Mouse Mammary Tumor Metastases in Lung: An Electron Microscopic Study

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SUMMARY

The ultrastructure of mammary carcinoma metastatic to the lungs of old, female (C57L X A/He)F1 hybrid and C3H/Crgl mice is described and illustrated. Metastatic mammary tumor nodules in lung were usually separated from lung tissue by a thickened basement membrane and loose connective tissue. Connective tissue reaction to the metastatic nodules was minimal and in some places completely absent. Where mammary tumor cells were seen within alveolar air spaces, the tumor cells shared the same basement membrane with extant alveolar lining cells. Type A alveolar cells could not be identified within tumor cell-filled alveoli, but surviving type B alveolar cells were observed, although most appeared to be degenerating. A narrow, compact zone of lung tissue surrounded the metastatic nodules. This zone was characterized by the presence of hyperplastic type B alveolar cells. The hyperplasia took the form of small acinar structures lined by type B cells. Metastatic mammary tumor cells grew in multiple layers within lung. The stroma of the outermost layers consisted, at least in part, of extant alveolar septal elements. Cells of the innermost layers appeared to grow around small, cyst-like spaces characterized by the presence of multiple layers of membranes resembling basal lamina.

INTRODUCTION

In the course of ultrastructural studies of the mouse lung tumor system, 2 older (C57L X A/He)F1 hybrid female mice, bearing large mammary tumors, were found to have multiple metastases to the lungs. Sections of the metastatic lesions were examined by electron microscopy. Type B alveolar cell (1) hyperplasia was found commonly in the immediate vicinity of mammary tumor metastases. In order to determine whether this alveolar cell hyperplasia occurred in other strains of mice, old, female C3H/Crgl retired breeders with large mammary tumors were examined. These mice also showed multiple tumor metastases to the lungs which on electron microscopic examination revealed, in many cases, similar type B alveolar cell hyperplasia immediately adjacent to metastatic nodules.

This paper describes and illustrates ultrastructural features of mammary tumors metastatic to the lungs of mice.

MATERIALS AND METHODS

Strain A and C57L mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. Male C57L and female A strain mice were mated to obtain F1 hybrids. Two untreated hybrid female mice bearing large mammary tumors were sacrificed when 14 and 16 months old. Hyperplastic alveolar mammary nodules, mammary tumors, lungs, and spleens were taken from these mice; and small blocks, about 1 cu mm in volume, were cut from these organs and tissues. For electron microscopy, tissue blocks were fixed for 15 min in an ice-cold solution containing 1.5% glutaraldehyde, 1% sucrose, and 50 mg/100 ml CaCl2 in 0.067 M cacodylate buffer, pH 7.4, and then postfixed for 2 hr in ice-cold 1% OsO4, 4.5% sucrose, and 0.25% UO2(NO3)2 in Veronal-acetate buffer, pH 7.4. C3H/Crgl mice were purchased from the Cancer Research Genetics Laboratory, University of California, Berkeley, Calif. Similar tissues and organs were taken from these mice and handled in the manner described for the (C57L X A/He)F1 hybrids except that fixation was carried out for 2 hr with the aldehyde solution. Tissue blocks were dehydrated with an ice-cold graded series of ethanol solutions and infiltrated and embedded with Araldite (4). Blocks were sectioned with glass or diamond knives with a Servall Porter-Blum II microtome. Sections were stained with 5% aqueous uranyl acetate and lead citrate (8) and examined with a Philips EM 200 electron microscope.

RESULTS

This report is confined primarily to observations on the lungs of these mice. Preliminary examination of the hyperplastic alveolar mammary nodules and primary mammary tumors indicates no unusual features. Both mammary lesions contain mammary tumor virus particles. Splenectomy of the mice are enlarged. On microscopic examination, spleen enlargement is diagnosed as reactive hyperplasia, and no metastases are observed. Numerous mammary tumor metastases are found in the lungs. A typical circumscribed group of metastatic nodules is seen in Fig. 1. A narrow zone of compact tissue surrounds
the nodules. At higher magnification (Fig. 2), some of the cell groups in the surrounding zone are seen to be smaller metastatic mammary tumor nodules; the remaining cells in the zone appear to be of lung origin.

Electron microscopic examination of the mammary tumor metastases reveals virus-containing cells arranged in an acinar pattern (Fig. 3). Virus particles are found in groups within cells or budding from cell surfaces. Viruses are also observed within luminal spaces formed by mammary tumor cells. These particles, both intra- and extracellular, correspond morphologically to the classical Bittner viruses. Viruses within the lumens have the typical type B appearance, with eccentrically located nucleoids (Fig. 3, inset). Many mammary tumor cells at the periphery of tumor acini lie circumferentially (Fig. 4). These peripheral cells, as well as some cells which are centrally located, do not contain virus particles. Wide, intercellular spaces occur at intervals between tumor cells. Such spaces are sparsely filled with a fine, irregularly branched fibrillar material.

