Changes in the Affinity of Phosphotungstic Acid and Positively Charged Colloidal Particles for the Surfaces of Malignant Human Transitional Epithelium of the Urinary Bladder

Gerald B. Dermer and William H. Kern

Department of Pathology, Hospital of the Good Samaritan Medical Center, Los Angeles, California 90017

SUMMARY

Phosphotungstic acid at low pH and colloidal iron hydroxide have been used to stain sialic acid residues at the surfaces of the transitional epithelium that covers the human urinary bladder. The staining of surfaces of normal epithelium was compared in the electron microscope to staining of surfaces of cells from noninvasive Grade I transitional cell carcinomas and from higher grades of invasive transitional cell carcinomas. Surfaces of normal epithelium and epithelium within the very-well-differentiated Grade I transitional cell carcinomas exhibit similar levels of affinity for each stain. The surfaces of cells of higher grade invasive bladder carcinomas exhibit about a 40% reduction in affinity for the stains. Also, the interdigita-
tions of the plasma membranes of epithelium from normal biopsies and Grade I carcinomas are absent in the invasive carcinomas. These observations indicate that surface sialic acid may have a role in intercellular adhesion and in stabilizing some surface structures of cells.

INTRODUCTION

It was recently reported (7) that malignant human breast epithelial cells have less PTA-positive material at their surfaces than does normal human breast epithelium. Since cell-surface staining by PTA at low pH appears to be due primarily to terminal sialic acid residues of glycoproteins (8), it was concluded that malignant human breast epithelium is deficient in surface sialic acid. Reductions in sialic acid levels (5, 11, 19-21, 26) have been demonstrated at the surfaces of some lines of virus-transformed cells in culture, which has led to the view that transformation results in the synthesis of incomplete surface polymers.

In this communication we report our observations of the surface staining of cells from normal human transitional epithelium of the urinary bladder, Grade I noninvasive transitional cell carcinomas, and higher grades of invasive, transitional cell carcinomas. In addition to acidic PTA, we have investigated the cell-surface affinity of another stain specific for sialic acid, CIH (9, 18, 25).

MATERIALS AND METHODS

This study deals with observations from 40 human urinary bladder biopsies, including 19 Grade I noninvasive papillary transitional cell carcinomas, 13 higher grade invasive papillary and nonpapillary transitional cell carcinomas, 1 squamous cell carcinoma, and 7 normal bladder mucosas. The cases were classified pathologically and cytologically by 1 observer (W. H. Kern). None of the cases that were classified as Grade I carcinomas showed evidence of invasion. Some might be considered to represent transitional cell papillomas but for the purposes of this study the Grade I carcinomas were not further divided.

Each biopsy was placed immediately in a 2.0% glutaraldehyde-0.2 M sodium cacodylate buffer solution (pH 7.3), cut into 1-cu mm pieces, and fixed for 1 to 2 hr. Some of the pieces were then embedded in the water-soluble plastic GMA (16) for subsequent staining of thin sections with PTA or CIH. Other pieces were postfixed for 1 hr in a solution of 1.0% OsO4 in cacodylate buffer and then rapidly dehydrated in increasing concentrations of acetone and embedded with Vestopal W for examination of cell ultrastructure. The Vestopal-embedded sections were stained with the standard combination of uranyl acetate followed by lead citrate.

The 1st step in the analysis of each biopsy was to prepare toluidine blue-stained sections from the GMA blocks until areas typical of the diagnosis were found. Some thin sections cut from these areas were stained with PTA or CIH. Other pieces were postfixed for 1 hr in a solution of 1.0% OsO4 in cacodylate buffer and then rapidly dehydrated in increasing concentrations of acetone and embedded with Vestopal W for examination of cell ultrastructure. The Vestopal-embedded sections were stained with the standard combination of uranyl acetate followed by lead citrate.

