Epithelial Cell Junctions in Primary and Metastatic Mammary Tumors of Mice

Dorothy R. Pitelka, Susan T. Hamamoto, and Barbara N. Taggart

Department of Zoology and Cancer Research Laboratory, University of California, Berkeley, California 94720

ABSTRACT

Spontaneous pulmonary metastases of mammary adenocarcinomas in mammary tumor virus-infected mice were compared with primary tumors, hyperplastic alveolar nodules, and normal mammary gland with respect to histological pattern, cell-cell relations in primary culture, and the ultrastructure and distribution of epithelial cell junctions. Metastases were detected at autopsy and histologically confirmed in 69 of 160 females examined from 7 strains. No characteristic differences were found between primary tumors in mice with and without metastases or between primary and metastatic tumors. Histologically, all three types ranged from alveolar to anaplastic; in culture, all formed contact-inhibited epithelial pavements. Freeze-fracture replicas of tumor tissue consistently revealed tight-junction belts, resembling those of nonlactating normal mammary cells, between cells lining alveolar or cystic lumina. A novel feature of the tumors was the presence of microlumina, not detectable histologically, in solid and anaplastic regions; cells forming microlumina were joined by short but complete tight junctions. Also different from normal was the common occurrence of macular tight junctions, both close to and distant from tight-junction belts. The abundance of belt and spot desmosomes varied widely. Gap junctions were about as common as in the normal gland. The propensity of malignant cells to form tight and other junctions indicates retention, with relatively moderate distortion, of a fundamental epithelial property. Since all of the junctions are adhesive, their abundance and wide distribution constitute strong evidence that reduced adhesive capacity is not a necessary correlate of epithelial cancer.

INTRODUCTION

Although most human cancers are of epithelial origin, the bulk of basic research on cancer has been performed with nonepithelial cells. Intrinsic molecular mechanisms of growth regulation are likely to be common to all cells, and nonepithelial cells, proving more manipulable and predictable under the rigorously controlled conditions required for such studies, have yielded most of our fundamental knowledge on cell cycle controls. With regard to the tumor properties responsible for invasion and metastasis, however, differences between epithelial and other cell types may be highly significant. Epithelial cells have unique adhesive and cohesive properties and morphological growth patterns, which, if retained through neoplastic transformation, could profoundly affect critical events in the spread of cancer.

The morphology of a simple epithelium is dictated by its primitive and primary function as a permeability barrier between 2 fluids of different composition. A continuous sheet of tightly joined cells fulfills this function, provided that the cells are physiologically polarized so that their metabolic exchanges with the 2 fluids are such as to maintain the difference (4). The basic epithelial phenotype accordingly comprises 3 important features: (a) transepithelial diffusion via intercellular spaces is restricted by continuous tight-junction belts (occluding junctions, zonulae occludentes) encircling the apical border of each cell and attaching it firmly to its neighbors; mechanical cohesion throughout the sheet is provided by these belts plus other kinds of specialized junctions (see Refs. 38 and 42 for reviews). Although the other junction types are present in many kinds of tissue, tight junctions are found only in tissues of epithelial organization (including endothelium and mesothelium); (b) the tight-junction belt defines the physiological differentiation of the plasmalemma into a laterobasal region of variable topography, where all exchanges with internal tissue fluids take place, and an apical region, typically bearing microvilli, which is the site of all interactions with luminal fluids; (c) between the epithelium and adjacent stromal cells and matrix is the basal lamina, a continuous layer of proteins and carbohydrates secreted by the epithelium and serving as its immediate substrate and boundary. Superimposed upon the basic phenotype (itself characteristically modified in stratified squamous, endocrine, and a few other specialized epithelia) are the variations in configuration of the epithelial sheet that define tissue architecture and the variations in cellular activity that determine organ-specific functions. Epithelial cells thus are generally in contact with one another throughout their lives; the contact is maintained by an abundance and variety of junctions not matched in other tissues, and its exclusiveness is guarded by the basal lamina.

