

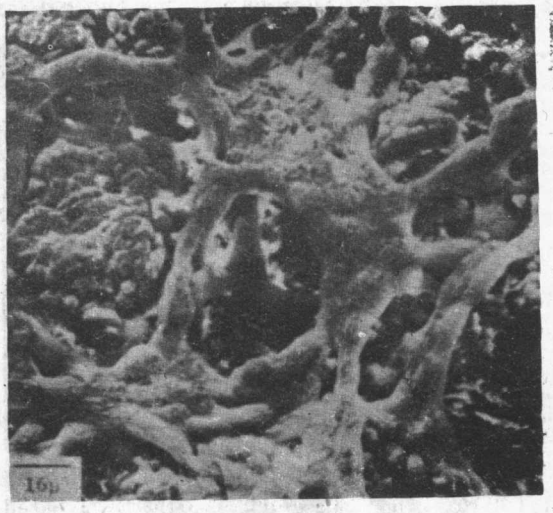


Fig. 4: Cyst wall containing branching papillae.

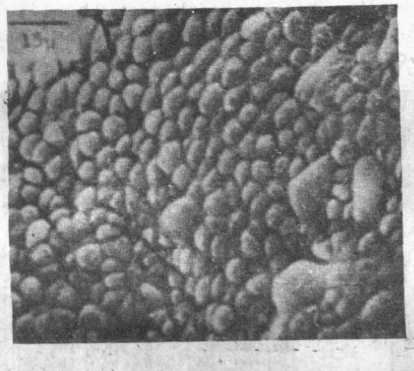


Fig. 2: Cells lining cyst walls often show moderate variation in size and shape.

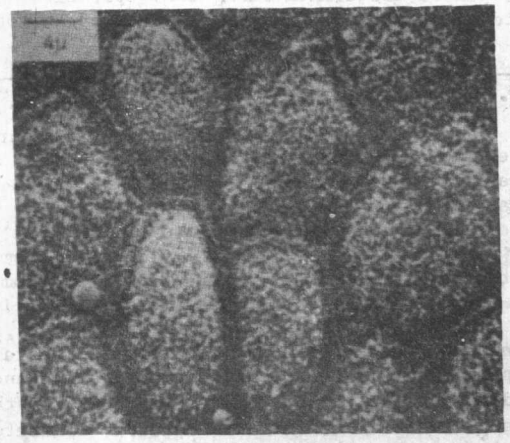


Fig. 3: A prominent band of microvilli encircles the terminal bar region of the epithelial cells in this cyst wall.

apocrine metaplasia have very few microvilli but are easily identified by their characteristic size and shape.

Duct hyperplasia, papillomatous projections and variations in cell size and shape also are present in breasts afflicted with infiltrating duct carcinoma, but the distribution and arrangement of the apical microvilli is different.

Infiltrating Duct Carcinoma - Ducts

Figures 5 and 6 show cells in histologically normal and hyperplastic ducts from breasts with infiltrating duct carcinoma. The apical surface of the duct epithelium may have some or all of the characteristics of ducts from noncancerous breasts, but duct cells from the cancerous breasts display greater variation in size and shape as well as the number, length and arrangement of microvilli. These cells almost invariably have fewer and shorter microvilli than duct cells from noncancerous breasts. In our experience, when these alterations are extensively distributed along the duct, there is good reason to suspect that the specimen came from a breast with carcinoma. Alterations that appear to be more specific for carcinomatous breasts are: (1) the partitioning of the surface microvilli into small groups or clusters of 3 or more microvilli clumped together at their tips (Fig. 7); (2) the presence of intercellular microvillus contacts, i.e., microvilli from adjacent cells touching at their apices (Fig. 8); and (3) a prominent clump of thickened, irregular microvillus-like projections in the center of the apical surface (Fig. 9).

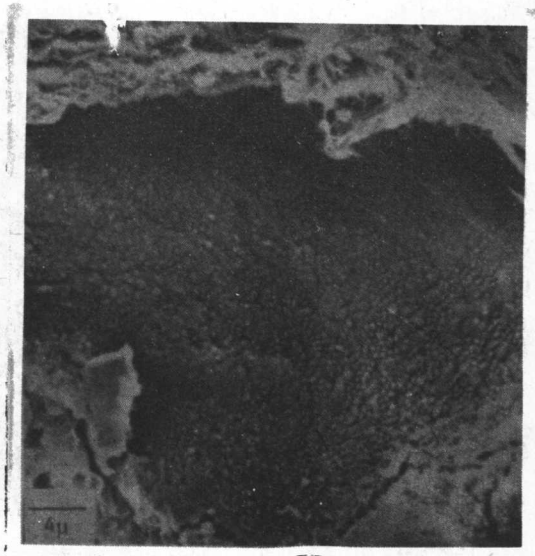


Fig. 5: Duct from so-called normal area of a breast with infiltrating duct carcinoma shows moderate variation in cell size and shape.

