Title
SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF HUMAN METASTATIC MAMMARY CARCINOMA

Permalink
https://escholarship.org/uc/item/60j0w5qv

Author
Hackett, A.J.

Publication Date
1979-02-01
SCANNING AND TRANSMISSION ELECTRON MICROSCOPY
OF HUMAN METASTATIC MAMMARY CARCINOMA

Adeline J. Hackett and E. Louise Springer

February 1979

Prepared for the U. S. Department of Energy
under Contract W-7405-ENG-48

TWO-WEEK LOAN COPY

This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. Division, Ext. 6782
SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF HUMAN METASTATIC MAMMARY CARCINOMA

Adeline J. Hackett and E. Louise Springer

Cell Culture Laboratory
School of Public Health
University of California, Berkeley
Berkeley, California 94720

Introduction

The biological behavior of breast cancer reveals variability manifested by the mode of appearance, and the nature of spread. These tumors metastasize by three routes: a) via the internal mammary chains of lymph-nodes b) subdermal lymphatics and c) the hematogenous route. The metastasis may be located in a single system such as bone or in multiple areas such as skin, lungs, and bones. The factors involved in the selection of sites are not understood, but it is known that prognosis is correlated with site: soft tissue metastasis has the best prognosis, osseous is intermediate, liver and brain have the poorest prognosis (1).

Ultrastructural studies have been done on various forms of breast carcinomas (2-4), but little has been done on the metastatic tumors. Surface structure as visualized by scanning electron microscopy (SEM) has been studied on a very limited number of tissue types, but it appears to be a promising new tool for analysis of malignant changes (5). We have embarked on a comparative study of mammary tumor cells metastatic to three sites, (soft tissue, bone, and visceral) in the hope that morphological characteristics can be correlated with dissemination pattern. This is the first report on that study and describes a bone metastasis of an infiltrating ductal carcinoma of the breast.

Procedures And Materials Used

Case History

This 54 year old caucasian women had a recurrent history of cancer. In 1963 she had a hysterectomy for carcinoma of the cervix; in 1973 a radical mastectomy for adenocarcinoma of the breast with axillary node involvement; in 1974 she had an oophorectomy and a total hip replacement because of metastatic bone disease. The patient died as a result of metastasis to the mediastinum in 1974.

The specimens for this study were obtained from the bone metastasis.
Electron Microscopy

Small pieces of tissue were taken from the internal portion of the tumor, fixed in 10% formaldehyde and stained with hematoxylin and eosin for light microscopy. Specimens for electron microscopy were fixed immediately after surgical excision in cold 2.5% glutaraldehyde in 0.1N sodium cacodylate buffer (pH7.3) for 3 hrs. The specimen was divided into 2 pieces while submerged in buffer and a sketch made of one cut surface to serve as a guide for orientation during embedment and thin sectioning.

The opposite and complementary cut surface was easily identified in the SEM. After 24 hrs. fixation with buffered 4% osmium tetroxide, the specimens were either: 1) critical point dried (6), mounted on specimen stubs with silver paint, then rotary coated with carbon, and gold (200Å) and examined in the Cambridge S4-10 Scanning Electron Microscopy or 2) dehydrated with ethanol, embedded in Epon, with the sections post stained with uranyl acetate and lead citrate then, examined in the Siemens Elmiskop 101 transmission electron microscope.

Cell Line

The established human breast tumor cell line, ALAB (7) was obtained from E. Lasfargues (Camden, N.J.). The cells were maintained in Falcon flasks in Eagle's basal medium supplemented with 20% fetal calf serum. The line was mycoplasma free. The cells were grown on glass coverslips for study by SEM. These methods have been described in detail elsewhere (8).

Results

Metastatic mammary cells (MMC) in bone were identified by transmission and scanning electron microscopy. Ultrastructural markers which differentiate the MMC from bone fibroblasts, and lymphoreticular cells are desmosomes, tonofibrils, and cytoplasmic organelles associated with secretory activity. Surface structures and cell-to-cell relationships which are distinctive for bone cells are layering of collagen bundles in a regular pattern, and the elongated spindle shape. MMC appear flat, cuboidal with smooth surfaces free of fibrils, blebs and microvilli by SEM. These characteristics were correlated with the appearance of the tumor by light microscopy.

Light Microscopy

The primary tumor was a well differentiated adenocarcinoma of the breast which showed a glandular pattern. Cells were oriented in tandem "Indian" file in an infiltration pattern typical of lobular carcinoma in situ. The metastatic tumor was classified as grade II. The extravascular cells were also observed in end-to-end arrangements, typical of breast carcinoma. The tumor cells were found in groups forming nodules surrounded by the osseous tissues.