The mammary gland of mice infected with MTV2 is one of the few organs in experimental animals that develop benign hyperplasia, carcinoma, and spontaneous metastasis with high frequency. Among overtly MTV-infected strains in our mouse colony, an overall average of 43% of females bearing mammary tumors develop macroscopic metastases in the lung. We have used histological and ultrastructural methods to examine the relationship between growth patterns and the 3 specified features of the epithelial phenotype in hyperplastic alveolar nodules, primary mammary tumors, spontaneous pulmonary metastases, and 3 transplanted mammary tumor lines. The present paper discusses tight and other junctions and cell polarity; a second paper (28) deals with the basal lamina and epithelial-stromal interface. We have described the basic epithelial features of normal mouse mammary tissue elsewhere (27) but will review them briefly for comparative purposes.

MATERIALS AND METHODS

Sources of Tissue. All tissues examined were taken at

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2 The abbreviation used is: MTV, mammary tumor virus.
autopsy or during Nembutal anesthesia from female Crl mice infected with MTV. Only primary lesions and normal mammary tissue were used, with the following exceptions. First-generation transplants of several hyperplastic nodules and 2 pulmonary metastases were used in order to obtain sufficient material from the same lesion for both sectioning and freeze-fracture, or for cell culture; also, 3 transplanted C3H mammary tumor lines, MT3, MT4, and MT6, now in their 13th, 18th, and 8th passages, respectively, were examined in vivo and in cell culture.

For the study of metastasis, a total of 160 mammary tumor-bearing females of 7 strains were isolated and allowed to live until debility or the size of primary tumors indicated imminent death, when they were killed by neck fracture. At autopsy, the lungs were examined for grossly visible metastatic tumors. If none was found, a 0.5% solution of trypan blue in 0.85% NaCl solution was injected through the trachea to expand the pulmonary air spaces; the trachea was then tied off and the lungs were removed. Tumor foci at or near the lung surface stood out as white spots against the predominant blue. Metastases were used in order to obtain sufficient material.

Histologically confirmed mammary tumor metastases were found in the lungs of 69 mice. Numbers of mice with metastases and total autopsied for each strain were: C3H, 36 of 96; C3Hf, 11 of 18; BALB/cfC3H, 3 of 7; BALB/cNIV, 5 of 8; A, 2 of 5; GR, 1 of 10; and RIII, 11 of 16.

Preparation for Microscopy. Large pieces of primary tumors or of lung for routine histological study were fixed in Bouin's fluid, and paraffin sections were stained with hematoxylin and eosin. For electron microscopy and critical light microscopy, small pieces of hyperplastic nodules and primary and metastatic tumors were fixed in 1% formaldehyde and 3% glutaraldehyde in 0.075 M sodium cacodylate (pH 7.4), postfixed in 1% OsO4 in the same buffer, stained in 0.5% uranyl acetate in Veronal-aceate buffer, dehydrated, and embedded in Epon. For some samples, 0.5 to 1% lanthanum nitrate was added to the aldehyde and osmium fixatives as an electron-opaque marker of intercellular space (32).

Epon sections (1 to 2 µm) were stained with Mallory's Azure II-methylene blue (33) for light microscope examination and photography. Thin sections for electron microscopy were stained with uranyl acetate and lead citrate.

For freeze-fracture, small pieces of tissue fixed in the same aldehyde fixative for 10 to 15 min were soaked in 20% glycerol, frozen in Freon cooled by liquid N2, and fractured and replicated at −115° in a Balzer's apparatus.

The numbers of individual mice from which one or more lesions were examined by the techniques specified are shown in Table 1.

Cell Culture. Tumors were minced and dissociated in saline A containing 0.025% EDTA and 0.05% trypsin. Hyperplastic nodules and normal mammary tissues were minced and dissociated with collagenase as described earlier (11). The cell suspensions were washed, filtered through fine-mesh cloth, and plated at 5 X 10^5 cells/sq cm on Falcon plastic Petri dishes or multiwell plates in Waymouth's Medium MB752/1 with 10% calf serum, insulin (10 µg/ml), hydrocortisone (5 µg/ml), and gentamicin (5 µg/ml). Cultures were incubated at 37° in 95% air-5% CO2.