The 1st step in the analysis of each biopsy was to prepare toluidine blue-stained sections from the GMA blocks until areas typical of the diagnosis were found. Some thin sections cut from these areas were stained with PTA, while others were stained with CIH. Five to 10 randomly chosen areas within these sections were then photographed at low magnification in the electron microscope, and the intensity of the surface-staining reactions was evaluated visually on the micrographs on a scale from 0 to 3+. The evaluation of surface staining was performed at 1 time after sets of micrographs from the 40 biopsies had been collected and in a manner that precluded knowledge of the pathological diagnosis of each biopsy.

GMA sections were stained with 3.0% PTA (7, 8) at a pH of 1.5 for 15 min, rinsed briefly in water, and air dried.
Other sections were stained with CIH prepared according to the method of Nicolson (18). The pH of the CIH solution was adjusted to 1.6. Thin sections were transferred with a wire loop and floated on the surface of the CIH solution. The optimal staining time was found to be 7 min. After staining, the sections were rinsed briefly in 12% acetic acid followed by 2 rinses in distilled water for a total of 30 min. Sections were then mounted on carboned, Formvar-coated grids and air dried. All sections were examined in an Hitachi 8S electron microscope.

RESULTS

A summary of the data concerning cell-surface staining by PTA and CIH is given in Table 1. Within each group the average level of staining of cell surfaces by PTA is similar to that exhibited by CIH. This is a reflection of the fact that within each biopsy the level of staining of cell surfaces by PTA or CIH is similar. The small differences in the levels of PTA and CIH staining in normal bladder mucosas and higher grade carcinomas are not significant. Surfaces of normal epithelium and epithelium within Grade I carcinomas exhibit a similar affinity for PTA or colloidal iron. Although there is some overlap, epithelium within Grades II, III, or IV invasive bladder carcinomas exhibits about 60% of the number of PTA or iron-binding sites at cell surfaces as do normal epithelium or Grade I carcinomas. Cells of the 1 squamous cell carcinoma, not included in the data in Table 1, exhibit less than 60% of these sites.

The ultrastructure of the intermediate layers of human bladder transitional epithelium found in normal biopsies and in Grade I carcinomas is similar, and is illustrated in Fig. 1a. Characteristically, the plasma membranes of the cells are deeply interdigitated. Similar areas from these biopsies within GMA sections stained with PTA (Fig. 1b) or CIH (Fig. 1c) show that all cell surfaces are covered with many binding sites for the stains. The reaction of cell surfaces with PTA is generally intense and sharp. Almost continuous reactive lines are found between cells. With CIH, many closely packed, positively charged colloidal particles are found at cell surfaces. Cytoplasmic structures such as Golgi sacculles, some vesicles, and lysosomes also react with PTA and CIH.

Generally, biopsies of higher grade carcinomas exhibit cells (Fig. 2a) the plasma membranes of which are not greatly interdigitated. Furthermore, the surfaces of these cells are poorly stained by PTA (Fig. 2b) or CIH (Fig. 2c).

DISCUSSION

It appears that cell-surface staining by PTA is due to the conversion at low pH of sialic acid nitrogen to ammonia (10), which reacts vigorously with PTA. At pH 1.6 the CIH-binding sites at surfaces of cells are predominantly carboxyl groups of sialic acid (9, 18, 25). The observation that in each biopsy cells exhibit similar levels of surface staining with PTA or CIH is further evidence that both are reacting with the same component, namely, terminal sialic acid residues of oligosaccharide chains of glycoproteins (10, 17) and possibly glycolipids (12) located at external surfaces of plasma membranes (3). Although the carbohydrate-containing portions of these macromolecules extend outward from the plasma membrane, other regions of these molecules free of carbohydrate are embedded within the membrane (3) and thus these molecules are integral membrane components.

The similar, high levels of surface staining of cells forming noninvasive papillary carcinomas and normal transitional epithelium is consistent with our observations and those of others (22) that cells from papillomas or well-differentiated noninvasive papillary carcinomas vary little, if at all, from normal bladder epithelium. With regard to levels of surface sialic acid revealed by PTA and CIH, Grade I noninvasive carcinomas appear normal.

