Changes in the Surface Coats of Neoplastic Human Breast Epithelium

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SUMMARY

Phosphotungstic acid at low pH has been used as a section stain to make visible the surface coats of benign and malignant epithelial cells from human breast biopsies in the electron microscope. For this purpose, tissue is fixed in glutaraldehyde and embedded in the water-soluble plastic glycol methacrylate.

Duct epithelial cells from all the biopsies containing benign tissue showed consistently more phosphotungstic acid-positive material at their surfaces than did the malignant cells. These results from an in vivo system are consistent with the observations of others, who have shown that there are lower amounts of sugars at the surfaces of transformed cells in culture. Thus, alterations of surface polymers, such as glycoproteins, may be important in the development and maintenance of the altered growth properties of malignant breast epithelial cells in vivo.

INTRODUCTION

In transformed mammalian cells in culture, there is no contact inhibition of cell division or of cell movement (10). These characteristics of transformation might result in part from alterations of cell surfaces (1, 5, 6). Other little understood surface changes detected in transformed cells in culture are the appearance of new antigens (3), cell agglutination by plant lectins (4, 12), and chemical changes (7, 20) that seem to indicate lower amounts of sugars, which are probably components of polymers such as glycoproteins or glycolipids at the surfaces of transformed cells. These chemical alterations may be due to changes in the ratio of glycosylating enzymes within the cells (11).

The binding sites for plant lectins such as concanavalin A are situated within the glycoprotein surface coat (2) external to the plasma membrane of cells, and it is possible that all of the properties of transformed cells mentioned above are due in part to changes within the surface coats of cells. Changes in the amount of surface coat material (8, 14, 15) have been described in contact-inhibited and transformed cell cultures, but to our knowledge this kind of information has not been obtained from an in vivo system. This communication describes initial results based on the use of PTA1 as an electron microscope section stain for surface coats of cells (17) in a limited number of human breast biopsies containing carcinomas or nonneoplastic tissue.

MATERIALS AND METHODS

The material studied to date consisted of portions of 15 breast biopsies, 10 of which contained infiltrating duct carcinomas. The remaining 5 showed evidence only of benign changes or exhibited normal parenchyma. The biopsies were fixed in 2.0% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.3) for 2 hr and embedded in the water-soluble plastic GMA (13). Thin sections were stained with 3.0% PTA, (pH 1.5) for 15 min and examined in an electron microscope.

RESULTS

In glutaraldehyde-fixed, GMA-embedded sections, the contrast of cells in the electron microscope is low due to the absence of osmium or other heavy metals used as section stains. Sections containing ducts cut from blocks of essentially normal breast biopsies or from those that exhibit benign changes, such as fibrocystic disease, show a similar distribution of stain after exposure to PTA (Fig. 1). Within ducts, electron-dense stain is confined to external surfaces of cells, Golgi sacculles and vesicles, other cytoplasmic vesicles, and dense bodies. Other cytoplasmic structures and nuclei generally do not stain.

Neoplastic cells from the 10 infiltrating duct carcinomas show consistent changes with respect to PTA staining when compared to the nonmalignant biopsies. Although the carcinomas differ greatly with respect to differentiation and cell ultrastructure, to a lesser or greater degree, all malignant cells examined exhibit a reduction of PTA-positive surface coat material (compare Fig. 1 to Fig. 2). On the other hand, the staining of cytoplasmic structures within malignant cells is similar to that observed within benign cells.

In benign biopsies, the staining of cell coats is intense at luminal surfaces of ducts where microvilli are covered with a continuous layer of stain (Fig. 3). Luminal contents are also sometimes stained. Luminal surfaces of duct-like structures within malignant cells are similar to that observed within benign cells.

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At lateral and basal surfaces of duct epithelium within benign biopsies, staining is generally less intense than at...
luminal surfaces, but sharp, almost continuous reactive lines are found between cells (Fig. 5). In contrast, lateral and basal surfaces of apposed cells from all the carcinomas examined exhibit a reduction or absence of stain (Fig. 6). When reactive material is present between cells, the sharp, almost continuous lines are replaced by less intense spots of stain.

DISCUSSION

When PTA at very low pH is used as a section stain for tissue that has been fixed only in glutaraldehyde and embedded in a water-soluble plastic such as GMA, it is thought that PTA stains glycoprotein (9, 17, 18). Staining is confined to surfaces of cells and to certain cytoplasmic structures such as Golgi sacules, some vesicles, and lysosomes. These cytoplasmic structures have been implicated in the metabolism of glycoproteins (19). Work that we have recently completed indicates that cell surface staining by PTA (pH 1.5) is due primarily to sialic acid residues present within glycoproteins.

Although our data differ from the results of others that have shown increased layers at the surfaces of transformed cells in culture (8, 14, 15), they are compatible with recent biochemical data. These data have shown that certain virus-transformed cells in culture have lower amounts of sialic acid (7, 11, 16, 20) and a marked decrease in both neutral and amino sugars per mg of protein at their surfaces (20) than do cells from the same line that were not transformed. Furthermore, spontaneously transformed cells exhibited similar changes, but contact-inhibited "revertant" cell lines from SV40-transformed mouse 3T3 cells (7) contained higher quantities of sialic acid than the transformed cells.

The changes in PTA staining that we have detected at the surfaces of malignant, human breast epithelium seem to indicate that there are lower amounts of sialic acid within the surface polymers of these malignant cells in vivo. Such changes may be important in the development and maintenance of lack of growth restraint, which is characteristic of these cells.

REFERENCES

Surface Coats of Breast Cells

Fig. 1. Essentially normal ductal epithelium from a human breast biopsy. Cellular structures exhibit little contrast because osmium fixation was not used. Exposure of sections to PTA results in a staining of lysosomes (Ly) and cell coats at all surfaces. At luminal surfaces, microvilli (Mv) are stained and lateral and basal cell surfaces exhibit an almost continuous layer of electron-dense stain (arrows). × 8,000.

Fig. 2. Infiltrating duct carcinoma 2912. PTA stains lysosomes (Ly) as in the benign biopsies, but surface coat staining is much reduced. Arrows are spots of reduced intensity stain. × 8,000.

Fig. 3. Luminal surfaces of a duct within a benign breast biopsy react strongly with PTA. Microvilli are heavily stained (arrows) as well as vesicles within the cytoplasm. × 54,000.

Fig. 4. Infiltrating duct carcinoma 10971. Free cell surfaces displaying microvilli, from this poorly differentiated carcinoma, exhibit a reduced amount of less intense PTA-positive material (arrows) when compared to luminal surfaces of benign cells seen in Fig. 3. Lysosome-like structures within the cell appear heavily stained. × 54,000.
Fig. 5. Lateral surfaces of nonmalignant duct epithelium exhibit a continuous layer of PTA-positive surface coat material (arrows). X 42,000.

Fig. 6. Infiltrating duct carcinoma 5487. Opposed cell surfaces are practically devoid of PTA-positive surface coat materials. A spot of stain is present where the cells are closely apposed (arrow), and stain is also present over some cytoplasmic vesicles. X 41,000.
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