RESULTS

Normal Mammary Gland. The mammary parenchyma of the adult female mouse comprises a single layer of polarized, cuboidal to columnar epithelial cells lining ducts and alveoli and a continuous (in major ducts) or discontinuous (small ducts and alveoli) basal layer of myoepithelial cells, all separated from the stroma by a continuous basal lamina. Cells of the epithelial layer bear an irregular fringe of short microvilli on their apical surfaces, and it is at this surface that both the normal constituents of milk and the virions of MTV are released into the lumen of the gland.

All cells of the lining layer are interconnected laterally by junctional complexes, which differ in composition and structure in lactating and nonlactating glands (25, 27). The tight-junction belt, which is always present, consists of a network of lines along which the outer leaflets of the 2 cell membranes are fused (38). Within each of the paired membranes, seen in freeze-fracture replicas (Figs. 1 and 3), the lines of the network appear as raised strands adhering to the inner (protoplasmic) leaflet of the split membrane and matching grooves in the outer (external) leaflet. A continuous smooth strand defines the luminal edge of the junction, whereas the abluminal edge is often more convoluted. During lactation (Fig. 1), the pattern of the network is compact, typically with 5 to 8 undulating strands oriented roughly parallel to the luminal border. In nonlactating glands (Fig. 3), the network is relatively disorderly. Below the continuous luminal border strands, numbers and orientation of strands vary widely, and free-ended strands often extend some distance from the abluminal edge; very rarely, short strands are present close to but not continuous with the network.

In mammary ducts at all times, the occluding junction is paralleled by a belt desmosome (intermediate junction, zonula adherens) and frequent spot desmosomes (maculae adherentes), making up a conventional epithelial junctional complex (see Figs. 5 and 19). Additional spot desmosomes interconnect epithelial and myoepithelial cells in all pair combinations. The desmosomal junctions do not involve membrane fusion or intimate contact; instead, the intercellular space is occupied by moderately dense material, and cytoplasmic filaments typically converge toward the inner surface of the junctional membrane. Both desmosomal junctions may be present in alveoli and ductules during pregnancy, but the spot desmosomes disappear around parturition and the belt desmosome is usually poorly developed. Gap junctions (communicating junctions, nexuses) are plaques of punctate fusions of membrane outer leaflets, seen as particle plaques in freeze-fracture replicas (see Figs. 8 and 26). They link mammary epithelial and myoepithelial cells in all pair combinations in all physiological states.

Hyperplastic Alveolar Nodules. Nodules and their transplants in gland-free mammary fat pads consist of branching ducts and alveoli, histologically resembling normal lobules of mammary glandular tissue.
pregnancy (compare Figs. 2 and 4). Cytoplasmic ultrastructure is also like that of prelactating cells but is commonly distorted by aggregates of cytoplasmic A particles of MTV or vacuoles containing mature B particles. Apical microvilli are more variable in distribution and shape than in normal tissue. Luminal cell borders are linked by junctional complexes with continuous tight-junction and desmosomal belts and occasional spot desmosomes (Fig. 5). Number and arrangement of intramembrane strands in the tight junctions are extremely variable (Figs. 6 and 7), as in nonlactating normal glands. Nodule transplants in lactating hosts did not develop the junctional modifications of normal lactating gland.

We have seen more gap junctions in both thin sections and freeze-fracture replicas of nodules than of mammary tissue in any other state; this is a subjective observation, since their average observed frequency per cell section is still too low to permit significant counts. Their structure is conventional (Fig. 8).

**Primary and Metastatic Tumors.** Mouse mammary carcinomas are known to vary widely, even within a single inbred strain, in degree of retention of glandular structure (9). Within our sample, the gamut of histological patterns expected of MTV-induced carcinomas appears among primary and large established metastatic tumors, and often within single tumors (Figs. 9 to 14). No differences have been detected in primary tumors of animals with and without metastases. Sections of large metastatic tumors often suggest a close resemblance to a primary tumor in the same mouse (Figs. 11 and 12) but almost as frequently do not. The histology of metastatic tumors is influenced to some degree by their relationship to surrounding lung tissue, which is discussed elsewhere (28). Otherwise, the organization and ultrastructure of mammary epithelial cells in the 2 sites show no characteristic differences, and they are described together here.

