SCANNING ELECTRON MICROSCOPY OF A PRIMARY AND A BRAIN METASTATIC BREAST CARCINOMA CELL LINE.

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INTRODUCTION

Bresst cancer cells have been studied both in vivo and in vitro by using the transmission electron microscope. Specific characteristics Were used with incomplete certainty to identify various types of cancer cells or cell lines (Arnold *et al.*, 1976; Buehring and Hacket, 1974; Domagala and Woyke, 1975; Ozello, 1971 and 1972 and Spriggs and Meek, 1961). Microvilli were frequently observed in cancer cells. Spring-Mills and Elias (1975) however, noticed that breast cancerous cells have fewer microvillo than non - cancerous cells. Microvilli Microvilli are known to present on the surface of some normal cells as the mesothelial lining of serous cavities (Andrews and Porter, 1973). However, shed cells in effusions were seen to lose their microvilli (Domagala and Woyke, 1975). The real significance of microvilli as a major difference between cancer and normal cells has been questioned (Kolata, 1975) and seems to need further clarification. The proper number, length, configuration and distribution of microvilli on the cell surface can only be visualized by the scanning electron microscope. The present study describes the surface ultra-structure of three human breast cell lines : 1- HBL-100 a nontumerogenic cell line (Gaffney *et al.*, 1976). 2 - ET-20, a primary ductal carcinoma (Lasfargues and Ozello, 1958). 3 - MDA-MB-361 (Blumenschein and Cailleau, 1976).

MATERIALS AND METHODS

HBT-100 cells were derived from a breast pump milk sample, of a normal lactating female that had two children, on 9/27/1974 (Investigator and laboratory of origin : Dr. Edwin V. Gaffney, Department of Biology, Pennsylvania State University, University Park, Pennsylvania). The cells have a chromosomal mode: 68, but they do not produce tumors in nude athymic mice. So. they are transformed nontumerogenic cells.

Egypt. J. Anat., Vol. 2, 77 - 86 (1979).

They were maintained in a spinner culture and the passage number 23 was transferred to coverglasses and fixed after 72 hours of incubation.

BT-20 cells were derived from the breast tissue of a 74 year old caucasian female on 3/28/1958. The diagnosis was a poorly differentiated infiltrating ductal carcinoma of non specific type. Their chromosomal mode is 50 and they are tumerogenic in nude athymic mice. The present cells are in a monolayer culture, passage number 208, transferred from a spinner culture and fixed after 72 hrs.

MDA-MB-361 cells are secondary breast carcinoma metastatic to the brain and obtained by craniotomy done for a 40 years old female on 2/5/1975. The primary disease was diagnosed as breast adenocarcinoma, and oophorectomy was done on 1/2/1975. The chromosomal mode of the cells is 56 with one extra long chromosome. The present cells are in a monolayer culture transferred from a spinner culture at the passage number 24 and fixed after 72 hours of incubation.

The three cell lines were cultured on one medium; L-15. The cells were washed thoroughly in cacodylate buffer and fixed in 2% glutaraldhyde in the same buffer (O.I.M) for one hour. They were then re-Washed and postfixed in O_s 0_4 for 20 minutes (1% aquous solution). Dehydration in ascending grades of ethyl alcohol was followed by three changes in absolute alcohol 20 minutes each. The specimens were then critical point dried using liquid C 0_2 and coated with gold palladium (2000 A° thickness). The cells were examined by the autoscann SEM operated at 20 K.V.

RESULTS

The nontumerogenic cell line (HBL-100) forms a monolayer sheet the cells of which are separated from each other by a variable distance (Fig. 1). The cells are generally flattened with a central bulging apparantly formed by the nucleus (Fig. 2). Although their outline is irregular, the cells have an almost equal size with an average diameter of 15 microns. The cell margins form thin cytoplasmic sheets with thin tapering processes, and the adjacent cells are confluent together (Fig. 2). The cell surface appears rounded, smooth and featureless. It is completely devoid of microvilli or other adornments except a few number of rounded blebs with a variable diameter between one and four microns (Fig. 2).