Transmission Electron Microscopy

The MMC in bone showed organizational and ultrastructural features typical of differentiated glandular epithelial cells of the breast. The cells were organized in two distinct patterns: infiltrating tandem or "Indian file" and multicellular aggregates or nodules. The cells in each organizational pattern had many ultrastructural features in common.
The infiltrating cells in tandem or "Indian file" were found in long rows surrounded by collagen containing un-mineralized bone stroma (Fig. 1). The nuclei were irregular in contour with clumped and margined chromatin. Numerous, irregularly shaped mitochondria with few cristae occupied most of the cytoplasm. The electron transparent matrix often contained rings and myelin figures. Small intracytoplasmic lumens containing fibrous elements were common. Tonofibrils were abundant in the cytoplasm but no perinuclear bundles were observed. Parallel microfilaments distinctive of myoepithelial cells were not identifiable in these cells. Basal laminae were not found on these smooth margined cells. Although these cells were closely apposed, no desmosomal junctions were observed.

The tumor cells in nodular formation were multilayered with cytoplasmic membrane invaginations between the cells similar to those found in normal mammary epithelium (9) (Fig. 2). The nucleus was lobulated with margined chromatin. The cytoplasm contained numerous distended mitochondria with disrupted cristae, and myelin figures. The perinuclear Golgi was well developed and dilated, even cystic. Pleomorphic electron-dense bodies consistent with lysosomes were occasionally observed. Lipid droplets and protein granules (probably casein) were more prominent than in the infiltrating tumor cells. Non-bundled tonofibrils were scattered throughout the cytoplasm, but the parallel microfilaments distinctive of myoepithelial cells were absent. Basal laminae were absent from these cells. Numerous desmosomes connected the nodular tumor cells (Fig. 3) which identified these cells as epithelial since neither osseus nor reticuloendothelial nor vascular cells have desmosomes. A consistent characteristic of both infiltrating and nodular tumor cells was the presence of "tubulofilamentous" structures within the electron transparent matrix of the mitochondria (Fig. 4). These structures are similar to those reported in epithelial cells of breast carcinoma (10).

Scanning Electron Microscopy (SEM)

An area adjacent to that examined by ultrathin section was surveyed by SEM. The bone tissue was easily distinguished from the infiltrating mammary tumor cells by the presence of elongated spindle-shaped cells and parallel bundles of collagen (Fig. 5). The mammary cells were flattened, cuboidal and relatively free of surface excrences. The two organizational patterns of tumor cells were very apparent in SEM. Fig. 5 shows the infiltration (tandem order) pattern and Fig. 6 the nodular pattern.

Some of the tumor cells in tandem were enfolded and curled forming "cytoplasmic towers" with a pseudopod affixed to the collagen surface (Fig. 5). Another cell with a smooth surface is thinly extended over several collagen bundles. A portion of the cell margin is deeply ruffled and creased. The plump erythrocyte is a good indicator of the quality of the preservation.

The arrangement of cells in intact tissue is an important identifying marker in the SEM. Osseus, reticuloendothelial and vascular cells are never arranged in squamae, but epithelial cells have this growth pattern. Squamae of metastatic mammary tumor cells that form nodules are characterized by the presence of microridges (Fig. 6). Some of the cells are in the actual process of sloughing. Since cells in culture resemble the tissue of origin, a metastatic mammary tumor cell line was examined for similarities to in vivo tumor cells. The size, shape, microvilli and
ruffled edges of the cultured cell (ALAB) shown in Fig. 7 is similar to the in vivo squamae-forming cells in Fig. 6. The column of cytoplasm (6 μ high and 3 μ in diameter) which rises above the surface of the cell (insert, Fig. 7) resembles the cellular conformations observed in vivo, Fig. 5. The "tower" appears to be hollow with thin, fluted plasma membrane walls.

Discussion

Extravascular infiltrating mammary tumor cells were observed in tandem (Fig. 1), but emboli of tumor cells were not found in or emerging from blood vessels. How these tumor cells arise in the bone or arrange themselves is not known and needs further exploration. Ultrastructurally, these tumor cells resemble those in the nodules with the exception of having fewer secretory products per cell and no desmosomes. Conceivably, more of the cell metabolism may be committed to processes involved with metastases than to secretion. Alternatively, infiltrating cells may not express differentiation until a duct or nodule is formed.