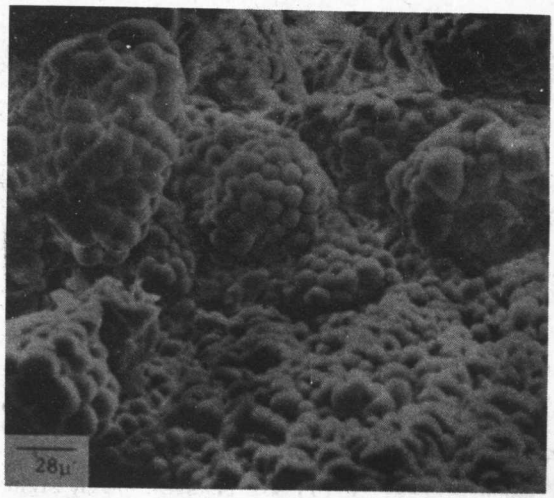
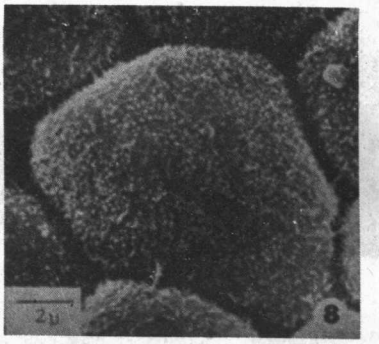


Fig. 6: Hyperplastic duct from so-called normal area of a breast with infiltrating duct carcinoma shows marked epithelial proliferation and variation in cell size and shape.



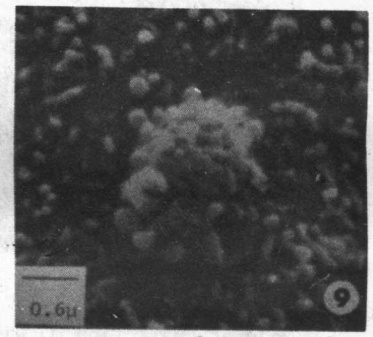


Fig. 7: Clumped microvilli are usually seen on the surface of duct cells from patients with infiltrating duct carcinoma. Fig. 8: Intercellular microvillus contacts are common among duct epithelial cells in breasts with infiltrating duct carcinoma. Fig. 9: Thickened, irregular microvilli are often found on duct cells in carcinomatous breasts.

Tissues from the last 29 women were processed and analyzed without knowledge of their clinical diagnosis. In every case there has been perfect agreement between the pathological diagnosis and the one we made using the SEM. Thus it appears that the distribution and arrangement of the apical microvilli on mammary duct epithelium can provide sufficient morphological information to allow us to predict whether the tissue came from a cancerous or non-cancerous breast. Whether the observed differences turn out to be either an accurate indicator of malignant change or an essential part of the neoplastic mechanism remains to be established.

One of the questions that arose from our observations on the biopsies from cancerous breasts was: Will ducts in so-called "normal" areas away from the primary lesion or biopsy site also show these changes? In an attempt to answer this, tissue samples were removed from mastectomy specimens at graded distances from the biopsy site. In all cases the microvilli on the duct epithelium throughout the breast resembled those within the mass of the initial sample. Ducts from so-called normal areas and at distances as great as 7 in. (18 cm) from the biopsy had the same surface features. Therefore, it appears that these surface characteristics extend throughout the affected breast (Figs. 10 and 11).

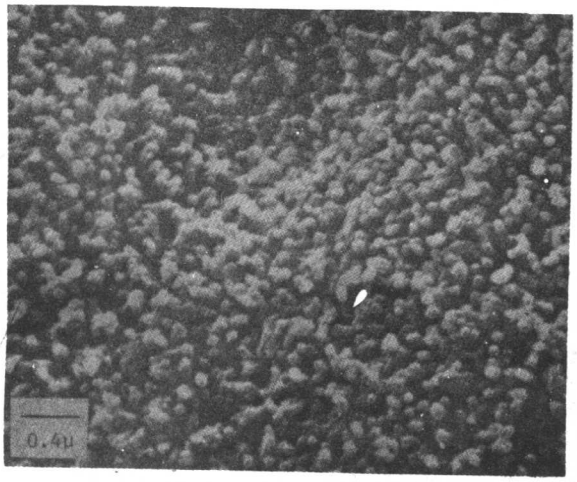


Fig. 10: The tissue shown here and in Figure 11 are from the same patient with infiltrating duct carcinoma. At biopsy, clumped short, thick microvilli were found on the duct epithelium.