The primary cancer cells «BT-20» appear as a continuous monolayer sheet of cells separated from each other by narrow slits of equal size (about 4*u*). probably due to slight shrinkage of the cells during preparation (Fig. 3). However, similar slits were seen in the living cells by light microscopy. The malignant

cells though flattened show a general roundness that conceals the nuclear bulging (Fig. 4). The cell outline is polygonal or fusiform, and the cell diameter vary considerably between 10 and 50 microns (Fig. 3). The cell surface exhibits numerous microvilli of nearly equal size (1u in length and 0.1 - 0.2u in diameter) (Fig. 4). The cell surface between the microvilli is completely smooth and featureless. The distance between two microvilli averages 2.5u.

The cells of the breast carcinoma metastatic to the brain (MDA-MB-361) are seen as scattered groups of cells with wide irregular spaces in between. The cell outline is irregular and adjacent cells have parallel margins (Fig. 5). The cells are strikingly large; thick and widely expanded (over 50u), and the general roundness of the cells conceals the nuclear bulging (Fig. 6). The surface features are mainly microvilli. They are numerous, densely packed and cover almost the whole exposed surface of the cell (Figs. 5 - 8). They exhibit a wide range of variation in length, diameter and surface distribution. Areas of smooth surface with very short and a few microvilli (Fig. 6 lower left) are irregularly intermingled with areas with long (1.8u.) microvilli that are densely packed with minimal or almost no space in between them (Figs. 6 & 8) and appear to be grouped in clusters or tufts. Big bullae with a completely smooth surface or with small blebs superimposed could be seen adjacent to areas of clusters of dense long microvilli (Fig. 8). Some cells show long curled pseudopodia like processes (Figs. 7&8).The microvilli on these processes are numerous but exhibit a gradually decreasing length distally towards the curled end which is smooth and devoid of microvilli. This end shows a wavy or wrinkled surface (Fig. 7). Parts of the cell margins at the edge carrying the pseudopodia like processes show thin expanded lamellae or ruffling attaching the cell to the underlying substrate (Fig. 6).

DISCUSSION

The significance of microvilli as a diagnostic criterion on the surface of cancer cells has been a point of discussion among investigators. Nemanic and Pitelka (1971) observed abundant irregularly scattered microvilli on the cells of normal lactating mammary glands in mice. The present HBL-100 cells are normal lactating mammary gland cells as they were originated from milk pump sample, however, their surface was strikingly smooth and devoid of any microvilli. The absence of microvilli from these cells is not likely to be due to the fact that they are transformed cells, on the contrary normally smooth cells exhibit extensive surface features and microvilli when become transformed (Borck & Fenoglio 1976). Possible

explanations of why normal lactating breast cells lose their microvilli in vitro are : 1- Microvilli are assigned to certain functions bound to the presence of the cells in their normal position in vivo. 2 - Cell-to-cell contact was found necessary for the appearance of microvilli in normally dividing cells (Rubin & Everhart, 1973), this could also be true during other activities as lactation. 3 - Cells in culture media have, as cells in effusion, a wider portion of their surface area exposed to the surrounding fluid and so need no microvilli.