In areas of predominantly alveolar or cystic histology (Figs. 9 and 10), electron microscopy confirms that most lumina are defined by a single-layered epithelium (Fig. 15), frequently with underlying myoepithelial cells. Less glandular areas appear in histological section as solid cords or lobes of mainly unoriented epithelial cells, separated by connective tissue strands enclosing capillaries or blood sinuses (Figs. 11 and 12). Occasional lumen-like cavities appear within these masses. Many such cavities prove on ultrastructural examination to be true lumina (Fig. 16); others are interstitial space, lined by basal lamina (28). Very common, even in some solid anaplastic areas, are microlumina, identifiable by tight junctions and apical microvilli but too small to be detectable by light microscopy (Figs. 17 and 18). Epithelial cells lacking any luminal frontage probably are present in most tumors; however, the abundance of microlumina makes the numbers of such cells much smaller than histological observation would suggest.

Complete separation of tumor lumina from nonluminal intercellular space by tight-junction belts (Figs. 15 to 21) is typical, in our experience, throughout parts of the tumor where the cells appear intact and healthy. Near necrotic zones, occasional tight junctions appear to have opened; Fig. 22 illustrates an example in which leakage of B particles from the lumen into lateral intercellular spaces has occurred. We have not identified such distinct discontinuities of the occluding belt in freeze-fracture replicas (Figs. 20, 21, and 23). The number of strands per transect of the junction is highly variable and may be reduced for short distances to a single one (Fig. 23). The configuration of the junctional network is typically disorderly, with many free-ended abluminal strands, like the tight junctions of nodules and nonlactating normal glands, and it remains so during lactation of the host.

In addition to these relatively normal occluding belts, macular tight junctions are common in some tumors. They range from single isolated strands to patches occupying more than 1 sq \( \mu m \) (Figs. 24 and 25). They are most frequent in lateral membranes of luminal cells, unconnected to the occluding junction, but in both replicas and thin sections they also appear at sites remote from any identifiable lumen.

The occluding belt may be the only junction at luminal cell borders, it may be accompanied by a belt desmosome, or spot desmosomes may also be present to complete the junctional complex (Fig. 19). Spot desmosomes are erratically distributed elsewhere; they are infrequent in some tumor samples but are exceptionally abundant and elaborate, with associated tonofilaments, in areas of epidermoid differentiation (Fig. 27). Gap junctions of normal structure (Fig. 26) also commonly join tumor cells, within or near tight junctions or scattered elsewhere in seemingly random fashion.

Cell sections and membrane fracture faces with no visible junctions are frequent enough in thin-sectioned and freeze-fractured tumor samples to suggest that many cells lack attachment to one or more of their neighbors and that some cells without any neighbor linkages do exist. Microscopic evidence is insufficient to confirm for any given cell the absence of junctions, since any one could be involved in a microlumen or other junctions outside of the plane of section or of fracture.

Intracellular polarization is indicated by the basal positioning of nuclei and supranuclear location of the Golgi complex in cells surrounding some lumina (Fig. 15), but often it is weak or lacking even in luminal cells. Surface polarization of luminal cells is more distinct. Apical microvilli are usually present, although they vary widely in abundance and shape, and release of MTV B particles is almost exclusively via the apical surface.

**Cell Culture.** Several large metastatic tumors or first-generation s.c. transplants of smaller ones were dissociated and plated at high density in cell culture. The behavior of these cells was indistinguishable from that described earlier (24) for primary mammary tumor and prelactating normal mammary cells similarly treated. Cells from any of these sources rapidly form a confluent pavement of epithelial cells linked by continuous tight-junction belts and by desmosomes; microvilli and budding MTV particles on the upper epithelial surface signify polarization toward the bulk medium as a lumen equivalent. Below the tight-junction zone, basolateral surfaces of the pavement cells are highly irregular in shape and often underlap neighboring cells extensively. Scattered or clustered stromal cells are typically present beneath the epithelial layer, along with some epithelial cells that form occasional desmosomes with pavement cells but apparently are not themselves integrated in the pavement.