Occasional macrophages are noted within metastatic nodules, but no leukocyte response is observed. Excessive fibroblastic or collagenous enclosure or infiltration of the mammary tumor metastases does not occur.

The tissue surrounding tumor nodules appears to originate from alveolar septal components. Thus, in Fig. 4, mammary tumor cells are separated from alveolar air space by a thick basal lamina, a loose connective tissue space containing collagen fibrils and fibroblasts, another basal lamina, and finally types A and B alveolar epithelial cytoplasm. Less often, a sector of tumor nodule is bordered by hyperplastic type B alveolar cells with only a basal lamina intervening (Fig. 5).

Of the several lung alveolar cell types, type B alveolar cells appear to react most markedly to the presence of metastatic mammary tumor. The reaction appears to be that of hyperplasia whereby small acinar structures are formed (Figs. 6 and 7). The lumens of such acini may be open and filled with material resembling discharged cytosomal contents (Fig. 6) or virtually closed and containing no substances (Fig. 7).

Many examples are found of type B alveolar cells in immediate contact with invading mammary tumor cells (Fig. 8). In such instances, type B cells appear to share the same basal lamina as the invading tumor cells and border on the same luminal space. Instances of type B cells interspersed among mammary tumor cells are seen. Such type B cells usually appear to be degenerating. Positive identification of type A alveolar cells within mammary tumor nodules is virtually impossible.

The closeness of virus-containing mammary tumor cells to type B cells may lead to viral uptake and possible infection of type B cells. Where the 2 cell types share a common lumen (Fig. 9), viruses discharged into the lumen are immediately accessible to type B cells. An example of viral particles within type B cells is shown in Fig. 10; however, the presence of virus in these cells is rare.

In some metastatic nodules, tumor cells border basally on large, irregularly shaped, cyst-like spaces filled primarily with fine fibrillar material resembling that found intercellularly between mammary tumor cells (Fig. 11). A basal lamina completely borders these spaces and is separated from the basal cell membrane of tumor cells by an electron-lucent space about the same width as the basal lamina. Within the cyst-like spaces, multiple, parallel membranes, closely resembling basal lamina, are found. At higher magnification (Fig. 12), the membranous material is seen to have periodic densities, about 350 to 400 Å apart. In addition, fine fibrillar material passes from membrane to membrane.

In some instances, collagen fibrils and cells considered to be fibroblasts are observed in some multiple membrane-containing spaces. Clumps of what appears to be cytoplasmic material frequently are found extracellularly within these spaces. The greatest quantity of this material is located just adjacent to the base of the mammary tumor cells.

DISCUSSION

In respect to the lung, the finding of greatest interest in the present study is that type B alveolar cells show a focal hyperplastic response to mammary carcinoma metastatic to lung. Ludatscher et al. (3), in an electron microscopic investigation of Morris hepatoma 5123 metastatic to rat lung, do not mention an alveolar cell response to the hepatoma metastases.

Proliferative lung cell response to focal lesions in human lung is known. However, because of the difficulty of lung cell identification at the light microscope level, a direct comparison to the type B cell hyperplasia observed in this study cannot be made. Local epithelial hyperplasia, in apparent response to the presence of tumors, has been described. The nature of this response is unclear. Moreover, different tumors may affect surrounding normal tissue elements differently. Therefore, the manner by which metastatic mammary tumor causes type B cell hyperplasia in mouse lung can be considered only speculatively.

Three ultrastructural studies of type B cell hyperplasia have been reported (2, 5, 9). In 2 of the 3 studies, hyperplasia appeared to be directly related to injury to type A alveolar cells. Alveoli with damaged type A cells became lined by hyperplastic type B cells. Possibly, some substance released from damaged type A cells stimulates type B cell proliferation. Damage to type A alveolar cells, in the case of metastatic mammary tumor cell growth pressure, may also be expected and could account for the type B cell hyperplasia observed in the present study. Virus particles in type B cells occur so rarely that it may be assumed that viral infection is not a cause of lung cell hyperplasia in this instance.

No information was derived from the present study concerning the manner by which mammary tumor cells invade the lung parenchyma. Vascular dissemination of mammary tumor cells is assumed, but neither tumor cell embolization nor invasion of blood vessels was recognized in the lung material examined.

One aspect of mammary tumor cell establishment in lung is of interest in relation to the question of metastatic tumor stroma. Reference is made to the membrane-containing cyst-like spaces around which many tumor cells grow. Examination of both light and electron microscopic sections...
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indicated that, in many cases, mammary tumor cells grow in multiple layers. The stroma of the outermost cell layers appears to be derived, at least in part, from remaining alveolar elements. The stroma of the innermost layers is apparently the cyst-like, membrane-containing spaces. This interpretation leads to the question of how the tumor cells became organized around cyst-like spaces. This cannot be answered by the observations; but if the interpretation is correct, then the involved tumor cells must, at some time, have been in a "free-floating" condition.