The complete smoothness and abscence of microvilli on the surface of the noncancerons HBL-100 cells are in marked contrast with the extensive covering of microvilli on the surface of the cells in the two malignant cell lines studied. The distribution of the microvilli is observed to be much dense and exhibit a wide range of variation in size, shape and density over the metastatic malignant cell line «MDA-MB-361» than on the primary malignant one «BT-20» (Figs. 4, 6 & 8). The difference between the surface features in the two malignant cell lines could possibly be due to the fact that one of them is primary cancer while the other is metastatic. It could also be due to the different pathological origin of the two cell lines, as one is ductal carcinoma and the other is adencarcinoma. Yet, one cannot conclude that metastatic cancerous cells exhibit more dense and widely variable microvilli than primary malignant cells, because the two cell lines are not derived from the same patient or even belong to the same pathological type. Also it is difficult to say that adenocarcinoma cells exhibit more surface microvilli than the cells of ductal carinoma from just these two cell lines. However, preliminary observations on a bigger number of primary and secondary breast cancer cell lines that are currently examined by the auther using the scanning microscope reveal similar supportive results. Moreover, although the present two malignant cell lines cannot be directly compared with each other, yet the marked difference between each of them and the noncancerous cells « HBL - 100 » gives a significant evidence that the appearance of microvilli is strongly suggestive of malignancy on normally smooth cells.

Recently the scanning electron microscope has been used to study the surface features of cancer cells (Ambrose and Elison, 1968; Boyde *et al.*, 1972; Jordan and Williams, 1971; Osumi *et al.*, 1974; Porter *et al.*, 1974; Vial and Porter, 1975 and Williams *et al.*, 1969 and 1975). Friedlander (1969) stated that ballooning of the nucleus in malignant cells causes surface changes with flattening of microvilli. More recently Spring-Mills and Elias (1975) mentioned that in ducts of mammary

glands, cancerous cells show a tendency to have fewer or rudimentary microvilli than noncancerous cells. However, many others observed microvilli on cancer cells (Fischer and Cooper, 1967; Porter *et al.*, 1974; Spriggs and Meek, 1961; Jordan and Williams, 1971 and Porter *et al.*, 1973).

The present observations show clearly the marked difference between the surface of the malignant cells that is densly covered with microvilli and the strikingly smooth surface of the noncancerous cells. The microvilli on the malignant cells were observed neither restricted to a specific stage of the cell cycle nor dependant on contact with adjacent cells (Porter *et al.*, 1973 and Kubin and Everhart, 1973).

Microvilli were observed on the surface of transformed cells, either by X-irradiation, virus or spontaneously induced. The present cells «HBL-100» though transformed are completely smooth. Microvilli are thus suggestive of malignancy rather than mere transformation.

Many possible functions were assumed to be carried out by microvilli; e. g. absorption, excretion, prevention of adhesion and increase of surface area of the cell ... etc. As microvilli are elicited by cell to cell contact and disappear when shed in effusions, they seem to be a response to external stimuli. However, the present observations on the metastatic cells (Fig. 8), where areas with dense tufts of long microvilli are close to areas of big smooth blebs, probably mean that these surface features are expressions of internal functions rather than responses to external environmental conditions. This also shows that various cytoplasmic regions of cell, no matter how close they are, could be involved in widely different functions.

Signs of malignancy other than microvilli could be also observed in the present malignant cell lines. Increased growth (Gey, 1955), was viscualized as increased cell size and roundness that concealed the nuclear bulging seen in the flattened noncancerons HBL-100 cells. Signs of loss of contact inhibition (Schutz & Mora, 1966) could also be detected as the cells were seen to overlap each other (Fig. 4).

The pseudopodia-like processes (Fig. 7) are suggestive of a possible mechanism of movements for the cells. These cytoplasmic processes flow forwards towards its curled end, and gradually withdraw their microvilli. The fluidity of the lipid phase of the plasma membrane may favour its motion and the rearrangement of its particulate enteties (Benedetti *et al., 1973).* These processes could expand later into thin lamellae or ruffles (Figs. 6 & 7) that attach themselves to the substrate. The role of lamellopodia in the locomotion of cells in culture has been

stated before (Abercrombie *et al.*, 1970 & 1971). These thin lamellae at the leading edge of the cell could expand, thicken and become filled with cytoplasm pulling the main bulk of the cell forwards. Further evidence of this mechanism could be given by cinematography of the living cells.