Development of domes (hemicysts) by the sequestration of fluid between the cells of the pavement and the substrate, provides evidence of transepithelial transport and of the functional integrity of the tight-junction permeability barrier (20, 24, 30). Domes are at least as abundant in primary and metastatic tumor cell cultures as in normal cultures under the same conditions. Cells in the epithelial pavement are electrically...
coupled (35), indicating that gap junctions are also functional.

Transplanted Tumors. Tumor line MT3, established from a typical adenocarcinoma, and MT6, from a spontaneous pulmonary metastasis, have become more anaplastic than most primary tumors but still have occasional lumina, and both continue to form confluent epithelial pavements and domes in culture. Metastatic foci were found in the lung of one fourth-generation host of MT3, but none have been observed in MT6 hosts.

Line MT4 was initiated by implantation of cells that had been dissociated from a mammary carcinoma and had failed to form a confluent pavement or domes in culture. Instead, epithelial cells in the original culture overlapped in loosely associated piles or formed small clusters within which microlumina were detected in thin sections (Fig. 28). The histological appearance of early transplant generations was similar to nonalveolar areas of primary tumors; later generations became very anaplastic (Fig. 29). We have found no true lumina in this tumor in vivo at any time, and the cells show no polarization. Freeze-fracture replicas have revealed no tight-junction belts, but macular tight junctions are numerous. Scattered single-strand fragments and elaborations networks are both present (Fig. 30). Gap junctions have been extremely rare, and desmosomal junctions are lacking in later generations. This tumor has not produced detectable metastases, even when host life span was increased by surgical removal of the tumor.

DISCUSSION

Our observations show that metastatic, as well as primary, mammary tumors of MTV-infected mice typically retain the propensity to form cavities lined by cells with apical specializations characteristic of epithelial lumina. They further retain the capability, both in situ and in cell culture, to form the several kinds of specialized cell junctions present in the normal mammary gland. Tight junctions are the most extensive of the cell junctions in both normal and malignant mammary gland, and they are the most critical element in the construction of an epithelial lumen. Since all of these junctions, whatever their other roles, are adhesive, it follows that their number and distribution in a tumor will largely determine the cohesiveness of the tissue and influence its morphological growth pattern.

Detachment of cells from the parent tumor mass is unquestionably a necessary step in metastasis and has been shown to be a common phenomenon in mammary and other tumors, whether they metastasize or not (14, 34). Since Coman (7, 8) reported that cells in carcinomas were more easily pulled apart with microneedles than those in normal epithelium, and because many transformed cell lines in culture lose the contact inhibition of locomotion shown by their untransformed counterparts, a generalized loss of cellular adhesiveness has been widely accepted as a property of malignant tumors. Not usually taken into consideration is the distinction between adhesion to a substrate and adhesion to other cells or the distinction between initial adhesion of surfaces on first contact and the equilibrium strength of adhesion (46) dependent largely on cell junctions. Weiss (43) has pointed out that Coman’s methods did not adequately test cell-cell adhesiveness. We and others (24, 41) have found that mouse mammary tumor cells in primary culture virtually always are contact inhibited, as is to be expected of cells that form stable circumferential junctions with each other. Furthermore, our transplanted tumor line MT4, which forms far fewer junctions than do typical mammary carcinomas and does not show contact inhibition in culture, is not exceptionally invasive and has not metastasized.

Published reports on the persistence of cell junctions in tumors (reviewed in Ref. 42) are fragmentary and conflicting and usually are based on small samples and incidental observations in studies not using the methods necessary for accurate junction identification. The inference from the accumulated data is that junctions are present in most tumors but often in reduced numbers as compared with normal tissues. More extensive, quantitative studies have shown fewer desmosomes in human invasive cervical squamous carcinomas than in normal cervix (17, 45) and fewer gap junctions in preinvasive and invasive squamous carcinomas of the same tissue than in benign dysplasia or normal cervix (16). Desmosomes were less frequent in invasive than in noninvasive transitional cell carcinoma of the human bladder (2), but in chemically induced bladder carcinomas of the rat (23) increase in desmosome size in squamous areas within the tumors more than compensated for decrease in number. Since transitional cell and squamous regions were equally invasive, this observation argued against a decrease in adhesiveness as a prerequisite for malignant behavior (23).