The nonmembranous, fine fibrillar material within the cyst-like spaces appears identical with that seen between mammary tumor cells and is probably synthesized by these cells. The membranous material within the spaces is very similar to basal lamina. Pierce (6) has shown that mouse mammary tumor cells in tissue culture can synthesize and secrete extracellular basal lamina material in small amounts. Therefore, it is likely that the membranous material within the cyst-like spaces is also synthesized by mammary tumor cells. It may be speculated that fibroblasts eventually grow into the mammary tumor-produced spaces, synthesize collagen, and form finally a more typical stroma into which capillaries proliferate.

Multiplicity of discrete basal lamina has been illustrated by Pierce and Nakane (7) in injured mouse testicular tube epithelium. The epithelium was injured by injection of small crystals of potassium permanganate into the testis. Multiplicity of basal lamina was interpreted by these investigators to be a response to injury by the testicular tubule epithelium.

In the case of basal lamina-like material in mammary tumor metastases in lung, its seemingly excessive synthesis may indeed be related to injury to some tumor cells. An alternative explanation is that basal lamina material is synthesized by the tumor cells and released into the cyst-like spaces to provide a pseudoconnective tissue framework.

The possibility that the membranous material in question is not identical to basal lamina must be considered. The regularly spaced dense spots along the membranes are not observed in typical basal lamina. In spite of this, the thickness, electron density, and location of the material strongly support the idea that it is similar to normal basal lamina. Also, careful examination of micrographs reveals, in many places, continuity between basal lamina and membranous material.

REFERENCES

Figs. 1 and 2 are light micrographs, and the remaining figures are electron micrographs of mouse mammary carcinoma metastatic to lung.

Fig. 1. A group of discrete mammary tumor nodules immediately surrounded by a "cuff" of lung cells. X 155.

Fig. 2. Higher magnification of a peripheral portion of nodule group in Fig. 1. A very small mammary tumor nodule (arrow) lies outside the large group. The "cuff" consists, in addition to connective tissue elements, of hyperplastic type B alveolar cells. X 390.

Fig. 3. A group of virus-containing mammary tumor cells. Virus particles are seen budding off from short microvilli as well as free within the lumens. A membrane-bound, vesicle-like structure appears in the lower lumen. X 16,200. Inset. Incomplete virus particles budding from microvilli and mature type B particles within the lumen are shown at higher magnification. X 39,000.

Fig. 4. Tissue separating mammary tumor (MT) cells from an alveolar air space (AAS). The separating components are basal lamina (BL), a connective tissue space containing collagen and fibroblasts (FB), and an epithelium made up of types A (A) and B (B) alveolar cells. X 14,300.

Fig. 5. Hyperplastic type B (B) alveolar cells bordering a mammary tumor nodule. A thick, multilayered basal lamina (BL) separates the mammary tumor (MT) from the type B cells. The most basal mammary tumor cell is usually oriented circumferentially in this situation. Intercellular spaces of variable sizes occur between mammary tumor cells. Such spaces contain a slight amount of fine, fibrillar material. X 14,900.

Fig. 6. Hyperplastic type B cells near a mammary tumor nodule. Two type B cells enclose a space, the lumen of which contains myelin-like material similar to that of discharged cytosomal contents. This acinar structure is surrounded by connective tissue elements. Arrows, junctions between type B cells. X 14,900.

Fig. 7. Hyperplastic type B cells adjacent to a mammary tumor nodule. Type B cells form a tight acinar structure almost completely lacking a lumen. Connective tissue elements surround the structure. X 14,600.

Fig. 8. Alveolar air space invasion by mammary tumor (MT) cells. A type B (B) alveolar cell and attenuated type A alveolar cells line alveolar air space (X) invaded by mammary tumor cells. A normal alveolar septum separates the invaded air space from an uninvaded alveolar air space (AAS). CAP, capillary. X 12,700.

Fig. 9. Type B cell adjacent to mammary tumor cells. Note the presence of free mammary tumor virus particles in lumen (L) shared by type B (B) and mammary tumor (MT) cells. X 17,100.

Fig. 10. Hyperplastic type B cells near a mammary tumor nodule. Rarely, virus particles (arrows) similar to mammary tumor virus are observed in type B cells. X 39,400.

Fig. 11. "Stromal" spaces in mammary tumor nodules. The basal portion of several mammary tumor cells adjoins cyst-like spaces. A basal lamina underlies the mammary tumor cells. Multiple, parallel, basal lamina-like membranes occur within the spaces as do widely dispersed, fine fibrils. Continuity between intraspace membranes and basal lamina is noted (arrow). X 14,900.

Fig. 12. "Stromal" space in mammary tumor nodule. At higher magnification, the membranes are seen to have periodic densities about 350 to 400 Å apart. Very thin fibrillar material extends between membranes at approximate right angles to their length. X 42,000.
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