SUMMARY

This is a study of the surface ultrastructure of MDA-MB-361 cells, a line derived from breast adenocarcinoma metastatic to the brain, by using the scanning electron microscope. The cell surface features are compared with those of a normal human breast cell line (HBL-100) and with a primary infiltrating ductal carcinoma cell line (BT-20). The malignant cells are larger and covered extensively with microvilli which exhibited marked variations in size, shape and distribution in the case of the secondary malignant cells. Some of the metastatic cells displayed pseudopodia - like processes which could play a role in the cell locomotion. The malignant cells differ markedly from the normal human breast cell line (HBL-100) which, although transformed, is nontumerogenic. The normal cells are smaller and have a smooth featureless surface. The presence of microvilli with variable size and distribution is strongly suggestive of malignancy and not mere transformation. ... ...

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LEGENDS

Fig. (1) : A scanning electron micrograph (X260) of HBL - 100 cells. They are separated from each other by a variable distance.

Fig. (2) : The marked area in fig. 1 (X 1120), the cells are smooth with a central bulging and a few number or rounded blebs.

Fig. (3) : A scanning electron micrograph (X 260) of BT-20 cells. They are separated from each other by narrow spaces about four microns.

Fig. (4) : The marked area in fig. 3 (X 2800). The cell surface exhibits numerous microvilli of equal size and homogeneous distribution.

Fig. (5) : A scanning electron micrograph (X 1360) of MDA-MB-361. The cell margins are parallel and the surface is covered with numerous microvilli of variable size.

Fig. (6) : A MDA-MB-361 cell (X 1600) showing big size of the cell and numerous microvilli of variable size and distribution.

Fig. (7) : Right box in fig. 6 (X 3400). Pseudopodia-like process.

Fig. (8) : Left box in fig. 6 (X 6400). Tufts of dense and long microvilli adjacent to a big smooth bleb.

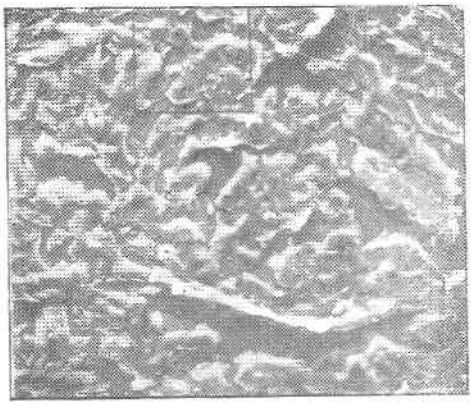
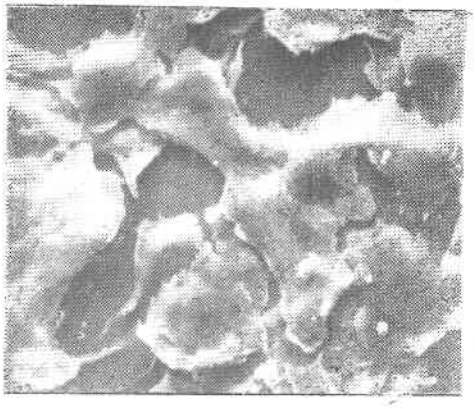


Fig. (1)



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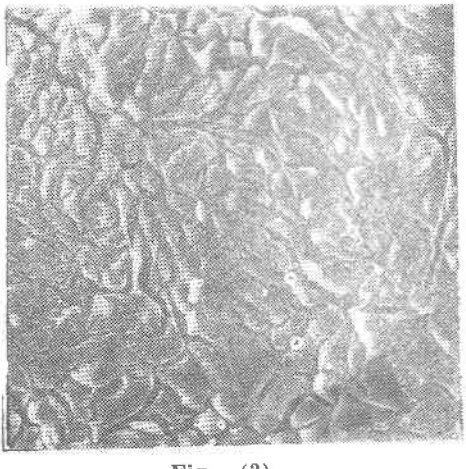