In the most extensive published ultrastructural survey and review of human breast neoplasia, Ahmed (1) describes and illustrates true lumina with tight junctions in thin sections of both benign and malignant tumor regions with glandular structure, and variable numbers of desmosomes in these and in solid tumors. These observations resemble ours on the mouse tumors, except that Ahmed (and other authors cited by him) has not reported microlumina and does not discuss gap junctions. In infiltrating carcinomas, however, Ahmed and others have illustrated rows or sheets of cells that are clearly identified as epithelial by the frequent presence of intracytoplasmic lumina but lack observable junctions; we have not found such configurations in the mouse.

In only a few studies have the extent and continuity of tight junctions in carcinomas been determined by the freeze-fracture technique. Weinstein et al. (42) and Merk et al. (19) observed persistence of the occluding belt but localized reduction in the number of strands in its network, often to a single one, in low-grade or early transitional cell carcinomas of human and rat bladder. Like the disorderly arrangements of strands that we have observed in malignant as well as normal and hyperplastic epithelium, this might be expected to affect the permeability of tight-junction belts but should not affect their adhesive strength. The same authors described increasing discontinuity of the belt, often reducing it to separated macular junctions, in more malignant bladder tumors (19, 42). Macular tight junctions have also been observed in freeze-fracture replicas of human prostatic carcinoma (37) and in human cranial germ cell tumor (39); we have found them in many tumors, including the transplantable MT4, which appears to have lost the capability to construct lumina and occluding belts. Among normal mature epithelia, macular tight junctions are present infrequently on lateral membranes of cells that also have intact occluding belts (12), where they may be related to junction turnover. They are frequent in some normal endocrine (22, 31) or keratinizing stratified squamous (10) epithelia that do not have lumina and associated tight-junction belts.
The assembled evidence indicates that a generalized reduction of adhesive capability attributable to junctions is not a necessary characteristic of malignant epithelial cells. However, the detachment of cells from the parent tumor may be facilitated by aberrant distribution of junctions in the tumor cell population. Whereas, in the normal adult mammary gland, every epithelial cell is firmly linked to all of its immediate neighbors, this probably is not consistently the case in most tumors. If cells lacking attachment to one or more neighbors are scattered through a tumor in sufficient numbers, they could collectively provide planes of least resistance for separation of clumps of linked cells.

Other factors thought to contribute to detachment of cells from tumors (29, 44) include lytic enzymes released from intact or necrotic tumor or host cells or macrophages and mechanical stress from body movements or external forces. Most desmosomal junctions are vulnerable to proteolytic enzymes that remove the intercellular adhesive material (38). Tight and gap junctions are more resistant, and the tight-junction area may even be increased by proteolytic treatment (21, 36). At present, chemical agents capable of causing the complete disruption of tight and gap junctions under physiological conditions have not been identified, but withdrawal of extracellular calcium is known to induce fragmentation of occluding belts into macular tight junctions (18, 26). Mechanical stress causes the tearing of perijunctional membranes rather than splitting of the junction (5, 6); it leaves one cell’s share of each junction attached as a bleb to its partner’s membrane (3, 5, 13), where it is removed by endocytosis. Presumably, the reannealing of torn membrane is more likely for a small patch than for an extended belt. If so, tumor cells joined by macular tight junctions, by microluminal tight junctions engaging only a minute part of the cell surface, or only by gap junctions and desmosomes could be mechanically torn apart with higher viability than can typical luminal cells.

An additional factor sometimes adduced in the literature to explain cell detachment is a rounding up and loss of contacts with neighbors by cells in mitosis. This does not apply to most epithelial cells in vivo, which can maintain their occluding belts through cytokinesis (15, 47).