The cells of higher grade invasive bladder tumors generally have surfaces that stain poorly with PTA or CIH and therefore seem to contain lower amounts of sialic acid. The 40% reduction in PTA or CIH binding that we detect morphologically at the surfaces of highly malignant human bladder cells is similar to changes in levels of surface sialic acid (11, 20) detected by chemical procedures in some lines of transformed cells. It is not known whether this change is due to reduced incorporation of sialic acid into surface components, to reduced levels at cell surfaces of a large

Table 1

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Stain intensity PTA</th>
<th>Stain intensity CIH</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>1.9 ± 0.5</td>
<td>1.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Grade I noninvasive carcinomas</td>
<td>19</td>
<td>1.7 ± 0.6</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Higher grade invasive carcinomas</td>
<td>13</td>
<td>1.2 ± 0.6</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

* With respect to the corresponding value of normal bladder mucosa.
moiety containing sialic acid (K. R. Stone, personal communication; Ref. 14), to an increased turnover of surface sialic acid (12), or to a combination of these factors. Changes in the activities of glycosyl transferases (6, 15, 17) within the cells could lead to reduced incorporation of sialic acid into surface components. On the other hand, increased levels of extracellular glycosidases (1) and proteases (2) could be responsible for an acceleration of degradation of surface material. Also, glycoproteins may be easily lost from surfaces of malignant cells (13).

Reduced levels of sialic acid at surfaces of highly malignant cells could be related to the observation that neighboring cells of Grade II or higher carcinomas rarely form complex surface interdigitations. A possible connection could be that, because carboxyl groups of sialic acid are some of the fixed anionic sites of plasma membranes, they may have a role in maintaining the anatomical form of cells by stabilizing membrane structures (23). Reduced levels of anionic sites due to reduced levels of sialic acid may therefore reduce membrane stability and rigidity (23) and prevent cells from maintaining some specialized surface structures.

It may be of some value to speculate on what changes in cellular behavior could in part be brought about by reduced levels of surface sialic acid. Virtually all bladder lesions that are poorly stained with PTA or CIH were diagnosed as invasive while all Grade I carcinomas were not invasive. Therefore, reduced levels of surface sialic acid might be associated with the invasive tendency of malignant cells, perhaps by reducing the adhesiveness of cells (4, 24), although it has been reported (27) that loss of sialic acid results in reduced invasion of transformed cells in vitro.

REFERENCES

Fig. 1. In a, the plasma membranes of the intermediate layers of normal human bladder mucosa and surfaces of cells from similar areas of Grade I noninvasive transitional cell carcinomas exhibit complex interdigitations. Tissue fixed in glutaraldehyde and osmium and embedded in Vestopal W. Uranyl acetate and lead citrate stains; × 4,000. In b, surfaces of normal bladder mucosa as well as surfaces of transitional epithelium from Grade I noninvasive carcinomas are intensely stained by PTA at low pH. Tissue fixed in glutaraldehyde and embedded in GMA. × 11,000. In c, surfaces of normal bladder mucosa as well as surfaces of transitional epithelium from Grade I noninvasive carcinomas are well stained by CIH. Lines of closely packed iron particles (arrows) are found at cell surfaces. × 48,000.

Fig. 2. a, similar to Fig. 1a but from a Grade III, invasive transitional cell carcinoma. Cell surfaces are not greatly interdigitated. × 4,000. b, similar to Fig. 1b but from a Grade III, invasive transitional cell carcinoma. Cell surfaces exhibit little affinity for PTA. Arrows, spots of surface staining. × 11,000. c, similar to Fig. 1c but from a Grade III, invasive transitional cell carcinoma. Cell surfaces (faint dense line down center of micrograph indicated by arrows) exhibit little affinity for the iron stain. × 48,000.
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