The tumor cells in the nodule did not form a ductal lumen. The arrangement of the cells however is suggestive of a ductule where no lumen is present. Since ductule (not ductal) epithelia would be expected to undergo exfoliation, and the SEM revealed the tumor cells in the process of exfoliation one might conclude that this tumor originated from ductule epithelial cells.

Since the basal laminae of non-neoplastic mammary tissue are always intact (11) and intraductal carcinomas have basal laminae interrupted by gaps of varying extent (12), a possible link may exist between the process of invasion and the condition of basal laminae in the primary tumor. These observations on a metastatic mammary adenocarcinoma where no basal laminae was found on the infiltrating tandem or nodular tumor cells (Figs. 1 and 2) suggests that either 1) metastatic mammary tumor cells in the bone are in an environment which inhibits basal laminae formation or 2) metastatic human mammary carcinoma cells may be deficient in this differentiated (normal) cell function. Studies are in progress on other metastatic mammary tumors to determine the state of the basal lamina.

The squamae of metastatic mammary tumors shown in Fig. 6 resembles the appearance of epithelial cells of rat vagina by SEM (10) in both shape and the presence of microridges. Microridges have been identified as the surface imprints of highly interdigitated cells (13) and would be consistent with the closely apposed cells observed in ultrathin section (Fig. 2). Although exfoliation of normal and malignant mammary cells occurs in the ductule and forms the basis for diagnostic aspiration cytology (14) it was surprising to find that exfoliation also occurred in the metastatic tumor. Since estrogen influences the sloughing-off of vaginal and mammary ductule epithelium, visualization of this process in tumor biopsies by SEM might indicate that the tumor is hormone responsive, but futher study will be required.

The "cytoplasmic towers" which were found in vivo (Fig. 5) and in vitro (Fig. 7) represent a bizarre cellular conformation of unknown significance. The "cytoplasmic tower" in the cultured cell (Fig. 7) is an eversion of the plasma membrane. It has been thought that
intracytoplasmic ducts which are frequently found in mammary carcinoma epithelial cells (2,3,4) and are shown in Figs. 1 and 2, are an inversion of the apical plasma membrane. Whether these are similar conformations and whether they are a response to the environment (i.e. tissue culture and bone), a manifestation of malignancy, or a previously unobserved capacity of all cells is at present unknown.

Summary

This combined ultrathin section and scanning electron microscopic (SEM) study of a human mammary carcinoma metastatic to the bone revealed two organizational patterns of tumor cells: 1) infiltrating and 2) nodular. Tumor cells comprising both patterns had characteristics of secretory mammary epithelial cells with the exception of the infiltrating cells where desmosomes were absent. Numerous dilated, pleomorphic, mitochondria containing tubulofilamentous structures occupied most of the cytoplasm and basal laminae were absent in all of the tumor cells. SEM also distinguished infiltrating from nodular patterns of organization. Infiltrating cells had complex membrane folding resulting in cytoplasmic "towers" that were attached to underlying collagen bundles by pseudopodia. Squamae of nodular tumor cells had microridges at cell junctions, and showed exfoliation. Cells of a line derived from a metastatic carcinoma of the breast, (ALAB), were grown on coverslips and by SEM showed characteristics in common with both the squamae and infiltrating tumor cells. These studies indicate that structure can be correlated with function in metastatic mammary tumor cells.

Acknowledgment

This work was conducted under the National Cancer Institute contract NO1-CP-53502. We thank James Johnston, M.D., (Kaiser Hospital, Oakland, California) for the histopathology.
Figure 1. Infiltrating mammary carcinoma in tandem are closely apposed with numerous distended mitochondria occupying most of the cytoplasmic space.

Figure 3. A portion of a cell from the tumor nodule illustrating the presence of well-developed desmosomes.

All markers on ultrathin section micrographs are 0.5μ.
Figure 2. Mammary carcinoma cells in nodules contain lipid droplets (L), protein granules (P), and many pleomorphic mitochondria with disrupted cristae.

Figure 4. High magnification electron micrograph showing tubulofilamentous structures in the distended mitochondria of both infiltrating and nodular mammary carcinoma cells.
Figure 5. Scanning electron micrograph of infiltrating mammary carcinoma cells in tandem. At the center of the illustration there is a cell formed into a cytoplasmic "tower." (Marker is 20µ).
Figure 6. Scanning electron micrograph showing squamae of mammary carcinoma cells with microridges. Some of the cells of sloughing. (Marker is 20μ).

Figure 7. Scanning electron micrograph of a cultured metastatic mammary carcinoma cell (ALAB). (Marker is 10μ). Inset: High magnification micrograph of cytoplasmic "tower." (Marker is 1μ).
References


This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.