The cohesive nature of a tumor mass in which most cells are linked by adhesive junctions imposes constraints on the pattern of tumor growth. If the factors responsible for separation of tumor cells act at the periphery of the mass, then direct invasion of neighboring tissues by individual cell migration and infiltration presumably can occur. We have found no evidence of single-cell infiltration of the stroma in invasive primary or spontaneous metastatic tumors. Expansion by proliferation or locomotion of tongues of cells in continuous contact (29, 40) is more consistent both with mouse mammary tumor histology and with the mode by which the developing mammary gland normally invades its adipose stroma.

We suggest that some of the characteristics commonly attributed to malignant tumors, general reduction in adhesive capacity, progressive loss of tissue architecture, and absence of contact inhibition of locomotion in cell culture, may in epithelial carcinogenesis in vivo represent secondary events occurring late in the course of malignant growth. Perhaps the development of these “malignant” characteristics, which are incompatible with the basic epithelial phenotype, requires a longer time or more cell generations than the life span of a mammary tumor-bearing mouse allows. Alternatively, the typical retention of basic epithelial properties by the mouse tumor could be specifically associated with its viral origin. In either event, the important point is that the possession of these properties is compatible with a high degree of malignancy and may influence the pattern of malignant growth.

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Fig. 1. Freeze-fracture replica of part of the tight-junction belt linking 2 mammary epithelial cells from a lactating C3H mouse. Top, P face (P) of an apical membrane, with microvilli (Mv) fractured away or extending into the lumen. The fracture plane shifts to the E face (E) of the membrane of the companion cell at the luminal border of or within the junction, which is thus seen as a pattern of anastomosing strands and furrows. Meshes of the network are mostly spindle shaped near the luminal edge and more rounded abuminally; the abluminal border is continuous, without free ends. × 45,000.

Fig. 2. Part of a normal lobule from a C3H female in late pregnancy. Many cells of the single-layered epithelium contain large fat droplets, and the lumina are filled with dense secretory material and additional fat droplets. photomicrograph. Epon thick section, Azure II-methylene blue. × 560.

Fig. 3. Freeze-fractured tight junction from the gland of a midpregnant C3H female. Below the apical cell membrane (Ap) at upper right are several parallel strands showing few anastomoses. Abluminal strands are convoluted, and a group of them extends for more than 3 μm over the lateral membranes. There are several free ends or loops (arrows) and one strand unconnected to the network (double arrow). × 30,000.

Fig. 4. Cluster of alveoli from a first-generation transplanted C3H hyperplastic alveolar nodule in the mammary fat pad of a virgin host. Secretory activity is evident, although less pronounced than in Fig. 2. Epon thick section, Azure ll-methylene blue. × 560.

Fig. 5. Thin section of a typical junctional complex from a C3H nodule transplant. Beginning at the luminal border, the 3 arrows demarcate the limits of the cross-sectioned tight junction and shallow belt desmosome; the cytoplasm underlying the membranes of both junctions is characteristically dense. A short distance below them is a small spot desmosome (D). In the lumen is an immature MTV B particle (B). × 40,000.

Figs. 6 and 7. Freeze-fracture replicas showing segments of tight-junction belts from a BALB/cC3H nodule transplant, illustrating the irregular width and pattern of the network. A long free end extends abuminally out of the field of view in Fig. 6, and the junction is locally reduced to a single strand in Fig. 7. Fig. 6, × 24,000; Fig. 7, × 30,000.

Fig. 8. Two gap junctions from a BALB/cC3H nodule transplant. Closely packed particles 8 to 9 nm in diameter form plaques on the membrane P face; in the top half of the upper junction, the fracture shifts to the E face of the membrane of the companion cell. × 45,000.

Figs. 9 to 14. Photomicrographs of Epon thick sections, stained with Azure ll-methylene blue.

Fig. 9. Alveolar primary mammary tumor in C3H ff mouse. Epithelium is predominantly single layered; luminal contours are irregular. × 560.

Fig. 10. Adenocystic area from a pulmonary metastasis in a different C3H female. × 220.

Figs. 11 and 12. Primary mammary tumor and lung metastasis, respectively, from a third C3H ff mouse. Both are composed of cords and nests of cells separated by slender connective tissue septa and by blood sinuses lined by endothelium. Arrows, putative lumina. × 560.

Fig. 13. Relatively anaplastic area, with some necrosis, in a C3H primary mammary tumor. × 560.