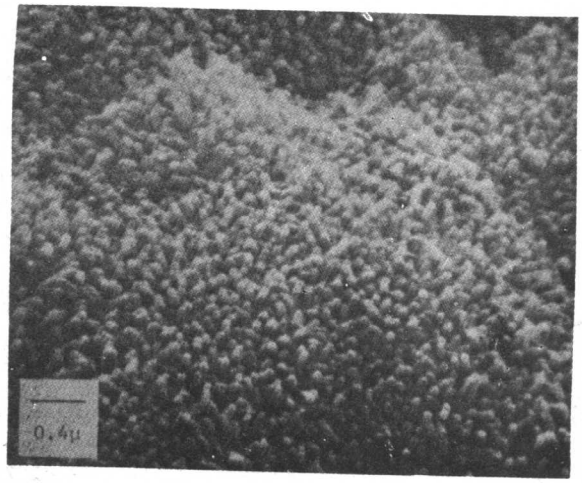


Fig. 11: Similar alterations in the apical microvilli of the duct epithelium were found at mastectomy in a region about 4 inches from the biopsy.

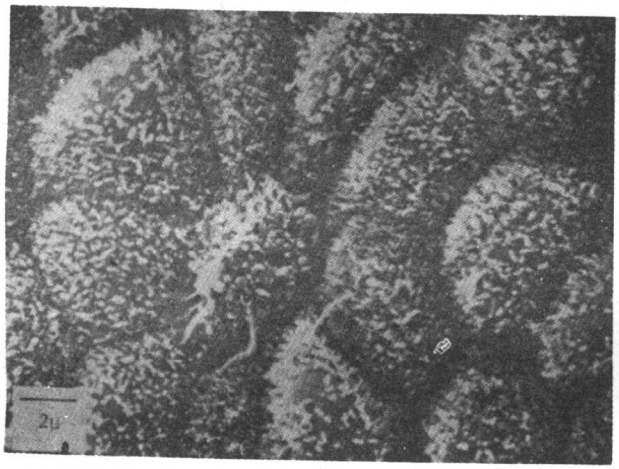


Fig. 12: Cyst wall from 32-year-old woman with dysplasia showing some alterations in the microvilli that are more commonly found in cancerous breasts.

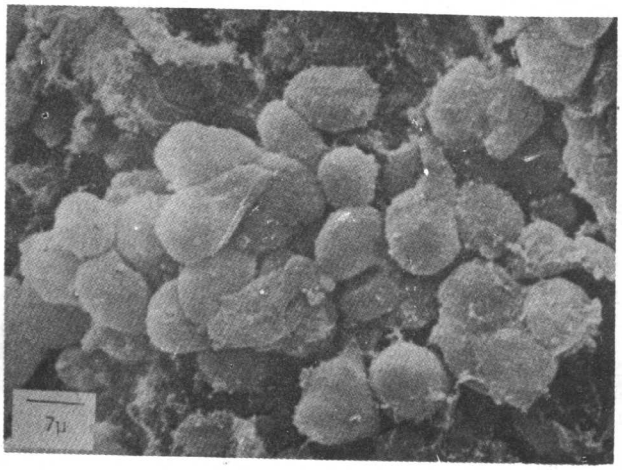


Fig. 13: Infiltrating duct carcinoma cells in the stroma of the breast have few microvilli.

When and how these alterations arise, their relationship to the multicentric or focal origin of carcinoma, and whether they are a preneoplastic lesion or the response of noncancerous duct cells to some influence exerted by the malignant breast cells or to systemic factors from the patient await clarification. Nevertheless, our findings corroborate and extend the histologic observations of others [6,7,8,9] that there are extensive alterations in the duct epithelium, even in areas not adjacent to invasive nodules, of all but a few of the breasts containing primary carcinomas. At the histologic level, the alterations range from minimal hyperplasia and disorganization of the duct epithelium to frank intraductal carcinoma, but there is no easily defined difference between extreme hyperplasia and early carcinoma. Therefore, it has become important to determine whether the SEM is revealing specific differences. To answer this question, we will examine and photograph with the SEM ducts from at least 100 patients with infiltrating duct carcinoma. Then, we will process these specimens for histology and transmission electron microscopy. This information plus that accumulated from nonmalignant breast lesions and reduction mammoplasties should provide a more definitive index to the frequency and specificity of the duct alterations.