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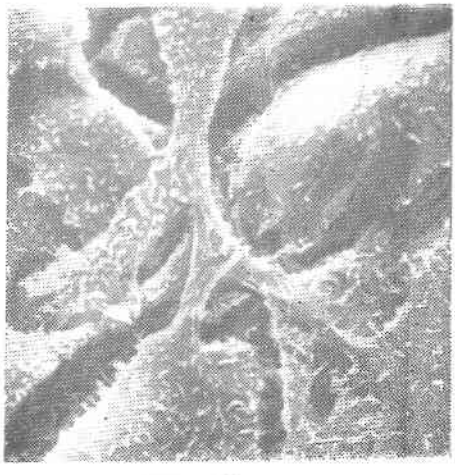

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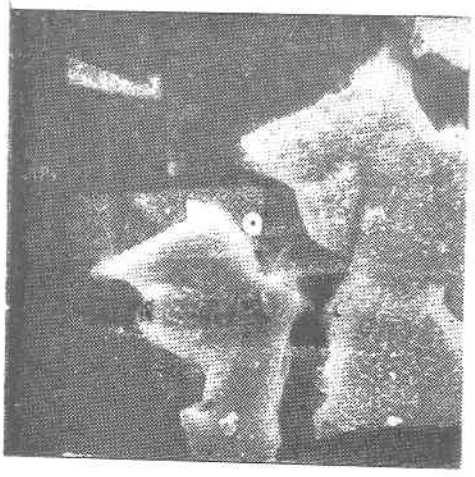

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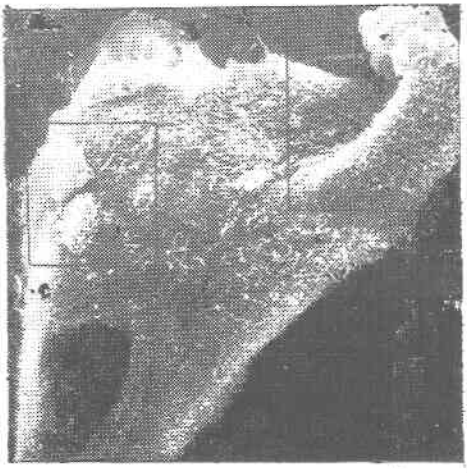

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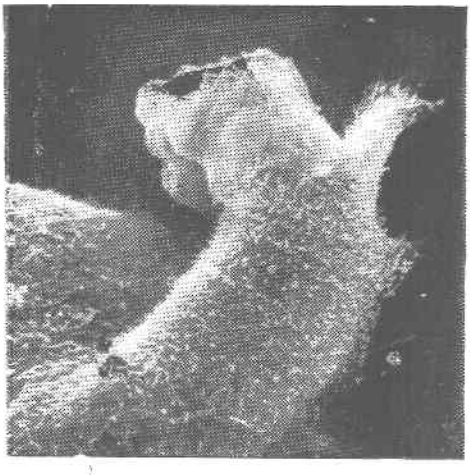

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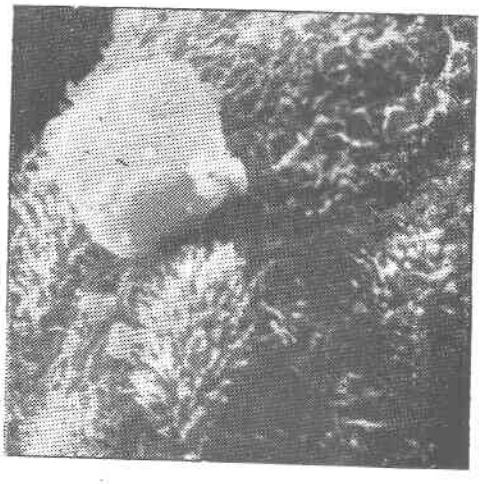

Fig. (8)