Fig. 14. Parts of 2 solid lobes of a lung metastasis in an RIII mouse. × 560.

Fig. 15. Part of an alveolar structure from the tumor in Fig. 9. The luminal surface is angular and bears relatively few microvilli; a bleb of vesicular cytoplasm appears to be in the process of sloughing. Obliquely sectioned tight-junction bells linking epithelial cells are visible at this low magnification only as dense bands (arrows). Nuclei are basal, and the bulk of cytoplasmic organelles is supranuclear. The nucleated cell and processes beneath the lining epithelium are probably myoepithelial. × 6,000.

Fig. 16. Edge of a lumen from a pulmonary metastasis in a C3H female. Alveolar microvilli are abundant and relatively normal in shape, with normal-looking microfilament cores. Between the arrows is a complete epithelial junctional complex, with tight junction and belt and spot desmosomes. MTV A particles (A) are present in the cytoplasm, and mature B particles (B) are present in the lumen. × 30,000.

Fig. 17. Two microlumina (arrows), about 15 μm apart, are included in this section from a solid primary tumor of a C3H mouse. The tissue was exposed to lanthanum nitrate during fixation, resulting in a black deposit filling all intercellular spaces except the lumen. Higher magnification showed that the lanthanum failed to permeate tight junctions between cells forming the lumen. In this section, 4 cells are involved in the lumen at the right but only 2 in that at the left. × 8,000.

Fig. 18. Microlumen in a small lung metastasis from a C3H animal. The 5 participating cells are linked by tight and desmosomal junctions. Microvilli protrude into the lumen, which also contains MTV B particles. × 20,000.

Fig. 19. Cross-section of a junctional complex from a primary tumor in a C3H ff mouse. From the luminal end at the left, the brackets demarcate the tight junction, belt desmosome, and spot desmosome. × 100,000.

Fig. 20. Freeze-fracture replica of the tight-junction-belt bordering a lumen in a pulmonary metastasis from a C3H female. The junction is irregular, with several abluminal free ends or loops, but is continuous. × 24,000.

Fig. 21. Freeze-fracture replica of a tight-junction belt surrounding a small lumen in a C3H primary mammary tumor. Except where interrupted by folds or tears in the replica, the entire junction is visible and continuous. It is disorderly in pattern and width, with frequent free ends. × 20,000.

Fig. 22. Disrupted junctional zone between 2 cells of a large lumen in a C3H primary mammary tumor. Some cells bordering the same lumen were severely necrotic. MTV particles from the lumen (left) have entered the lateral intercellular space (arrows). × 24,000.

Fig. 23. Part of an occluding belt from a metastasis in the lung of a C3H mouse. The junction is very shallow, consisting in most areas of only 2 to 3 strands; at the arrow, it appears to be reduced to a single strand. × 45,000.

Figs. 24 and 25. Macular tight junctions from primary mammary tumors in 2 C3H females. Fig. 24, × 45,000; Fig. 25, × 35,000.

Fig. 26. Gap junction in a C3H pulmonary metastasis. It is not distinguishable from gap junctions in normal mammary tissue. × 60,000.

Fig. 27. Primary mammary tumor from a BALB/cN manager. Cells on the upper side of the lumen show epidermoid differentiation, with well-developed spot desmosomes (D) and many tonofilament bundles; microvilli are rudimentary. On the lower side are cells with more frequent but pleomorphic microvilli and no evident epidermoid development. × 11,500.

Fig. 28. Culture of cells from a C3H primary tumor; sister cultures were implanted s.c. to initiate tumor line MT4. This section, cut perpendicular to the Petri dish surface, shows a small internal lumen (Lu) within a cluster of epithelial cells. The lumen is filled with dense material not present in nearby intercellular spaces. A cluster of MTV A particles is visible in the cell at the right. × 15,000.

Fig. 29. Photomicrograph of an Epon thick section of an anaplastic tumor from the 16th passage of tumor line MT4. × 560.

Fig. 30. Macular tight junction in a tumor from passage 13 of MT4. At the arrow is a small cluster of particles suggesting a gap junction. × 50,000.
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